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Original article

# Serum bile acids and leptin interact with glucose metabolism in patients with liver cirrhosis

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SUMMARY

*Background & aims:* We investigated possible involvements of bile acids (BA) and leptin in hepatogenous insulin resistance being present in up to 90% of cirrhotic patients.
*Methods:* Blood was analysed in 10 cirrhotic patients (8m/2f, 48 ± 10.4 yrs) and 10 controls (8m/2f, 43 ± 9.3 yrs) after oral nutrition and during 1 h of parenteral feeding. In patients, leptin was additionally analysed from mesenteric and arterial blood.
*Results:* Cirrhosis patients showed typical signs of hepatogenous insulin resistance (hyperinsulinaemia, normoglycaemia, hyperglucagonaemia). Both fasting BA ($r = .714$, $p = 0.047$) and fasting leptin ($r = .867$, $p = 0.001$) correlated to HOMA and predicted insulin response after oral feeding ($R^2$adj = .783, $p = 0.002$). But during parenteral nutrition only leptin predicted insulin response ($p = 0.005$). The prandial glucose response was negatively correlated to the BA increase after oral nutrition ($r = -.733$, $p = 0.028$) and to the change in leptin during parenteral nutrition ($r = -.738$, $p = 0.037$) pointing towards a nutritional route-dependent positive impact on glucose tolerance of both substances. Prandial glucagon response was correlated to BA under both feeding conditions ($p < 0.05$). We found no relevant intestinal release of leptin during fasting or feeding conditions.
*Conclusion:* Our results suggest a substantial involvement of BA and leptin by improving postprandial glucose tolerance related to liver cirrhosis.


## 1. Introduction

Abnormal glucose tolerance (hepatogenous insulin resistance) is frequent in patients with liver cirrhosis (LC) and a predictor of 30-day mortality in patients with decompensated LC.[1] Hepatogenous insulin resistance is characterised by mainly peripheral insulin resistance in the skeletal muscle and fat tissue, while uptake of glucose in the liver is normal or even enhanced.[2] The peripheral glucose disposal in LC patients is approximately half of that reported in healthy subjects (e.g.[3,4]). Even in the early stages of LC the incidence of glucose intolerance is high, and varies from 60 to 80% depending on the degree of liver damage.[5] The underlying causes and molecular mechanisms of hepatogenous insulin resistance and its control are still unclear.[5]

During the last few years the perceived role of bile acids (BA) underwent a substantial modification away from their constricted classic role as solvents for dietary fats to potent regulators of glucose and lipid metabolism.[6] In the present report, we were especially interested in exploring a possible impact of high circulating BA levels on glucose metabolism in LC patients. Our interest was triggered by two recent observations. First, increased BA levels after Roux-en-Y gastric bypass surgery indicate an improved glucose tolerance.[7] Second, fasting BAs are negatively associated with insulin sensitivity in adults with and without type 2 diabetes.[8]

*Abbreviations:* LC, liver cirrhosis; BA, bile acids; CA, cholic acid; DC, deoxycholic acid; CDC, chenodeoxycholic acids; HOMA, homeostasis model assessment; TIPS, transjugular intrahepatic portosystemic shunt.

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In previous investigations we showed that free leptin concentrations in LC correlate to insulin levels as well as insulin resistance.[9] Other groups investigated total leptin and found either increased (e.g.[10–12]) or normal leptin concentrations in LC (e.g.[13,14]). None of these reports focused, however, on the association of leptin and hepatogenous insulin resistance. Therefore, we also included leptin measurements in our present study.

We selected for well characterised stable LC patients with chronically elevated concentrations of circulating BA due to LC per se but additionally enforced through to the previous implantation of a transjugular intrahepatic portosystemic shunt (TIPS). The study of TIPS patients not only allowed us to evaluate the impact of significantly increased BA on glucose intolerance, but also enabled us to access the mesenteric venous circulation directly to measure viscerally released leptin.

We hypothesized that both leptin and BA are involved in basal or postprandial regulation of glucose metabolism in LC patients.

In an explorative approach we investigated a potential correlation between plasma levels of BA, leptin and glycaemic parameters in patients with LC. In comparison to healthy controls we tested these associations in 1) in the basal state, 2) after oral nutrition with an expected postprandial increase of BA and 3) during parenteral nutrition with an expected prandial inertness of BA response. Our experimental set-up allowed us to evaluate differences in the venous, arterial and mesenteric blood and to estimate intestinal uptake versus peripheral release of leptin. A better understanding of the control of glucose metabolism in LC may bear direct significance for patient care by possibly stimulating further clinical studies, drug development or nutritional strategies.

**Table 1**
General characteristics of patients and controls.

| | Patients ($n = 10$) | Controls ($n = 10$) | $p$-value |
|---|---|---|---|
| | Mean (SD) | Mean (SD) | |
| *General characteristics* | | | |
| Male/female | 8/2 | 8/2 | |
| Age (years) | 48 (10.4) | 43 (9.4) | .256 |
| Height (cm) | 175 (9.9) | 180 (8.2) | .226 |
| Weight (kg) | 78.5 (7.6) | 85.3 (9.6) | .064 |
| BMI (kg/m$^2$) | 25.7 (2.9) | 26.3 (3.5) | .733 |
| ASAT (U/L) | 29.0 (14.1) | 36.1 (10.3) | .197 |
| ALAT (U/L) | 22.5 (8.0) | 21.3 (6.6) | .807 |
| Bilirubin (mg/dL) | 1.42 (.7) | .56 (.3) | **0.003** |
| Prothrombin time (%) | 68 (11.6) | 89 (1.9) | **<0.001** |
| Cholesterol (mg/dL) | 199 (11) | 203 (31) | .786 |
| Body cell mass (kg) | 29 (5.5) | 35 (4.9) | **0.027** |
| Fat mass (kg) | 18 (7.9) | 20 (8.1) | .773 |
| *Glucose metabolism* | | | |
| HOMA | 3.58 (2.29) | 1.59 (.99) | **0.016** |
| Glucose (mg/dL) | 83.3 (15.2) | 76.8 (8.4) | .063 |
| Insulin (μIU/L) | 18.3 (11.6) | 6.6 (4.0) | **0.016** |
| Glucagon (ng/L) | 87.1 (56.6) | 39.8 (14.2) | **0.014** |
| C-peptide (ng/mL) | 1.91 (.90) | 1.39 (.27) | .353 |
| Free fatty acids (mg/dL) | 31.7 (10.4) | 16.4 (4.9) | **0.002** |
| *Bile acids and leptin* | | | |
| Total BA (μmol/L) | 99.9 (78.2) | 2.77 (2.34) | **<0.001** |
| Sum CAs (μmol/L) | 46.9 (45.6) | .55 (.67) | **<0.001** |
| Sum CDCs (μmol/L) | 41.6 (28.5) | 1.25 (1.03) | **<0.001** |
| Sum DCs (μmol/L) | 11.1 (9.9) | .96 (.89) | **<0.001** |
| Total leptin (ng/mL) | 15.3 (18.8) | 8.9 (8.5) | .327 |

BMI: body mass index, ASAT: aspartate amino transferase; ALAT: alanine amino transferase; BA: bile acids; CAs: conjugated and unconjugated cholic acids; CDC: conjugated and unconjugated choledeoxycholic acids; DCs: conjugated and unconjugated deoxycholic acids. Statistically significant values are shown in bold face.

## 2. Materials and methods

### 2.1. Methods

#### 2.1.1. Subjects

All subjects gave written informed consent before entering the study and the study protocol was approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin.

*2.1.1.1. LC patients (see Table 1).* Eight men and two women with diagnosed biopsy-proven LC and in situ porto-systemic shunting via TIPS due to previous intractable complications (recurrent variceal bleeding or refractory ascites) were recruited while in hospital for a routine control of TIPS patency. We purposely selected a subgroup of patients with stable clinical condition (defined by lack of gastrointestinal bleeding in the last two months, absence of ascites and ASAT/ALAT levels lower than three times the upper normal limit) to increase group homogeneity and diminish a possible metabolic interference caused by different stages of hepatic decompensation. All patients had an alcohol-dependent pathogenesis of liver disease and were abstinent from alcohol for at least six months. Routine controls for TIPS patency allowed access to the mesenteric vein. Patients were on average 33 ± 31 months after TIPS insertion (range 5.7–83.4 months). Portosystemic pressure gradients were measured during routine TIPS portography immediately before the study as previously described.[15] Patients with diagnosed diabetes or thyroid dysfunction were excluded from the study. None of the patients were on beta-blocker therapy or received any other drugs that potentially affect insulin resistance. Child-Pugh status was A in 9 patients and B in 1 patient.

*2.1.1.2. Controls (see Table 1).* Eight men and two women (43 ± 9 years of age) were recruited as volunteers. Health was defined by physical examination, absence of acute or chronic disease or any acute or chronic medication. All routine blood levels were within the normal range.

#### 2.1.2. Study implementation (see also Supplemental Figure 1)

Body composition was determined in all subjects one day prior to the catheterisation study and all subjects had fasted overnight when the study started. Patients were investigated immediately after TIPS angiography with the mesenteric catheter still in place. All subjects were kept in the supine position for at least 30 min before sampling baseline blood until the end of the investigational period. Subjects were not allowed to drink or eat except nutrition according to the protocol.

Blood was drawn at the same intervals from the cubital vein in controls and in patients simultaneously from the cubital vein, upper mesenteric vein, and radial artery. Blood was sampled at the oral baseline, and thereafter in either 15 min or 30 min intervals until 60 min after oral stimulus. At 240 min of the study protocol we sampled blood for the parenteral baseline, thereafter started the 60 min infusion of parenteral nutrition and continued blood sampling either at 15 min or 30 min intervals until the end of the infusion (details see Supplemental Figure 1).

*Oral nutrition*: After blood sampling at 0 min, subjects drank 200 ml of an oral supplement (Biosorb®, Fresenius-Kabi, 300 kcal, 12.0 g protein, 36.8 g carbohydrates, and 11.6 g fat).

*Parenteral nutrition*: A 60 min infusion of parenteral nutrition (Fresenius-Kabi, Trimix perikal®) started immediately after blood sampling at 240 min of the study protocol. The energy supplied was 150% of previously measured resting energy expenditure divided by 24 h to receive the requirements for 1 h (90–150 kcal).

See online supplement for information on catheterisation techniques, body composition analysis and biochemical analyses.

**Statistics**: The data were analysed using PASW v18.0 software (SPSS, Chicago, IL, USA). $P$ values below .05 were considered

statistically significant. Since the nature of this study was exploratory rather than confirmatory, no precautions for multiple testing (e.g. Bonferroni adjustment) were taken.[16] Data are presented as mean ± standard deviation unless indicated otherwise. Data showed skewedness for some parameters, therefore, we consistently used non-parametric tests (Mann–Whitney's rank sum test for unpaired data, i.e. comparison of patients and controls; Wilcoxon test for paired data, i.e. comparison between different points in time). We used Spearman correlation coefficient to evaluate associations between two parameters. Areas under the curve (AUC) were calculated using the trapezoidal rule.

## 3. Results

### 3.1. Baseline characteristics (Table 1)

Compared to healthy controls patients had higher bilirubin levels, lower coagulation capacity (prothrombin time) and decreased body cell mass (BCM). Fasting glucose was normal in patients but HOMA, insulin, glucagon and free fatty acids were increased. These changes are typical for hepatogenous insulin resistance.[5] Liver parameters (ALAT, ASAT, bilirubin, prothrombin time) did not correlate to HOMA (data not shown). As expected, all serum bile acids (BA) were significantly higher in patients. Baseline total leptin concentration was normal.

### 3.2. Baseline correlations (Table 2)

At baseline in patients both BA and leptin correlated to HOMA, while only leptin was significantly associated with insulin concentration and insulin release as estimated by C-peptide. Only BA correlated significantly with glucagon levels which showed the expected positive relation to fasting free fatty acids ($r = .745$, $p = 0.021$). We found interrelations between leptin and BA between $r = .571$ and $r = .714$ depending on the BA subgroup. BA or leptin did not correlate to glycaemic values in controls.

### 3.3. Postprandial course (Figure 1)

As compared to controls the glucose response of patients was impaired after a standardised oral nutritional stimulus (Fig. 1A). In both feeding protocols insulin showed an excessive increase (Fig. 1B), while pancreatic beta–cell activity estimated from C-peptide levels was similar to controls except for an attenuated increase with carry-over effect to baseline before the parenteral stimulus (Fig. 1C). The initially increased glucagon concentration rapidly decreased within 15 min after oral feeding (Fig. 1D). High baseline free fatty acids dropped sharply after the oral nutritional stimulus in patients (Fig. 1E) and were not statistically different from controls during parenteral nutrition. As expected, BA levels (Fig. 1F) postprandially increased after an oral stimulus both in patients (15 min and 30 min, $p < 0.05$ from baseline) and controls (30 min, $p < 0.05$ from baseline) and remained unchanged during parenteral nutrition. We observed no significant change in leptin concentration during both feeding periods (Fig. 1G). There was an unexplained high leptin concentration in one patient of 66 ng/mL underlying the slightly higher mean leptin concentrations in the patient group.

The prandial response to most measured glycaemic markers was significantly weaker during parenteral nutrition compared to oral nutrition in both patients and controls (1A–E). This was in contrast to BA or leptin, where response to oral versus parenteral nutrition was not significantly different, (1F–G) although a cumulative BA increase was noted in 6 patients after oral stimulus and in 2 patients following parenteral nutrition.

### 3.4. Postprandial correlation

We next explored if baseline BA or leptin related to the prandial response (AUC) of glycaemic markers in patients (Table 3-1). We found significant relations of baseline BA and leptin to AUC insulin after oral nutrition, while during parenteral nutrition only leptin showed a borderline association with AUC insulin. C-peptide showed borderline relations to leptin ($p = 0.060$) and deoxycholic acid ($p = 0.058$) in the oral period and interrelated significantly to leptin in the parenteral period (in which deoxycholic acid was not analysed) pointing towards an axis of action on pancreatic β-cells for both molecules. AUC glucagon was related to baseline BA after oral nutrition and there was a trend for a similar relationship during parenteral nutrition. However, we did not observe the same evidence in healthy controls (Supplement Table 1).

**Table 2**
Spearman correlation matrix (2-tailed) of baseline values.

| | HOMA | Glucose | Insulin | Glucagon | C-peptide | FFA | BA total | CA | CDC | DC |
|---|---|---|---|---|---|---|---|---|---|---|
| **Patients** | | | | | | | | | | |
| BA total | .714* | .060 | .619 | .750 | .214 | .599 | | | | |
| CA | .738* | .168 | .595 | .786* | .238 | .335 | .833* | | | |
| CDC | .595 | -.084 | .524 | .750 | .048 | .455 | .952** | .792* | | |
| DC | .643 | .000 | .595 | .464 | .282 | .563 | .905** | .595 | .857** | |
| leptin | .867** | -.261 | .867** | .583 | .745* | .097 | .667 | .690 | .571 | .714* |
| **Controls** | | | | | | | | | | |
| BA total | .195 | .584 | .188 | -.104 | .147 | .139 | | | | |
| CA | .043 | .480 | .128 | -.031 | .331 | .115 | .903** | | | |
| CDC | .006 | .134 | .036 | .239 | .086 | .248 | .709* | .685* | | |
| DC | .383 | .523 | .255 | -.337 | .160 | .067 | .745* | .612 | .236 | |
| leptin | .361 | -.477 | .408 | -.265 | .074 | .251 | .008 | .025 | -.192 | .243 |

Grey areas show either *: $p \leq 0.05$; **$p \leq 0.01$ or borderline significance (grey only, $p < 0.09$).
FFA = free fatty acids, BA = bile acids, CA = cholic acids, CDC = chenodexoycholic acids, DC = deoxycholic acids.

We then performed linear regression analysis with BA and leptin to identify independent predictors of insulin response (Fig. 2, lower part). For the oral period, F-test indicated that 78.3% of the variance in AUC insulin was explained by baseline BA or baseline leptin values. Although leptin missed statistical significance as an independent predictor, these results point towards a role of BA and potentially also leptin in the regulation of the postprandial insulin increase in LC patients. However, in the absence of a BA increase during parenteral nutrition the only independent predictor for the insulin response was leptin (Fig. 2, lower right part).

Next we investigated if the prandial changes (delta AUCs) of BA or leptin correlated with the prandial changes (delta AUCs) in glycaemic markers in patients (Table 3-2) and controls (Supplement Table 1). Interestingly, then not insulin but glucose correlated to BA in the oral period and to leptin in the parenteral period, both in an inverse fashion. The association between BA and glucagon levels was again evident. Controls failed to show these correlations (Supplement Table 1). The findings in patients are shown in Figs. 2 and 3.

### *3.5. Differences between mesenteric, arterial and venous leptin concentration*

No significant intestinal release was observed for leptin.

## 4. Discussion

The **first main result** of the present study suggests that BA are either directly or indirectly involved in the counterregulation of peripheral insulin resistance targeting glucose, insulin and glucagon metabolism. Interestingly the enteral nutritional route is apparently necessary for the BA effects on glucose and insulin, while the relation to glucagon even exists during parenteral nutrition. The **second main result** is the direct or indirect involvement of leptin in the regulation of glucose and insulin metabolism in a fashion similar to BA. However, during enteral nutrition the effects of leptin seemed to be partly obscured by the effects of BA and were only clearly visible during parenteral nutrition in the absence of BA release and any intestinal surface contact of nutrients. Furthermore, we found no relevant intestinal release of leptin. **In summary** our results reveal a surprising and complex interrelationship between BA, leptin and glycaemic parameters that suggest a substantial involvement of BA and leptin in the control of glucose metabolism in LC.

LC patients in our study expressed typical signs of **hepatogenous insulin resistance** exemplified by hyperinsulinaemia, normoglycaemia, and hyperglucagonaemia.[5] Normal baseline C-peptide indicated unaltered pancreatic beta cell release of insulin, which was confirmed by a normal 60 min postprandial increase of

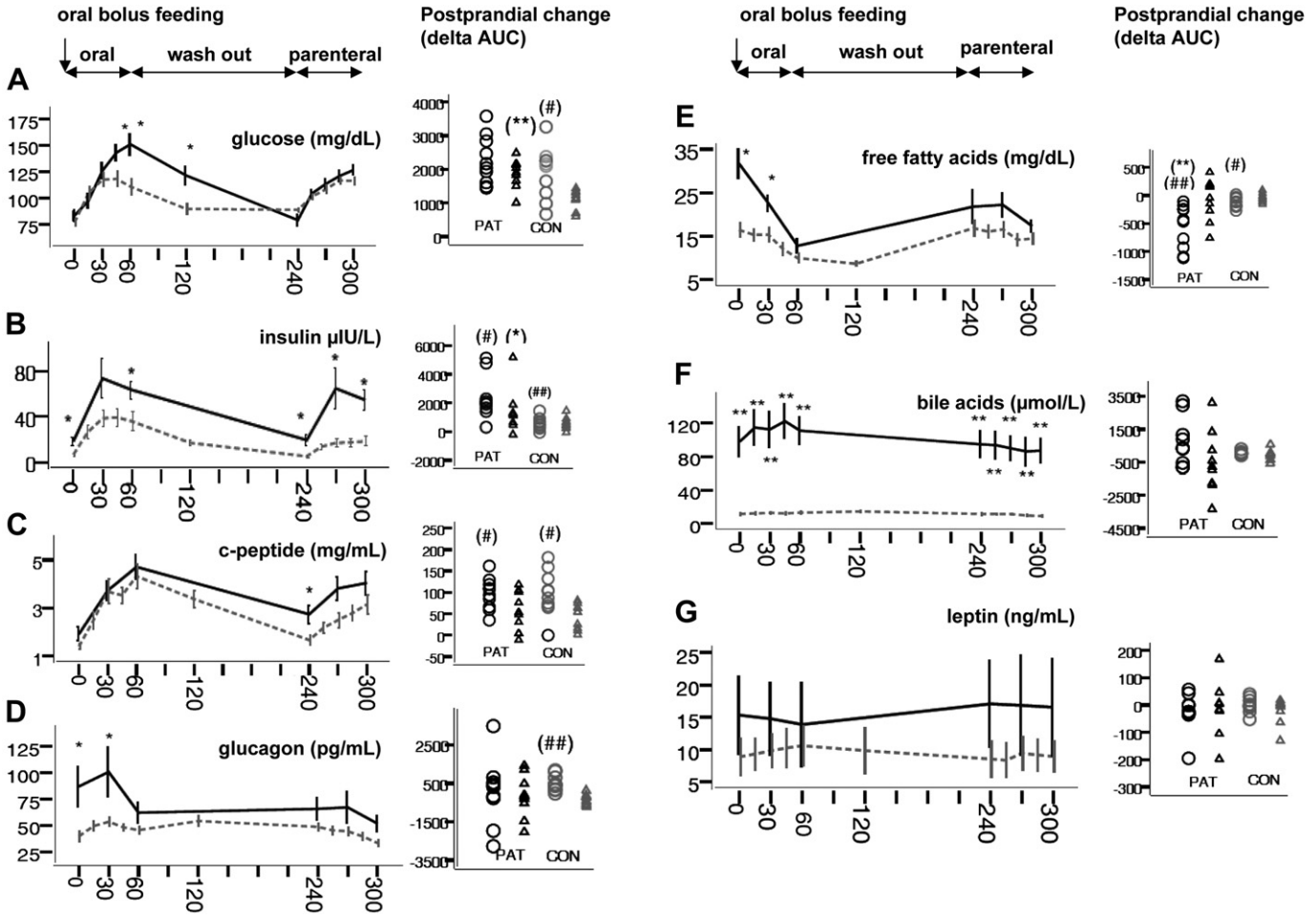



**Fig. 1.** Displays the course of glycaemic parameters, leptin and bile acids after oral intake of a 300 kcal nutritional supplement (min 1–60) and continuous parenteral nutrition (min 240–300). Bold lines: patients; broken lines: controls. The integral of prandial change is depicted in the adjacent right plots: black circles: oral period in patients; grey circles: oral period in controls; black triangles: parenteral period in patients; grey triangles: parenteral period in controls. #, ## = $p < 0.05$, <0.01 between oral and parenteral period; *, ** = $p < 0.05$, <0.01 between patients and controls.

**Table 3**
Prandial relations of BA or leptin to glycaemic markers.

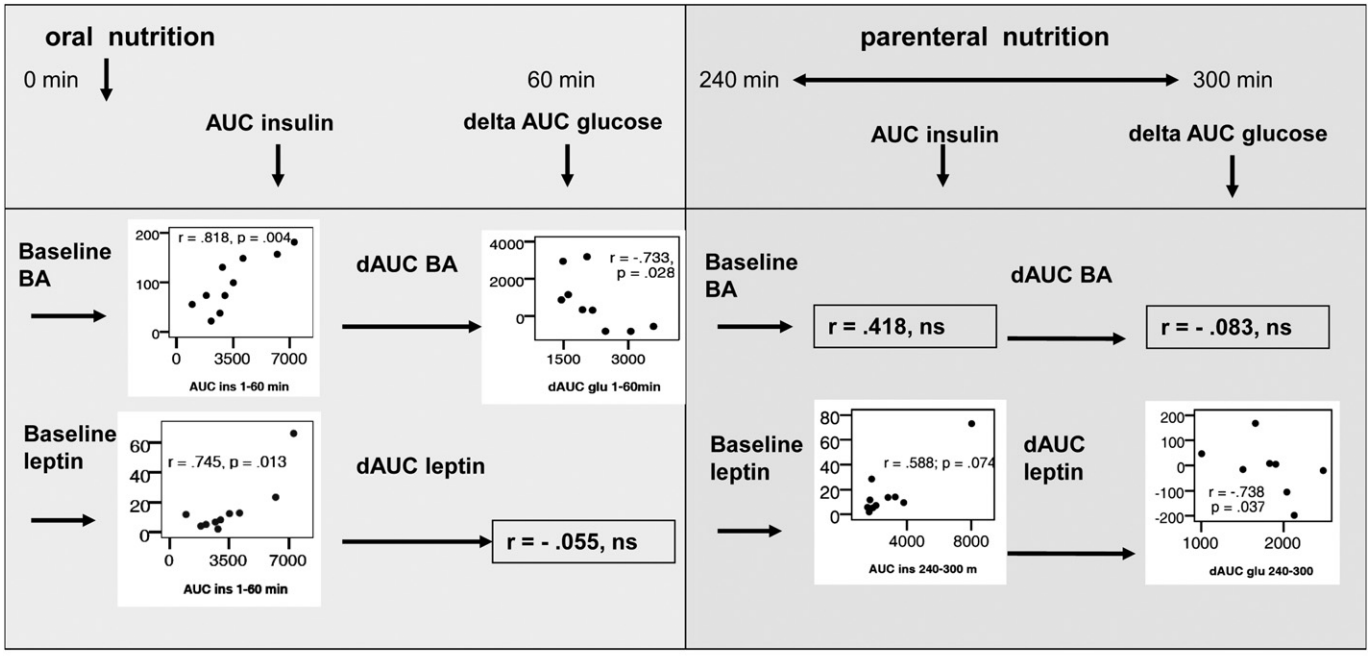
1) Do baseline levels of BA or leptin relate to the prandial AUC of glycaemic markers?

| | Oral period | | | | | | | | Parenteral period | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AUC glucose | | AUC insulin | | AUC C-peptide | | AUC glucagon | | AUC glucose | | AUC insulin | | AUC C-peptide | | AUC glucagon | |
| | ρ | p | ρ | p | ρ | p | ρ | p | ρ | p | ρ | p | ρ | p | ρ | p |
| Fasting Total BA | −.321 | ns | .818 | .004 | .491 | ns | .667 | .050 | −.248 | ns | .418 | ns | .367 | ns | .663 | .067 |
| Fasting CA | .048 | ns | .786 | .021 | .452 | ns | .786 | .036 | | | | | | | | |
| Fasting CDC | −.357 | ns | .857 | .007 | .548 | ns | .857 | .014 | | | | | | | | |
| Fasting DC | −.286 | ns | .881 | .005 | .690 | .058 | .536 | ns | | | | | | | | |
| Fasting Leptin | −.079 | ns | .745 | .013 | .612 | .060 | .467 | ns | .273 | ns | .588 | .074 | .700 | .036 | .483 | ns |

2) Does the prandial change in BA or leptin relate to the prandial change in glycaemic markers?

| | Oral period | | | | | | | | Parenteral period | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | dAUC glucose | | dAUC insulin | | dAUC C-pept. | | dAUC glucagon | | dAUC glucose | | dAUC insulin | | dAUC C-pept. | | dAUC glucagon | |
| | ρ | p | ρ | p | ρ | p | ρ | p | ρ | p | ρ | p | ρ | p | ρ | p |
| dAUC Total BA | −.733 | .025 | −.067 | ns | .000 | ns | .762 | .028 | .083 | ns | −.467 | ns | −.217 | ns | .810 | .015 |
| dAUC CA | −.762 | .028 | .190 | ns | .000 | ns | .714 | .071 | | | | | | | | |
| dAUC CDC | -.762 | .028 | .048 | ns | .167 | ns | .857 | .014 | | | | | | | | |
| dAUC DC | −.667 | .071 | .000 | ns | .143 | ns | .786 | .036 | | | | | | | | |
| dAUC Leptin | −.055 | ns | −.079 | ns | .600 | .067 | .533 | ns | −.738 | .037 | −.476 | ns | −.190 | ns | .238 | ns |

ρ = Spearman correlation coefficient; Statistically significant values ($p < 0.05$) or values with borderline significance ($p < 0.08$) are shown in bold face. AUC ORAL refers to the total area under the curve until 60 min after oral intake (time 1–60 min). AUC PARENTERAL refers to the total area under the curve for the 60 min of continuous parenteral nutrition (time 240–300 min). dAUC = delta area under the curve and refers to the nutrition-induced cumulative increase of the respective parameter. ORAL refers to the first 60 min after oral intake (time 1–60 min). PARENTERAL refers to the total area under the curve for the 60 min of continuous parenteral nutrition (time 240–300 min).




**PREDICTION OF INSULIN RESPONSE (AUC insulin)**

**Oral**

| | R | R² adj | F-test | β-weight | p |
|---|---|---|---|---|---|
| **BL BA** | | | | .520 | **.040** |
| BL Leptin | | | | .482 | .052 |
| | .912 | .783 | **.002** | | |

**Parenteral**

| | R | R² adj | F-test | β-weight | p |
|---|---|---|---|---|---|
| BL BA | | | | .099 | .707 |
| **BL Leptin** | | | | .950 | **.007** |
| | .882 | .779 | **.005** | | |

**Fig. 2.** Both fasting leptin and BA predict insulin response after oral nutrition with the postprandial delta AUC of BA but not leptin correlating negatively to the postprandial response of glucose pointing towards better glucose tolerance with increased postprandial BA response. During parenteral nutrition, however, where prandial increase of BA did not occur, relations are focused on leptin only, both for prediction of prandial insulin response and negative relation of postprandial delta AUCs between leptin and glucose.

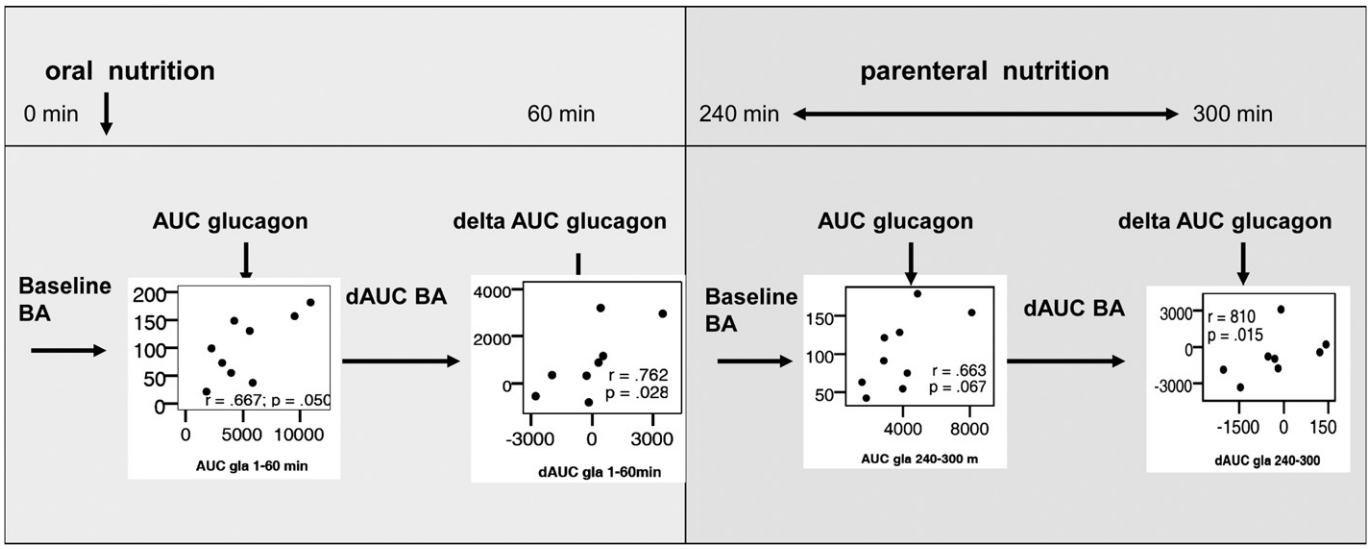



**Fig. 3.** In contrast to insulin, relations to glucagon were focused solely on BA and where unaffected by the nutritional route of administration. Both baseline BA and delta AUC of BA correlated to AUC and delta AUC of glucagon during both feeding periods pointing to a direct link of BA and glucagon excretion.

C-peptide compared to controls. Interestingly, 4 h after oral nutrition, C-peptide concentration was still significantly higher in patients compared to controls indicating a more sustained release of insulin. These findings are in line with previous studies reporting that hyperinsulinaemia in LC patients is mainly caused by diminished peripheral uptake and degradation, reduced hepatic insulin extraction and portosystemic shunts and less by increased pancreatic insulin production.[5] As observed in liver disease before[17] our results suggest a dominant effect of hyperglucagonaemia on tissue lipolysis in patients. Summarised, patients showed metabolic pathologies typical for LC patients and provided a good model for investigating interactions with BA and leptin.

With regard to our **main findings** the following molecular signal transductions might explain the associations we discovered.

**In regard to BA** there is interesting new evidence that activation of the G-protein coupled BA receptor TGR5 contributes to the maintenance of glucose homeostasis and insulin sensitivity, preservation of liver and pancreatic functions, and resistance to weight gain and steatosis.[6] These effects are due to enhanced mitochondrial function in enteroendocrine cells, muscle, and brown adipose tissue, leading to increased incretin secretion and energy expenditure.[6,18,19] In the human intestine, BA binding to TGR5 induces the release of the incretin glucagon-like peptide −1 (GLP-1) from enteroendocrine L cells after nutrient ingestion[20] by the closure/opening of the $K_{ATP}/Ca_v$ channels through modulation of mitochondrial oxidative phosphorylation and a subsequent change in the ATP/ADP ratio.[6] GLP-1 enhances postprandial insulin secretion and thus improves insulin sensitivity.[20] Increased postprandial insulin secretion measured by C-peptide was not observed in our patients in the immediate 1 h postprandial period, but notably there was the significant higher C-peptide level at baseline before the parenteral feeding period 4 h after oral intake suggesting a sustained stimulation of insulin production and a carry over effect from the oral nutritional period. Stimulation of GLP-1 secretion might explain this observation and might also account for the association between BA and insulin or glucose only after enteral (oral) nutrition but not during parenteral nutrition in our patients. Interestingly, two previous studies show that induction of GLP-1 secretion by direct access of increased BA concentrations to L-cell-rich regions of the intestine may contribute to the rapid remission of type 2 diabetes after gastric bypass surgery.[7,21] Furthermore and in line with our results, one of the two studies[7] demonstrates an inverse relationship between total BA and the 2 h postprandial glucose levels in patients, further indicating that BA may be involved in the glucose homeostasis in humans. Also activation of BAs second main receptor, the nuclear receptor FXR, was previously reported to improve insulin sensitivity in humans.[22,23] FXR-mediated actions of BA appear mainly to affect liver metabolism by increasing glycogen production and decreasing lipogenesis and VLDL production, thereby reducing hepatic glucose and fatty acid output.[22] But recent studies demonstrate 1) insulin resistance and hyperglycaemia in FXR knockout mice,[23] 2) decreased whole-body glucose disposal in FXR-deficient mice[24] and 3) decreased serum glucose and improved insulin sensitivity in diabetic db/db mice fed with a synthetic FXR agonist[25] thus suggesting also a role of FXR in regulating peripheral glucose metabolism.

Next we enquired if there is a molecular link between **BA and glucagon** that could explain the correlations we observed in LC patients (see Fig. 3). The glucagon receptor (GR) belongs to the B family of G protein-coupled receptors and is up-regulated by glucose and down-regulated by glucagon as well as agents that increase intracellular cAMP.[26,27] Interestingly, BA increase intracellular cAMP by binding to TGR5. *In vitro* studies showed that foremost dihydroxy BA like chenodeoxycholic acid can stimulate phosphorylation, heterogenous desensitisation and thus degradation of GR via a potential PKCα activation[28] leading to reduced cAMP production. This desensitisation weakens the effects of glucagon, and should decrease cytoplasmatic internalisation and degradation of this hormone.[26] Thereby, glucagon plasma levels remain high dependent on circulating BA levels. But the BA effect is dose-dependent and BA concentrations of higher than 10 μM are necessary to exhibit the effect, which likely explains the absent correlation in our healthy controls where CDCA concentrations were approximately 1 μM and thus too low.[26] On the other hand, most cell studies were done with 50 μM,[26] which is close to the plasma concentration of chenodeoxycholic acid (CDCA) in our patients. In addition, glucagon seems to regulate hepatocellular uptake, transport, and secretion of BA in the liver through a cross-talk between the same signal-transduction pathways mentioned above, exerting effects on calcium mobilisation, cAMP synthesis and protein kinase C (PKC) activation.[29]

**Leptin** is known to exert direct actions on the pancreas and leptin receptors are present in pancreatic β-cells.[30] Leptin specifically inhibits glucose-stimulated insulin secretion via the cyclic

adenosine monophosphate (cAMP)/protein kinase A (PKA) or phospholipase C/protein kinase C (PKC) pathways.[30,31] We, however, found positive (and not negative) associations between leptin and insulin in our patients under fasted and fed conditions. This can be explained by complementary mechanisms. Not only does leptin downregulate insulin, but insulin itself stimulates both leptin biosynthesis and secretion from adipose tissue establishing a classic endocrine adipo-insular feedback loop that might be more prominent in the presence of hyperinsulinaemia.[32] Furthermore, leptin resistance[9] and elevated bound leptin[33] were previously reported in LC patients and might contribute to inactivate the leptin-induced reduction of insulin secretion in β-cells. In fact, in mice the acute disruption of leptin signalling resulted in hyperinsulinaemia and insulin resistance as were present in our LC patients.[34]

We found no intestinal release of leptin at baseline or under nutritional stimulus, which parallels the results of Wiest and colleagues, the only other study that simultaneously explored adipokine concentration differences in arterial, hepatic venous, portal venous and peripheral blood in LC and healthy humans so far.[13]

## 5. Strength and limitation

Investigating a homogeneous group of TIPS patients with normal portal pressure and compensated LC allowed us not only to investigate splanchnic blood concentration through mesenteric access laid during routine TIPS controls immediately before investigation. It also provided a model characterised by controlled shunting of the liver. Insulin clearance or glucose levels do not change after TIPS.[35] Thus our patients present with metabolic changes typical for LC in general. Because patients were similar in the severity and origin of disease, the impact of these parameters could not be evaluated, but our results and previous authors[13] confirmed that the concentration of leptin is not correlated with portal-caval pressure gradient or with Child Pugh stage.[9,13] We evaluated subgroups of BA only in the enteral period, changes and associations during parenteral nutrition should be the subject of future studies. In addition, GLP-1 was not measured in the present study to support the hypothesis outlined above. Although so far there is limited evidence for the role of chronic inflammation in the development of hepatogenous insulin resistance we cannot exclude a causal involvement of inflammation in this process.

## 6. Conclusions

In LC according to our results BA and leptin seem to be independently involved in glucose metabolism. From our results and as suggested by previous authors[7,30] BA and leptin seem to stimulate postprandial insulin sensitivity. According to our results BA may to act dominant over leptin. Normally, improving peripheral insulin resistance is a protective mechanism. However, in LC we cannot exclude the contrary. In LC peripheral insulin resistance leading to prolonged postprandial hyperglycaemia could also be an adaptive mechanism to channel glucose to the liver, an essential substrate to limit hepatic gluconeogenesis, to enable glycogenesis, and many other biosynthetic and regenerative processes. This latter speculative scenario could contribute to explain the normal or even increased hepatic glucose uptake consistently found in LC patients (e.g.[2,36]).

Based on our results with associations seen also to cholic and deoxycholic acid we hypothesise that BA in our study exerted their effects more likely through the TGR-5 receptor than through the FXR-receptor in the liver, or possibly through the contribution of both. Since BA was only associated to glycaemic parameters after oral nutrition, but not during parenteral nutrition, BA signalling in the intestine or postprandial BA increase may be necessary to achieve these effects.

Our data suggest that the effects of BA and leptin on glucose metabolism in LC are complex. A further delineation of the underlying mechanisms and signalling pathways would require additional in-depth pre-clinical approaches.

## Statement of authorship

All authors have made substantial contributions and approved the final version of the manuscript.

Role of each author: JO = conception and study design; TS, SG, AO, EK, TK, UJFT = generation and collection of data; LV = Analysis and interpretation of data; LV, JO, UJFT, JDS, GB, HL = drafting or revision of the manuscript.

## Conflict of interest

The authors reported no conflict of interest.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clnu.2012.06.006.

## References

1. Hagel S, Bruns T, Herrmann A, Stallmach A, Schmidt C. Abnormal glucose tolerance: a predictor of 30-day mortality in patients with decompensated liver cirrhosis. *Z Gastroenterol* 2011 Mar;**49**(3):331–4.
2. Johansson U, Wahren J, Eriksson LS. Splanchnic and peripheral glucose metabolism in cirrhosis. *J Hepatol* 1994 Jun;**20**(6):760–7.
3. Iversen J, Vilstrup H, Tygstrup N. Kinetics of glucose metabolism in relation to insulin concentrations in patients with alcoholic cirrhosis and in healthy persons. *Gastroenterology* 1984 Nov;**87**(5):1138–43.
4. Petrides AS, Groop LC, Riely CA, DeFronzo RA. Effect of physiologic hyperinsulinemia on glucose and lipid metabolism in cirrhosis. *J Clin Invest* 1991 Aug;**88**(2):561–70.
5. Garcia-Compean D, Jaquez-Quintana JO, Maldonado-Garza H. Hepatogenous diabetes. Current views of an ancient problem. *Ann Hepatol* 2009 Jan;**8**(1):13–20.
6. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab* 2009 Sep;**10**(3):167–77.
7. Patti ME, Houten SM, Bianco AC, Bernier R, Larsen PR, Holst JJ, et al. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. *Obes (Silver Spring)* 2009 Sep;**17**(9):1671–7.
8. Cariou B, Chetiveaux M, Zair Y, Pouteau E, Disse E, Guyomarc'h-Delasalle B, et al. Fasting plasma chenodeoxycholic acid and cholic acid concentrations are inversely correlated with insulin sensitivity in adults. *Nutr Metab (Lond)* 2011;**8**(1):48.
9. Ockenga J, Tietge UJ, Boker KH, Manns MP, Brabant G, Bahr MJ. Distinct roles of free leptin, bound leptin and soluble leptin receptor during the metabolic-inflammatory response in patients with liver cirrhosis. *Aliment Pharmacol Ther* 2007 Jun 1;**25**(11):1301–9.
10. Celinski K, Konturek PC, Slomka M, Cichoz-Lach H, Gonciarz M, Bielanski W, et al. Altered basal and postprandial plasma melatonin, gastrin, ghrelin, leptin and insulin in patients with liver cirrhosis and portal hypertension without and with oral administration of melatonin or tryptophan. *J Pineal Res* 2009 May;**46**(4):408–14.
11. Krawczyk K, Szczesniak P, Kumor A, Jasinska A, Omulecka A, Pietruczuk M, et al. Adipohormones as prognostic markers in patients with nonalcoholic steatohepatitis (NASH). *J Physiol Pharmacol* 2009 Oct;**60**(Suppl. 3):71–5.
12. Kalaitzakis E, Bosaeus I, Ohman L, Bjornsson E. Altered postprandial glucose, insulin, leptin, and ghrelin in liver cirrhosis: correlations with energy intake and resting energy expenditure. *Am J Clin Nutr* 2007 Mar;**85**(3):808–15.
13. Wiest R, Moleda L, Farkas S, Scherer M, Kopp A, Wonckhaus U, et al. Splanchnic concentrations and postprandial release of visceral adipokines. *Metabolism* 2010 May;**59**(5):664–70.
14. Shiraki M, Terakura Y, Iwasa J, Shimizu M, Miwa Y, Murakami N, et al. Elevated serum tumor necrosis factor-alpha and soluble tumor necrosis factor receptors

correlate with aberrant energy metabolism in liver cirrhosis. *Nutrition* 2010 Mar;**26**(3):269–75.
15. Holland-Fischer P, Vilstrup H, Frystyk J, Nielsen DT, Flyvbjerg A, Gronbaek H. The IGF system after insertion of a transjugular intrahepatic porto-systemic shunt in patients with liver cirrhosis. *Eur J Endocrinol* 2009 Jun;**160**(6):957–63.
16. Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998 Apr 18;**316**(7139):1236–8.
17. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011 Jun 24;**332**(6037):1519–23.
18. Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006 Jan 26;**439**(7075):484–9.
19. Ockenga J, Valentini L, Schuetz T, Wohlgemuth F, Glaeser S, Omar A, et al. Plasma bile acids are associated with energy expenditure and thyroid function in humans. *J Clin Endocrinol Metab* 2012 Feb;**97**(2):535–42.
20. Parker HE, Wallis K, le Roux CW, Wong KY, Reimann F, Gribble FM. Molecular mechanisms underlying bile acid-stimulated glucagon-like peptide-1 secretion. *Br J Pharmacol* 2012 Jan;**165**(2):414–23.
21. Nakatani H, Kasama K, Oshiro T, Watanabe M, Hirose H, Itoh H. Serum bile acid along with plasma incretins and serum high-molecular weight adiponectin levels are increased after bariatric surgery. *Metabolism* 2009 Oct;**58**(10):1400–7.
22. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile Acid receptors in metabolic regulation. *Physiol Rev* 2009 Jan;**89**(1):147–91.
23. Li T, Chiang JY. Bile Acid signaling in liver metabolism and diseases. *J Lipids* 2012;**2012**:754067.
24. Cariou B, van HK, Duran-Sandoval D, van Dijk TH, Grefhorst A, Abdelkarim M, et al. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. *J Biol Chem* 2006 Apr 21;**281**(16):11039–49.
25. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sci U S A* 2006 Jan 24;**103**(4):1006–11.
26. Authier F, Desbuquois B. Glucagon receptors. *Cell Mol Life Sci* 2008 Jun;**65**(12):1880–99.
27. Vuguin PM, Charron MJ. Novel insight into glucagon receptor action: lessons from knockout and transgenic mouse models. *Diabetes Obes Metab* 2011 Oct;**13**(Suppl. 1):144–50.
28. Ikegami T, Krilov L, Meng J, Patel B, Chapin-Kennedy K, Bouscarel B. Decreased glucagon responsiveness by bile acids: a role for protein kinase Calpha and glucagon receptor phosphorylation. *Endocrinology* 2006 Nov;**147**(11):5294–302.
29. Bouscarel B, Kroll SD, Fromm H. Signal transduction and hepatocellular bile acid transport: cross talk between bile acids and second messengers. *Gastroenterology* 1999 Aug;**117**(2):433–52.
30. Yarandi SS, Hebbar G, Sauer CG, Cole CR, Ziegler TR. Diverse roles of leptin in the gastrointestinal tract: modulation of motility, absorption, growth, and inflammation. *Nutrition* 2011 Mar;**27**(3):269–75.
31. Lee YH, Magkos F, Mantzoros CS, Kang ES. Effects of leptin and adiponectin on pancreatic beta-cell function. *Metabolism* 2011 Dec;**60**(12):1664–72.
32. Kieffer TJ, Habener JF. The adipoinsular axis: effects of leptin on pancreatic beta-cells. *Am J Physiol Endocrinol Metab* 2000 Jan;**278**(1):E1–14.
33. Ockenga J, Bischoff SC, Tillmann HL, Rifai K, Widjaja A, Boker KH, et al. Elevated bound leptin correlates with energy expenditure in cirrhotics. *Gastroenterology* 2000 Dec;**119**(6):1656–62.
34. Levi J, Gray SL, Speck M, Huynh FK, Babich SL, Gibson WT, et al. Acute disruption of leptin signaling in vivo leads to increased insulin levels and insulin resistance. *Endocrinology* 2011 Sep;**152**(9):3385–95.
35. Holland-Fischer P, Nielsen MF, Vilstrup H, Tonner-Nielsen D, Mengel A, Schmitz O, et al. Insulin sensitivity and body composition in cirrhosis: changes after TIPS. *Am J Physiol Gastrointest Liver Physiol* 2010 Aug;**299**(2):G486–93.
36. Schmueli E, Walker M, Alberti G, Record CO. Normal splanchnic but impaired peripheral insulin-stimulated glucose uptake in cirrhosis. *Hepatology* 1993;**18**:86–95.