Section of Gastroenterology and Hepatology
Department of Internal Medicine
Sahlgrenska University Hospital
Göteborg University
Göteborg, Sweden

# The Role of Gut Dysfunction and Nutritional Factors in Liver Cirrhosis

**Evangelos Kalaitzakis, MD**


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# ABSTRACT


## The Role of Gut Dysfunction and Nutritional Factors in Liver Cirrhosis

Evangelos Kalaitzakis, MD
Section of Gastroenterology and Hepatology
Department of Internal Medicine
Sahlgrenska University Hospital, Göteborg University, Göteborg, Sweden

Malnutrition is a common finding in patients with liver cirrhosis. Malnutrition has been shown to be associated with increased morbidity and mortality. Its pathogenesis remains unclear but both poor dietary intake and increased energy expenditure have been reported.

Spontaneous bacterial peritonitis is an important clinical problem in cirrhotics. It may occur as a consequence of repeated access of bacteria from the intestinal lumen (translocation) to the mesenteric lymph nodes. One of the mechanisms proposed to explain bacterial translocation in cirrhosis includes increased intestinal permeability.

The aims of the present study were to evaluate GI symptoms in cirrhotic patients and their possible relation to nutritional status, to assess whether gastric sensorimotor dysfunction or metabolic disturbances are associated with reduced food intake, and to investigate the role of ascites in intestinal permeability in patients with liver cirrhosis.

Gastrointestinal symptoms and health-related quality of life (HRQOL) were assessed with the aid of two questionnaires. Gastric sensorimotor function was measured by means of an electronic barostat. Food intake, as assessed with a food diary, was related to fasting and postprandial glucose, insulin, leptin, and ghrelin concentrations. Intestinal permeability was evaluated by a $^{51}$Cr-EDTA permeability test.

Cirrhotics were found to have increased severity of GI symptoms compared with reference values from the general population. A relationship between GI symptoms and compromised HRQOL as well as weight loss was observed.

Proximal stomach relaxation to a meal was increased in patients with liver cirrhosis as compared with healthy controls but the relation between gastric accommodation and energy intake was found to be disturbed in these patients. Gastric sensitivity to distension was shown to be related to GI symptom severity and to liver cirrhosis severity scores.

Patients with liver cirrhosis exhibited higher postprandial insulin and glucose concentrations compared to controls. Cirrhotics had higher fasting leptin that fell significantly postmeal and they showed an attenuated increase of ghrelin before the next expected meal. Altered glucose and hormonal levels in patients with cirrhosis were associated with poor food intake.

Only a few patients with cirrhosis had increased intestinal permeability, as assessed by a $^{51}$Cr-EDTA test, which was not influenced to a major extent by ascites.

**Conclusions:** In patients with liver cirrhosis GI symptom severity is high and it is associated with impaired HRQOL and weight loss. Gastric accommodation is not involved in the poor food intake observed in cirrhotics and gastric sensitivity seems to be a relevant factor for GI symptom generation in these patients. Altered postprandial glucose, leptin, and ghrelin levels are correlated to reduced energy intake in this patient group. Increased intestinal permeability is probably of limited importance in the pathophysiology of bacterial infections in patients with liver cirrhosis and ascites.



**Keywords:** liver cirrhosis; malnutrition; gastrointestinal symptoms; health-related quality of life; food intake; energy expenditure; gastric accommodation; gastric barostat; insulin resistance; leptin; ghrelin; intestinal permeability
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*To the misunderstood patients with alcoholic liver cirrhosis*

## Ιθάκη

Σα βγεις στον πηγαιμό για την Ιθάκη,
να εύχεσαι νάναι μακρύς ο δρόμος,
γεμάτος περιπέτειες, γεμάτος γνώσεις.
Τους Λαιστρυγόνας και τους Κύκλωπας,
τον θυμωμένο Ποσειδώνα μη φοβάσαι,
τέτοια στον δρόμο σου ποτέ σου δεν θα βρεις,
αν μεν' η σκέψις σου υψηλή, αν εκλεκτή
συγκίνησις το πνεύμα και το σώμα σου αγγίζει.
Τους Λαιστρυγόνας και τους Κύκλωπας,
τον άγριο Ποσειδώνα δεν θα συναντήσεις,
αν δεν τους κουβανείς μες στην ψυχή σου,
αν η ψυχή σου δεν τους στήνει εμπρός σου.
Να εύχεσαι νάναι μακρύς ο δρόμος.
Πολλά τα καλοκαιρινά πρωϊά να είναι
που με τι ευχαρίστησι, με τι χαρά
θα μπαίνεις σε λιμένας πρωτοειδωμένους,
να σταματήσεις σ' εμπορεία Φοινικικά,
και τες καλές πραγμάτειες ν' αποκτήσεις,
σεντέφια και κοράλλια, κεχριμπάρια κ' έβενους,
και ηδονικά μυρωδικά κάθε λογής,
όσο μπορείς πιο άφθονα ηδονικά μυρωδικά,
σε πόλεις Αιγυπτιακές πολλές να πας,
να μάθεις και να μάθεις απ' τους σπουδασμένους.
Πάντα στον νου σου νάχεις την Ιθάκη.
Το φθάσιμον εκεί ειν' ο προορισμός σου.
Αλλά μη βιάζεις το ταξείδι διόλου.
Καλλίτερα χρόνια πολλά να διαρκέσει
και γέρος πια ν' αράξεις στο νησί,
πλούσιος με όσα κέρδισες στο δρόμο,
μη προσδοκώντας πλούτη να σε δώσει η Ιθάκη.
Η Ιθάκη σ'έδωσε τ' ωραίο ταξίδι.
Χωρίς αυτήν δεν θάβγαινες στον δρόμο.
Άλλα δεν έχει να σε δώσει πια.
Κι αν πτωχική την βρεις, η Ιθάκη δε σε γέλασε.
Έτσι σοφός που έγινες, με τόση πείρα,
ήδη θα το κατάλαβες οι Ιθάκες τι σημαίνουν.

Κωνσταντίνος Π. Καβάφης (1911)

## Ithaca

When you set out on your journey to Ithaca
hope your road is a long one,
full of adventure, full of knowledge.
The Lestrygonians and the Cyclops,
the angry Poseidon – don't be afraid of them:
You will never find such as these on your path,
if your thoughts remain lofty, if a fine
emotion touches your spirit and your body.
The Lestrygonians and the Cyclops,
the fierce Poseidon you will never encounter,
if you do not carry them within your soul,
if your soul does not set them up before you.
Pray that the road is long.
That the summer mornings are many, when,
with such pleasure, with such joy
you will enter ports seen for the first time;
stop at Phoenician markets,
and purchase fine merchandise,
mother-of-pearl and coral, amber and ebony,
and sensual perfumes of all kinds,
as many sensual perfumes as you can;
visit many Egyptian cities,
to learn and learn from scholars.
Always keep Ithaca in your mind.
To arrive there is your ultimate goal.
But do not hurry the voyage at all.
It is better to let it last for many years;
and to anchor at the island when you are old,
rich with all you have gained on the way,
not expecting that Ithaca will offer you riches.
Ithaca has given you the beautiful voyage.
Without it you would have never set out on the road.
It has nothing more to give you.
And if you find it poor,Ithaca won't have fooled you.
Wise as you have become, with so much experience,
you must have understood what Ithacas mean.

Constantine P. Cavafy (1911)

The Role of Gut Dysfunction and Nutritional Factors in Liver Cirrhosis


evangelos.kalaitzakis@vgregion.se

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# LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

**I.** **Gastrointestinal symptoms in patients with liver cirrhosis. Associations with nutritional status and health-related quality of life**.
Evangelos Kalaitzakis, Magnus Simrén, Rolf Olsson, Pia Henfridsson, Irene Hugosson, Maria Bengtsson, Einar Björnsson
Scand J Gastroenterol2006. In press.

**II.** **The role of gastric sensorimotor dysfunction in gastrointestinal symptoms and energy intake in liver cirrhosis**
Evangelos Kalaitzakis, Magnus Simrén, Hasse Abrahamsson, Einar Björnsson
Scand J Gastroenterol2006. In press.

**III.** **Altered postprandial glucose, insulin, leptin and ghrelin levels in cirrhosis: correlations with energy intake and resting energy expenditure**
Evangelos Kalaitzakis, Ingvar Bosaeus, Lena Öhman, Einar Björnsson
Submitted

**IV.** **Intestinal Permeability in Cirrhotic Patients with and without Ascites**
Evangelos Kalaitzakis, Jan-Erik Johansson, Ingvar Bjarnason, Einar Björnsson
Scand J Gastroenterol 2006;41:326-30

# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| AUC | Area under the curve |
| BMI | Body Mass Index |
| CI | Confidence interval |
| GI | Gastrointestinal |
| GSRS | Gastrointestinal Symptom Rating Scale |
| HOMA-IR | Insulin resistance expressed as homeostasis model assessment index |
| HRQOL | Health-related quality of life |
| IQR | Interquartile range |
| MCS | Mental component summary |
| MDP | Minimal distending pressure |
| MELD | Model for end-stage-liver disease |
| NS | Non-significant |
| PCS | Physical component summary |
| QOL | Quality of life |
| REE | Resting energy expenditure |
| SD | Standard deviation |
| SF-36 | Short Form 36 |
| SPECT | Single-photon emission computed tomography |
| vs | Versus |

# INTRODUCTION

Liver cirrhosis is defined histologically as a diffuse process with liver cell necrosis/apoptosis, fibrosis and regenerative nodules [1]. There are several causes of liver cirrhosis, the most common being high alcohol consumption, hepatitis C, hepatitis B, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, and non-alcoholic steatohepatitis [1]. Cirrhosis, apart from other features peculiar to the cause, results in two major events: hepatocellular failure and portal hypertension. Important complications of liver cirrhosis include, but are not limited to, esophageal varices, ascites, hepatic encephalopathy, hepatic failure with jaundice, hepatocellular cancer, and cholangiocarcinoma [1]. In recent years it has become widely recognized that liver cirrhosis may affect several organ systems such as the cardiovascular system [2, 3], the respiratory system [4], the kidneys [5, 6], and the skeletal system [7, 8]. Cirrhosis has also been associated with varying degrees of malnutrition [9] as well as with alterations in the gastrointestinal (GI) tract [10]. Apart from liver transplantation, no specific cure exists for liver cirrhosis to date.

## 1. MALNUTRION IN LIVER CIRRHOSIS

Malnutrition is common in patients with liver cirrhosis with a reported prevalence as high as 80% depending on the patient population studied and disease severity [9, 11-14]. It has been reported to correlate with etiology of liver disease (higher in alcoholic than in non-alcoholic cirrhosis) [13], but some controversy exists [15]. Malnutrition has been shown to be associated with increased morbidity and mortality [9] and it can severely compromise liver transplantation results [12].

The mechanisms of malnutrition in liver cirrhosis are not fully understood but poor dietary intake [9, 16], increased energy expenditure [12, 17-19], and malabsorption [9, 20] have been reported. Potential reasons for low energy intake include reduced appetite, possibly associated with increased brain tryptophan availability [21], early satiety especially in the presence of ascites [22], poor palatability of low-sodium diets, and hepatic encephalopathy [9]. Increased basal energy expenditure, although not a constant feature of cirrhosis,

has also been reported to contribute to a negative energy balance in cirrhotic patients [9, 12, 17-19, 23-25]. Furthermore, disturbances in macronutrient metabolism, with increased lipid oxidation and decreased carbohydrate oxidation (starvation-type metabolism), have also been described in these patients [16, 17, 23, 26, 27] and might be involved in the development of malnutrition. Last, fat malabsorption has been reported to be frequent in cirrhotics (especially in those with evidence of malnutrition) [20]. A reduction in the area of the intestinal absorptive surface has been proposed in cirrhotis by some previous studies [28] and could, theoretically, contribute to malabsorption. However, not all studies have found defective active absorption in these patients [20, 29].

# 2. THE GASTROINTESTINAL (GI) TRACT IN LIVER CIRRHOSIS

## 2.1 GI symptoms in liver cirrhosis

GI symptoms are considered to be common in cirrhotics [30, 31] and may potentially lead to reduced energy intake. However, data on their prevalence are scarce. Previous studies on cholestatic liver disease have found increased GI symptom severity in patients with primary sclerosing cholangitis [32] and primary biliary cirrhosis [33] compared to controls, but only a few patients in these studies had cirrhosis. In a previous report only published in abstract form, abdominal pain, nausea, bloating, and early satiety were found to occur more frequently in patients with chronic liver disease compared to healthy controls [30]. According to another study, dyspeptic symptoms without any apparent organic cause were reported by 28/62 patients with cirrhosis [31]. To date, no study has evaluated GI symptoms in cirrhotics using a validated questionnaire and the possible association of GI symptoms with malnutrition and weight loss has not been investigated in this group of patients.

## 2.2 Structural changes of the GI tract

The effects of liver cirrhosis on the GI tract have been considered to be mainly associated with portal hypertension. A major endoscopic finding is varices most commonly located in the esophagus and/or the fundus of the stomach. Occasionally varices may be found in “ectopic” locations such as in the

duodenum or in the rectum [34]. Esophageal varices develop in the majority (90%) of patients with alcoholic cirrhosis and the same is probably true for cirrhotics with other etiologies provided the follow-up period is long enough [35, 36]. They can be the site of GI bleeding, a potentially lethal complication of liver cirrhosis despite modern treatment [35, 36].

Mucosal changes are also frequently encountered upon endoscopic examination of the GI tract in patients with liver cirrhosis [10, 37]. Portal hypertensive intestinal vasculopathy is a term used to describe the fundamental structural change in the intestine, a vasculopathy due to changes in the intestinal microcirculation secondary to longstanding portal hypertension [10]. Signs of portal hypertensive intestinal vasculopathy may be observed in all parts of the GI tract [10]. The prevalence of portal hypertensive gastropathy, with its characteristic mosaic appearance, has been reported in 11 - 94% of cirrhotic patients [10]. The stomach has also been found to be significantly thickened on ultrasound examination in patients with cirrhosis and portal hypertension [38].

Compared to healthy controls, patients with liver cirrhosis have been reported to have higher increased plasma gastrin [39] and higher prevalence of peptic ulcers [40, 41]. In an endoscopic study, the annual incidence rate of peptic ulcer observed in 140 patients undergoing endoscopic follow-up was 4.3% [42]. Ulcers are associated with decompensated cirrhosis [43] but are asymptomatic in up to 2/3 of cases [42]. In a meta-analysis, the prevalence of helicobacter pylori infection has been found to be higher in cirrhotics with peptic ulcer disease than in those without [44].

## 2.3 Gastric sensorimotor function

### 2.3.1 Gastric accommodation

During fasting, the smooth muscle of the proximal stomach maintains a tonic contractile activity [45-47]. During and after ingestion of food, a relaxation of the proximal stomach occurs, providing the meal with a reservoir and enabling a volume increase without a rise in pressure (gastric accommodation reflex) [45, 46, 48-51]. However, volume increase also occurs in the distal stomach [52].

Gastric accommodation involves a vagovagal reflex pathway influencing the balance maintained by cholinergic excitatory drive and non-adrenergic non-cholinergic inhibitory input. The afferent signal involves stretch-sensitive mechanoreceptors in the esophagus and the stomach as well as osmo- and chemo-receptors in the stomach and the duodenum [46, 48, 51, 53]. Gastric tone is also influenced by sympathetic stimuli. Animal studies have shown that stimulation of $\alpha$-adrenoreceptors in smooth muscle generally produces relaxation due to a direct action on postjunctional $\alpha_1$-adrenoreceptors and due to an indirect action on prejunctional $\alpha_2$-adrenoreceptors located on cholinergic nerve terminals of enteric neurons [54]. Thus, clonidine, an $\alpha_2$-agonist, has been reported to induce stomach relaxation in humans [54]. On the other hand, the efferent signal of the gastric accommodation reflex involves nitric oxide as the principal neurotransmitter at the neuromuscular junction [49, 50].

Impaired gastric accommodation has been associated with upper gastrointestinal symptoms including early satiety, bloating, epigastric pain, in patients with functional dyspepsia [55, 56], diabetes [57], prior surgery including fundoplication [58], vagotomy and partial gastrectomy [59]. In functional dyspepsia, impaired gastric accommodation has been demonstrated in 40% of patients in whom it was found to be associated with early satiety and weight loss [56]. In the same group of patients treatment with a fundus relaxing drug improved early satiety [56]. The measurement of gastric accommodation would therefore potentially be of value in the assessment of patients with GI symptoms and in the evaluation of therapeutic interventions.

Several techniques have been used to evaluate gastric accommodation in humans. Gastric barostat studies, using a polyethylene balloon placed in the gastric fundus, are generally regarded as the gold standard [51]. The balloon is connected to an electronic barostat device. The barostat keeps the balloon in apposition with the fundic wall, allowing isobaric volume fluctuation of the balloon. The intraballoon pressure is kept constant and fluctuations in the intraballoon volume reflecting changes in tone are recorded [51].

This technique is, however, invasive, cumbersome to the patients and labour-intensive. Therefore, there has been a search for easily applicable non-invasive tools to assess gastric accommodation. A slow caloric satiety drinking test has been suggested to correlate to proximal stomach relaxation as measured with a

gastric barostat in healthy and dyspeptic subjects, and it has been used to predict impaired accommodation and early satiety in dyspeptics [56, 60]. The subject is asked to consume a liquid meal at a constant rate, scoring their satiation level at 5-min intervals. The test is terminated when the subject reaches maximal satiety. The amount of calories ingested is used as a surrogate marker for gastric accommodation [51, 56, 60]. Other techniques used to measure proximal stomach accommodation to a meal include imaging tests such as abdominal ultrasound, magnetic resonance imaging, and single-photon emission computed tomography (SPECT) [51].

Gastric accommodation has been found to be impaired in patients with alcoholic cirrhosis in an ultrasonographic study [61] and in cirrhotic patients with tense ascites in a study involving SPECT compared to healthy controls [62]. However, gastric accommodation and gastric sensory function have not been investigated with a gastric barostat in liver cirrhosis and data on the effects of altered gastric motility and sensitivity on energy intake and gastrointestinal symptoms are lacking in this group of patients. Furthermore, the relationship between the satiety drinking test and the gastric accommodation has not been evaluated in cirrhosis.

### 2.3.2 Gastric sensitivity to distension

Gut stimuli, specifically gastric distension by food ingestion, may induce GI symptoms. It has been reported that gastric tone is important in determining the sensitivity of the stomach to distension [47, 63]. A subsequent study in healthy subjects, aiming to define whether perception of gastric distension is dependent on intragastric volume, pressure, or gastric wall tension, showed that gastric wall tension determines perception of gastric distension at least below nociception [64]. The presence of nutrients in the small intestine increases the mechanosensitivity of the stomach [65].

Hypersensitivity to gastric distension, defined as enhanced sensitivity to balloon distension of the proximal stomach is present in a subset of functional dyspepsia patients [65, 66] and it is associated with postprandial pain, belching, and weight loss [66]. It is therefore conceivable that the occurrence of postprandial GI symptoms in dyspeptics with hypersensitivity to gastric distension leads to decreased food intake, thus resulting in weight loss. This has, however, not been

tested in studies involving quantification of calorie intake. Hypersensitivity to gastric distension has also been reported in patients with postsurgical gastroparesis [47] but not in other patients with organic causes of dyspepsia [67]. To date, no studies have investigated gastric sensitivity to distension and its relevance for generation of gastrointestinal symptoms in liver cirrhosis.

### 2.3.3 Gastric emptying

Another way of assessing gastric motor function is measurement of gastric emptying. Delayed gastric emptying has traditionally been considered a mechanism that contributes to symptom generation in patients with GI motility disorders and systemic diseases affecting the GI tract [68]. Previous studies have shown that gastric emptying is abnormally slow in approximately 30-50% of outpatients with long-standing diabetes mellitus, although the magnitude of this delay is modest in many cases [69]. Other diseases associated with delayed gastric emptying include patients with functional dyspepsia [70], systemic sclerosis [71], Parkinson's disease [72], and chronic renal failure [73].

In patients with liver cirrhosis, gastric emptying has been found to be delayed [74-77], normal [78-80], or accelerated [81]. Several factors may account for the divergence of results hitherto published, including selection of patient groups with different characteristics, selection of small patient groups or small control groups and use of different measurement methods. In a recently published study from our group a newly developed radiological procedure using radiopaque markers was used to assess gastric emptying in cirrhotics with portal hypertension [74]. Delayed gastric emptying was found in male patients with cirrhosis but no correlation could be found with variceal pressure as measured with a small pressure-sensitive capsule attached to a gastroscope [74]. Measurement of gastric emptying was considered relevant in the present study because of its potential interrelation with gastric accommodation.

## 1.4 Intestinal permeability

The intestinal tract constitutes a large interface between the outside environment and the human body. This interface has two critical functions: As a filter, it allows movement of selected nutrients from the intestinal lumen into the internal milieu. As a barrier, the gut prevents the permeation of potentially harmful

micrororganisms or substances such as luminal antigens and luminal proinflammatory factors [82, 83]. The gut barrier function include both immunogenic (such as mucosal lymphocytes, dendritic cells, immunoglobulins) and non-immunogenic mechanisms such as selective intestinal permeability [82, 83].

Non-invasive methods have been used to assess the barrier function of the intestine by measuring the urinary excretion of orally administered test substances such as monosaccharides, disaccharides, and $^{51}$Cr-EDTA [82, 84]. The urinary excretion of orally administered test markers can be influenced by several premucosal (such as gastrointestinal dilution and transit, bacterial degradation, digestion hydrolysis) or postmucosal factors (such as metabolism, tissue distribution, renal function) apart from intestinal permeability itself. This has led to the formulation of the principle of differential urinary excretion of test substances, which provides an index of intestinal permeability [84].

Bacterial infections are one of the most important clinical problems in patients with liver cirrhosis with spontaneous bacterial peritonitis being the most relevant [85]. Spontaneous bacterial peritonitis may occur as a consequence of repeated access of bacteria from the intestinal lumen (translocation) to the mesenteric lymph nodes thereby gaining access to the ascitic fluid [86]. Furthermore, permeation of intestinal bacterial products such as endotoxin and bacterial DNA may have implications for the activation of the immune system, and the derangement of the hyperdynamic circulatory status, the induction of renal failure in patients with liver cirrhosis [86, 87]. Several mechanisms have been proposed to explain bacterial translocation in liver cirrhosis such as intestinal bacterial overgrowth, intestinal motility disturbances [88], impairment of the intestinal barrier function, and alterations in the local immune defenses [87, 89].

Studies have shown that alcohol misuse in patients with liver disease is associated with increased intestinal permeability [90-92] and endotoxemia [92]. This suggests that a “leaky” gut may play a pathogenic role in the development of chronic liver injury. Increased intestinal permeability is evident in a number of conditions associated with bacterial translocation and/or endotoxemia [82-84]. Intestinal permeability in liver cirrhosis has been variously reported as increased or normal [28, 29, 93-98]. However, limited data exists on the state of

intestinal permeability in patients with cirrhosis and ascites (who are at risk of developing spontaneous bacterial peritonitis) or the effect of paracentesis.

# 3. METABOLIC DISTURBANCES IN LIVER CIRRHOSIS

## 3.1 Impaired glucose tolerance and insulin resistance

Diabetes mellitus is common in liver cirrhosis. In most cases, diabetes seems to follow cirrhosis and it is called hepatogenous diabetes [99, 100]. Impaired glucose tolerance and insulin resistance are also common in cirrhotic patients [9, 23, 101, 102]. Both diabetes mellitus and impaired glucose tolerance have been reported to be negatively associated with survival in these patients [99]. Although the pathophysiology of impaired glucose tolerance and insulin resistance is unclear there are published data suggesting that they might be associated with the impairment of nutritional status in cirrhotics [101, 102]. Insulin resistance has been shown to correlate with resting energy expenditure (REE) [101] and it has also been proposed as the main mechanism of the starvation-type metabolism of cirrhosis [101]. Furthermore, elevated postprandial insulin levels have been proposed as a possible factor mediating a satiety cascade resulting in reduced energy intake in this group of patients [101].

## 3.2 Leptin

Leptin is a hormone involved in the endocrine regulation of energy metabolism and food intake [103]. It is produced mainly by differentiated adipocytes, acts on the hypothalamus suppressing food energy intake and stimulating energy expenditure [103], and circulates in free and protein-bound forms [104]. However, leptin is also produced by other tissues as well, such as the fundus of the stomach, the skeletal muscle, the liver, and the placenta. Leptin is considered to be a hormonal factor that informs several hormonal circuits and biological peripheral functions of the nutritional status of the organism [105, 106].

Decreased leptin levels are observed in several energy deprivation states, such as anorexia nervosa and exercise-induced amenorrhea, being at least partly responsible for the decrease in reproductive hormones, the fall in thyroid hormones, the increase in stress hormones and the rise in insulin-like growth factor-1 seen in these patients. These neuroendocrine alterations have adaptive

value by mobilising needed energy stores and diverting limited resources [106]. Conversely, obesity is associated with high leptin concentrations and resistance to the catabolic effect of leptin to suppress appetite and heighten energy expenditure [103, 106]. Although plasma leptin can be acutely increased by physiological insulinemia [107] there is no universal agreement in the literature as to whether leptin levels rise [107, 108], remain unchanged [109, 110], or fall [111] in the immediate postprandial phase in non-cirrhotic individuals.

Leptin levels have been found to be high in patients with alcoholic [112, 113] and with post-hepatitic [113-115] cirrhosis and to be positively correlated to insulin levels [113]. However, only few studies are available on the relations of leptin to energy intake and REE in these patients [25, 116]. In a previous report, no correlation was found between total leptin concentration and REE in cirrhotics with adequate food intake [116]. Also, bound (but not free) leptin has been shown to be increased and positively correlated to REE in patients with postviral cirrhosis on a weight-maintaining diet [25]. The relation of leptin to spontaneous energy intake and REE in patients with cirrhosis of various etiologies has not been previously investigated. Furthermore, postprandial leptin concentrations and their possible relation to food intake in cirrhotics have not been previously studied.

## 3.3 Ghrelin

Ghrelin is a novel hormone produced mainly by epithelial cells lining the fundus of the stomach [117] with only small amounts being synthesized in the placenta, kidney, pituitary, and hypothalamus [103]. Ghrelin activates pituitary and hypothalamic neurons stimulating growth hormone release [103]. In addition, the activation of neuropeptide Y-producing hypothalamic neurons by ghrelin results in stimulation of appetite and food intake in humans [103]. It also exerts other central and peripheral actions, including stimulation of lactotroph and corticotroph secretion, influence of pancreatic action as well as control of gastric motility and acid secretion [118].

In cases of negative energy balance such as cancer and cardiac cachexia ghrelin concentrations are elevated [119]. Fasting ghrelin levels have been reported to be elevated in Child-Pugh C cirrhotic patients as well as in patients with complications of liver disease [120]. Elevated fasting ghrelin have also been

shown to identify a group of cirrhotics with decreased energy intake and malnutrition [121].

Plasma ghrelin secretion is blocked by food intake and, therefore, ghrelin concentrations are higher just before a meal [103, 118]. Insulin has been reported to be essential for meal-induced ghrelin suppression [122-124] and glucose is proposed to have an additional effect [122]. Furthermore, an inverse relationship between leptin and ghrelin has been observed and although experimental evidence for a possible negative feedback control between them is conflicting it has been proposed that leptin could be of importance for suppression of basal ghrelin in normoinsulinemic subjects [109]. To date, postprandial changes of plasma ghrelin concentrations have not been investigated in patients with liver cirrhosis. However, in order to study the potential importance of insulin, leptin, and ghrelin for energy intake and REE they need to be investigated together, a study not previously undertaken in liver cirrhosis.

# 4. HEALTH-RELATED QUALITY OF LIFE (HRQOL)

## 4.1 Overview

Modern medicine has had an important impact on mortality from chronic diseases, which however still impose a considerable burden on families, health care, and society. HRQOL is meant to give the patients' perspective on the burden of disease and its measurement is usually done using multi-item questionnaires to estimate daily function. Questionnaires are completed by patients themselves thus reflecting the patient's subjective experience of the impact of disease on daily activities and well-being [125]. HRQOL has become an important tool in assessing and explaining disease outcomes [125, 126]. Generic HRQOL instruments may be used in any population irrespective of underlying disease, whereas disease specific instruments are constructed for a particular disease. Combining generic and disease specific instruments is recommended as it allows comparisons between diseases and within disease groups [125].

## 4.2 HRQOL in liver cirrhosis

HRQOL has been shown to be impaired in patients with cirrhosis [127-129]. Several factors such as severity of liver disease, symptoms of cirrhosis (muscle cramps and pruritus), and psychological factors have been implicated in HRQOL impairment in patients with chronic liver diseases [127-129]. Gastrointestinal symptoms have been reported to be associated with impaired HRQOL and psychological distress in patients with functional gastrointestinal disorders [130, 131], primary sclerosing cholangitis [33], and primary biliary cirrhosis [32] as well as in patients with inflammatory bowel disease [132]. However, it is unknown whether gastrointestinal symptoms influence HRQOL in patients with liver cirrhosis.

# AIMS OF THE PRESENT STUDIES

The limited knowledge on gut function as well as on nutritional and metabolic alterations in liver cirrhosis raised the following questions:

1. Is gastrointestinal symptom severity increased in patients with liver cirrhosis? Is there an association between gastrointestinal symptoms and nutritional status and/or HRQOL in this group of patients?

2. Do patients with liver cirrhosis have altered gastric sensorimotor function in comparison to healthy individuals? Is there a relation between gastric sensorimotor function and nutritional status, energy intake, or GI symptoms in these patients?

3. Are basal and postprandial levels of plasma glucose, insulin, leptin, and ghrelin related to energy intake and REE in cirrhotics? Are postprandial changes in these hormones interrelated in liver cirrhosis?

4. Is intestinal permeability altered in cirrhotic patients with ascites? Does therapeutic paracentesis influence intestinal permeability?

# SUBJECTS AND METHODS

The studies were performed according to the Declaration of Helsinki and were approved by the Göteborg University Ethics Committee. The studies II and IV were also approved by the radiation committee of the Sahlgrenska University Hospital. All the participants in the studies gave informed consent. The methods used are introduced and commented on in this chapter. For further details, see separate papers (I-IV).

## 1. SUBJECTS

The studies were carried out in our hospital between 2003 and 2005 in patients with liver cirrhosis. The diagnosis of liver cirrhosis was established histologically or based on the presence of at least 2 of the following: characteristic imaging features, esophageal or gastric varices, ascites, increased INR that could not be attributed to any other cause. The severity of the liver disease was assessed according to the Child-Pugh classification and the model for end-stage liver disease (MELD) score [133]. Hepatic encephalopathy was assessed clinically and graded on a scale from 0 to 4 according to the West-Haven criteria [134]. Most patients participating in study I and all patients participating in studies II-IV had undergone gastroscopy in the previous 6 months.

**Paper I**

A total of 128 consecutive adult patients with liver cirrhosis (in- or out-patients) were prospectively enrolled in the study. Patients unable to understand Swedish as well as those unable to complete the questionnaires due to severe comorbidities such as dementia and psychosis, or debilitating hepatic encephalopathy were excluded. Patients hospitalized because of acute diseases or complications related to liver disease were evaluated when stable clinical conditions were reached. Out of 142 consecutive patients who fulfilled the inclusion criteria and were approached, 128 patients (90%) agreed to participate in the study and completed the questionnaires. Patient data were collected from medical records, including etiology of liver disease, continued alcohol abuse in the case of alcoholic cirrhosis, previous variceal bleeding, existing esophageal or fundic varices, comorbidities potentially compromising nutritional status, and

daily use of gastrointestinal drugs (including lactulose). The presence of ascites was evaluated by means of abdominal ultrasonography or clinical assessment upon completion of the questionnaires. Basic patient characteristics are shown in table 1.

*Table 1*. Basic data in patients with liver cirrhosis (n=128) (I)

| | |
|---|---|
| Age | 57.2 (11.5) |
| Female/Male | 50 / 78 (39 / 61%) |
| Outpatients/inpatients | 103 / 25 (20 / 80%) |
| Etiology of liver cirrhosis | |
| Alcoholic or mixed[a] | 55 (43%) |
| Viral[b] | 22 (17%) |
| Cholestatic[c] | 20 (16%) |
| Cryptogenic | 18 (14%) |
| Other[d] | 13 (10%) |
| Previous variceal bleeding | 36 (28%) |
| Esophageal and/or fundic varices | 84 (66%) |
| Ascites | 48 (38%) |
| Tense ascites | 20 (16%) |
| Hepatic encephalopathy[e] | 29 (23%) |
| Hepatocellular carcinoma | 11 (9%) |
| MELD score | 13.2 (5.6) |
| Child-Pugh score | 8.6 (2.3) |
| Child-Pugh class A/B/C | 28/57/43 (22 / 44 / 34%) |
| Malnutrition[f] | 37 (30%) |
| Weight change[g] in the previous 3 months | -1.8% (4.3) |
| Weight change[g] in the previous 6 months | -2.1% (6.2) |

Data are presented as mean (SD) or n (%) as appropriate

[a] Alcoholic or mixed: 37 (29%)- alcoholic, 16 (12%)- alcoholic and chronic hepatitis C, 1 (1%)- alcoholic and primary biliry cirrhosis (PBC), 1 (1%)-alcoholic and primary sclerosing cholangitis (PSC)

[b] Viral: 20 (16%)- chronic hepatitis C, 2 (1%)- chronic hepatitis B

[c] Cholestatic: 10 (8%)- PSC, 9 (7%)- PBC, 1 (1%)- unclassifiable cholestatic disease

[d] Other: 4 (3%)- autoimmune hepatitis, 4 (3%)- non-alcoholic steatohepatitis, 3 (2%) overlap syndrome, 1 (1%)- a1-antitrypsin deficiency, 1 (1%)- allograft hepatopathy

[e] Hepatic encephalopathy: 27 (21%)- grade 1, 2 (2%)- grade 2, none with grade 3 or 4

[f] Malnutrition: skinfold thickness and/or mid-arm muscle circumference < $10^{th}$ percentile, according to standard tables for the Swedish population based on age and sex, and/or BMI < 20 $kg \cdot m^{-2}$

[g] Mean (SD) dry weight change expressed as a percentage of actual body weight during the last 3 or 6 months (negative values represent weight loss)

## Paper II

Sixteen patients with liver cirrhosis were enrolled in the study. Patients with malignancy, infections, known gastrointestinal or renal disease, significant respiratory or cardiac dysfunction, known diabetes mellitus, hepatorenal syndrome, untreated thyroid dysfunction, hepatic encephalopathy grade II-IV, gastric varices, or previous gastrointestinal surgery other than appendectomy were excluded. Patients with any medication (including lactulose) affecting gastrointestinal motility and sensitivity were asked to interrupt it at least 24 hours before the barostat studies and the satiety drinking test. Patients with

alcoholic cirrhosis had been abstinent for at least 6 months at inclusion. One patient was found to have diabetes mellitus upon blood sampling for purposes of this study. He had normal HBA1c, complained of no GI symptoms and required only dietary interventions (instituted after completion of the study protocol) for diabetes control. Three patients had mild ascites detectable by ultrasonography at inclusion and were treated with spironolactone. None had peripheral edema. Fifteen age-, sex- and body mass index (BMI)-matched healthy weight-stable volunteers acted as controls. Basic subject characteristics are shown in table 2.

*Table 2.* Basic characteristics in all subjects (II)

| | Cirrhotics (n=16) | Healthy Controls (n=15) | p-value[a] |
|---|---|---|---|
| Age (years) | 56 (48-61) | 52 (49-62) | 0.57 |
| Sex (M/F) | 13/3 | 10/5 | 0.35 |
| BMI ($kg/m^2$) | 25.9 (24.4-29.7) | 25.6 (24.1-26.6) | 0.29 |
| Weight Change (%) | 0 ( 0 - 5.8) | | |
| Etiology of cirrhosis | | | |
| Alcoholic | 7 | | |
| Viral | 2 | | |
| Cryptogenic | 4 | | |
| Other[b] | 3 | | |
| Esophageal varices (n) | 9 | | |
| Portal hypertensive gastropathy (n) | 11 | | |
| Ascites (n) | 3 | | |
| Hepatic encephalopathy grade I (n) | 2 | | |
| MELD score | 9.5 (7.5-13.8) | | |
| Child-Pugh score | 7 (6-9) | | |
| Child-Pugh A/B/C (n) | 7/6/3 | | |

Data expressed as median (IQR)
[a] p-value for significance calculation between cirrhotics and healthy controls
[b] Other: 1- autoimmune hepatitis, 1- non-alcoholic steatohepatitis, 1- primary biliary cirrhosis
Weight change (%), dry weight change expressed as a percentage of actual body weight during the last 6 months (negative values would represent weight loss)

## Paper III

Thirty-one consecutive outpatients with liver cirrhosis were enrolled. Patients with malignancy, infections, known gastrointestinal or renal disease, significant respiratory or cardiac dysfunction, insulin-dependent diabetes mellitus, hepatorenal syndrome, untreated thyroid dysfunction, and hepatic encephalopathy grade II-IV were excluded. Patients with alcoholic cirrhosis had been abstinent for at least 6 months at inclusion. All had normal serum creatinine. Twenty-six out of 31 had endoscopic evidence of esophageal varices and 20/31 of portal hypertensive gastropathy. Two patients were found to have diabetes mellitus upon blood sampling for purposes of the study. Six patients had mild ascites detectable by ultrasonography at inclusion and were treated with spironolactone. None had peripheral edema. Ten age-, sex- and body mass index (BMI)-matched healthy weight-stable volunteers acted as controls.

**Paper IV**

Twenty in- or out- patients with liver cirrhosis were enrolled. Ten patients had no ascites and no clinical signs of portal hypertension such as esophageal varices or splenomegaly (A- group) and ten had clinically severe ascites (A+ group). Twenty sex- and age-matched healthy volunteers acted as controls for the intestinal permeability estimations. Subjects with malignancy, infections (current or in the previous four weeks), known GI or renal disease, evidence of hepatorenal syndrome, those admitted with GI bleeding at the time of the study, as well as those unable or unwilling to give informed consent were excluded. Also excluded were patients receiving substances known to affect intestinal permeability [84] such as non-steroidal anti-inflammatory drugs in the previous 2 weeks. Most patients with alcoholic cirrhosis had been abstinent for several months and a patient had to be abstinent for at least 2 weeks for inclusion in the study. Bjarnason et al. have shown that intestinal permeability of alcoholic patients abstinent for more than 2 weeks is not significantly different compared to healthy individuals [91]. In 6 patients with ascites intestinal permeability was performed before and after therapeutic paracentesis (at least 48 hours apart). Subject characteristics are shown in table 3.

*Table 3.* Subject characteristics (IV)

| | A- group (n=10) | A+ group (n=10) | Healthy controls (n=20) | |
|---|---|---|---|---|
| Median (range) age | 58 (43-76) | 63 (45-83) | 55(43-69) | NS |
| Female/Male | 3/7 | 5/5 | 9/11 | NS |
| Etiology | | | | |
| Alcohol | 5 | 4 | | NS |
| Viral* | 1 | 2 | | |
| Other† | 4 | 4 | | |
| Child-Pugh class (A/B/C) | 8/2/0 | 0/0/10 | | p<0.001 |
| Child-Pugh score Median (IQR) | 6 (5.7-6.25) | 11 (10-12.25) | | p<0.001 |
| MELD score Median (IQR) | 10 (7.5-12.5) | 15 (13-19.75) | | p=0.06 |
| Creatinine (mmol/l) Median (IQR) | 69 (58.5-86) | 73.5 (54-114) | | NS |

A-: Cirrhotics without ascites, A+: Cirrhotics with ascites
*Viral etiology: A- group (HCV-1), A+ group (HCV-1, HBV-1)
†Other etiology: A- group (cryptogenic cirrhosis-3, primary sclerosing cholangitis-1), A+ group (cryptogenic cirrhosis-3, primary billiary cirrhosis-1)

## 2. QUESTIONNAIRES (I, II)

Two types of self-administered questionnaires were used in the studies in order to assess GI symptoms (I, II) and HRQOL (I). The results were compared between the sub-groups within the cirrhotic patients (II) and normal values from

the general population (I) [135, 136]. The two questionnaires are summarised in Table 4.

**Gastrointestinal Symptom Rating Scale (GSRS) (I,II)** This measure of perceived severity of gastrointestinal symptoms was initially developed as an interview-based rating scale [137] and was later modified into a self-administered questionnaire [138]. The GSRS uses a seven-grade Likert scale and includes 15 items which are grouped into five domains: reflux, abdominal pain, constipation, indigestion, and diarrhea. The higher the scores, the more pronounced the symptoms. The questionnaire has been previously validated [138]. One item, eating dysfunction, which was developed previously in a manner analogous to the GSRS [139], was also considered clinically relevant for this study. Eating dysfunction is a question concerning early satiety, difficulties in eating normal portions, and postprandial pain. GSRS data are presented as a total score, as domain scores, and as a separate score for eating dysfunction. The results from the GSRS were compared with normal values from the Swedish general population obtained in a previous study in which 2162 healthy subjects were enrolled [135].

**Short-Form 36 (SF-36) (I)** This generic HRQOL instrument was developed as a comprehensive measure of general health status for use in the Medical Outcomes Study, and has been thoroughly tested for validity and reliability [136, 140-142]. This questionnaire assesses the extent to which an individual's health limits physical, emotional, and social functioning. It consists of 36 items organised in eight domains (physical functioning, role limitations caused by physical health problems, bodily pain, general health perceptions, vitality, social functioning, role limitations caused by emotional problems and mental health) and a separate item regarding perceived change of health status. The SF-36 is scored from 0 to 100, with higher scores indicating better health-related quality of life. Two comprehensive indices of HRQOL can also be computed: physical component summary (PCS), summarizing the first four domains, and mental component summary (MCS), summarizing the last four domains. SF-36 has previously been used for the assessment of QoL in patients with chronic liver disease [127-129]. Normative data from the Swedish general population are available, as well as thorough assessment of validity and reliability of the Swedish version of SF-36 [136]. An age- (2-year age interval) and gender-

matched reference sample (n=299), randomly drawn from the Swedish SF-36 normative database (n=8930), was used as a control group [136].

*Table 4.* Questionnaires used in this thesis.

| Questionnaire Dimensions | Items | Contents |
|---|---|---|
| SF-36 | | |
| Physical functioning | 10 | Physical function |
| Role physical | 4 | Role limitations caused by physical health problems |
| Bodily pain | 2 | Effects of pain on well-being and disability |
| General health | 5 | Perceived physical and mental health status |
| Vitality | 4 | Physical and mental well-being |
| Social functioning | 2 | Social disability caused by mental and/or physical health problems |
| Role emotional | 3 | Disability caused by emotional problems and mental health |
| Mental health | 5 | Mental function and well-being |
| Health status change | 1 | Perceived change in health status |
| GSRS | | |
| Reflux | 2 | Acid regurgitation, heartburn |
| Abdominal pain | 3 | Abdominal epigastric pain, sucking sensation in the epigastrium, nausea and vomiting |
| Indigestion | 4 | Borborygmus, abdominal distentsion, eructation, increased flatus |
| Constipation | 3 | Decreased passage of stool, hard stools, feeling of incomplete evacuation |
| Diarrhea | 3 | Increased passage of stool, loose stools, urgent need for defecation |
| Eating dysfunction | 1 | Early satiety, difficulties in eating normal portions, postprandial pain |

## 3. NUTRITIONAL STATUS ASSESSMENT (I, II, III)

Weight was measured in light clothing without shoes in all subjects. Among patients with ascites every effort was made to calculate dry weight by review of medical records (weight after last paracentesis or before ascites development). Body mass index was calculated and unintentional weight change of 1kg or more that could not be explained by ascites or edema during the previous 3 and 6 months was noted (after careful review of medical records or patient recall). Dry weight change was expressed as a percentage of actual body weight. Triceps skinfold thickness and mid-arm muscle circumference were measured by one of three experienced dieticians. It has been demonstrated that triceps skinfold thickness and mid-arm muscle circumference can be measured fairly accurately in patients with advanced liver disease and that they are only mildly affected by fluid retention [9]. Anthropometric measurements have been proposed as the most practical objective indices of nutritional depletion in chronic liver disease [9] and they are widely used in the nutritional evaluation of patients with cirrhosis [9, 11-14]. In study III, skinfold thickness at the biceps, subscapular, and suprailiac sites were also measured and percent body fat was calculated [143], a method shown to have comparable results with dual energy

x-ray absorptiometry in cirrhotics without overt fluid retention [144]. Patients were considered malnourished when triceps skinfold thickness and/or mid-arm muscle circumference were below the $10^{th}$ percentile (I) or the $5^{th}$ percentile (II,III), according to standard tables for the Swedish population based on age and sex [145], and/or if BMI was < 20 $kg \cdot m^{-2}$ [146].

## 4. DIETARY INTAKE ASSESSMENT (II, III)

All cirrhotics and healthy controls were instructed by a research dietician to complete a 4-day food diary (recording intake for 3 weekdays and 1 weekend day). Upon return of the diaries, the dietician interviewed the subjects to check for incomplete recordings and to estimate serving sizes. Estimation of serving sizes and conversion to weight units were aided by a previously validated meal model [147]. Intake of energy and nutrients were calculated using a computerized dietary analysis program (Dietist, Kost och Näringsdata AB, Sweden). The nutrient database was the National Food Composition Tables [148] which takes into account average nutrient loss during food preparation. In paper II, daily energy intake was compared with the recommended intake for cirrhotics according to the European Society of Parenteral and Enteral Nutrition guidelines [149]. Total daily energy intake is reported per kilogram body weight (energy intake/kg) (II, III) or as a ratio of REE (III).

## 5. GASTRIC BAROSTAT STUDIES (II)

Gastric barostat studies were performed in all subjects as previously described [56, 65]. Following an overnight fast, a balloon catheter, consisting of a highly compliant balloon made from polyethylene, finely folded and attached to a double lumen polyvinyl tube (Sherwood Medical, Tullamore, Ireland), was introduced through the mouth and secured to the subject's cheek with adhesive tape. To unfold the balloon, it was inflated manually with a fixed volume of 300 ml of air with the subject in a recumbent position. Then it was withdrawn gently to be positioned in the gastric fundus and again deflated. The procedure was performed under fluoroscopic guidance. The subject was then positioned in a sitting position with knees bent (80°) and trunk upright. The polyvinyl tube was connected to a programmable electronic barostat (Dual Drive Barostat, Distender Series II, G&I Electronics Inc., Toronto, Ont., Canada). Subsequently, subjects were allowed a 30-minute adaptation period, before minimal distending

pressure (MDP) was determined by increasing intrabag pressure by 1 mm Hg every three minutes until a volume of 30 ml or more was reached. This pressure level equilibrates the intra-abdominal pressure. Subsequently, isobaric distensions were performed in stepwise increments of 2 mm Hg starting from MDP, each with a duration of 2 minutes, while the corresponding intragastric volume was recorded. Subjects were instructed to grade their upper abdominal sensations at the end of every distending step, using a keypad linked to the main barostat. A graphic rating scale that combines verbal descriptors on a scale graded 0–6 (1=first perception, 5=discomfort, 6=pain) was used [56, 63]. The endpoint of each sequence of distensions was established at an intrabag volume of 1000 ml or when the subject reported discomfort or pain (score 5 or 6). Subsequently, the balloon was completely deflated and a 30-minute adaptation period was allowed, before pressure was set at MDP + 2 mm Hg for at least 90 minutes. After 30 minutes, a liquid meal (200 ml, 300 kcal, 16% proteins, 49% carbohydrates, 35% lipids; Nutridrink, Nutricia) was administered. Subjects were asked to consume the liquid meal within 2-3 minutes. Gastric tone measurement was continued for 60 minutes after the meal.

## 6. ANALYSIS OF BAROSTAT DATA (II)

Thresholds for perception and discomfort were assessed. Perception threshold was defined as the first pressure level and the corresponding volume that evoked a perception score of ≥ 1. Discomfort threshold was defined as the first pressure level and the corresponding volume that provoked a score of ≥ 5. Pressure thresholds were expressed relative to MDP [65, 66]. Gastric compliance was calculated as the slope of the pressure-volume curve during the isobaric distensions [56]. Gastric tone was assessed as the area under the volume-time curve (AUC) before and after administration of the meal [65]. Gastric accommodation was calculated as the difference between the AUC before and after the meal. Maximal gastric balloon volumes (Vmax) were assessed as well as the time from meal administration until a maximal balloon volume was reached.

## 7. SATIETY DRINKING TEST (II)

Satiety drinking test was performed in all cirrhotic patients participating in study II after an overnight fast as previously described [60]. A peristaltic pump filled

one of two beakers at a rate of 15 ml/min with a liquid meal (Nutridrink; Nutricia Nordica, Stockholm, Sweden). Patients were requested to maintain intake at the filling rate, thereby alternating the beakers. At 5 minute intervals, they scored their satiety on a scale graded 0–5 (1=threshold, 5=maximum satiety). Patients were instructed to cease meal intake when a score of 5 was reached. The endpoint of the satiety drinking test was the amount of calories ingested until the occurrence of maximum satiety (score 5).

## 8. GASTRIC EMPTYING TEST (II)

After an overnight fast, all the cirrhotic patients participating in study II had a standardised breakfast of 480kcal consisting of oatmeal porridge and one cheese sandwich. Twenty radiopaque markers with a density of 1.27g/mm$^3$ and a diameter of 4mm were added to the meal. Fluoroscopic control with counting of radiopaque markers in the stomach was performed 4h after ingestion and was repeated at 5h and 6h unless all the markers had left the stomach. Gastric emptying was then assessed by calculating the individual mean percentual gastric retention of markers 4 to 6 hours postmeal. Scintigraphic studies of indigestible solids have previously used remaining contents in the stomach after 2h and 4h to test for gastric emptying [150, 151]. Indigestible markers are emptied with a time delay of 1.5h to 2h compared to digestible solids [152]. Therefore we focused on the period 4 to 6 hours after the meal to test for delayed gastric emptying. Previous studies have demonstrated gender differences with a slower emptying among women [150, 152]. According to the reference values of our laboratory obtained in 131 healthy subjects (74 women, 57 men), 65% gastric retention was the upper reference value for women and 25% for men.

## 9. RESTING ENERGY EXPENDITURE (III)

REE and non-protein respiratory quotient were determined for all subjects in the morning after an overnight fast (10h) by indirect calorimetry (Deltatrac; Datex, Helsinki, Finland) at 7:30-8:30am.

## 10. TEST MEAL (III)

In study III, about one week apart from indirect calorimetry, at 7:30-8:00am, after an overnight fast, a subgroup of 18 cirrhotics (group A) and all healthy controls had a 480 kcal test meal of oatmeal porridge and one cheese sandwich with set amounts of macronutrients (55% carbohydrate, 31% fat, 14% protein). The subjects were instructed to eat the meal within 10 min. Blood samples for serum insulin and plasma glucose were drawn from an indwelling cannula at baseline and at 30min, 60min, 90min, 2h, and 4h after the meal. In a subgroup (of group A) of 13 cirrhotics (group B) and all healthy controls, blood samples were also drawn for plasma leptin and ghrelin analysis at the same intervals.

## 11. BLOOD SAMPLE ANALYSIS (III)

Blood samples for glucose, insulin, and leptin were drawn after an overnight fast Insulin resistance was expressed as homeostasis model assessment index (HOMA-IR) [153]. Plasma was immediately separated by centrifugation at 1000g (4$^{o}$C) and then stored at -80 $^{o}$C until subsequent leptin and ghrelin analysis. Plasma total ghrelin levels were measured by commercial RIA (Linco Research, Inc., St. Louis, MO), using $^{125}$I-labeled ghrelin as a tracer and ghrelin antiserum specific for total ghrelin. The detection limit for the assay was 93 pg/ml. Ghrelin was expressed in absolute values. Plasma leptin concentrations were measured by using commercial ELISA (Quantikine human leptin, R&D Systems, Oxford, UK). The detection limit for the assay was 15.6 pg/ml. Leptin was expressed in absolute values, as a ratio of weight (leptin/body weight), of BMI (leptin/BMI), and of fat in kg (leptin/fat).

## 12. ASSESSMENT OF INTESTINAL PERMEABILITY (IV)

Gastrointestinal permeability was assessed by the $^{51}$Cr-EDTA permeability test [154]. The test solution consisted of 4 MBq of $^{51}$Cr-EDTA (specific activity 1-2 mCi/mg chromium, Amersham International, UK), in 50ml of water. At 08:00am after an overnight fast, the subjects emptied their bladders and then drank the test solution. They remained fasting for a further 1h, after which normal food and fluid intake, except for alcohol [91], was permitted. Urine was collected for the next 24h. Three milliliters of the 24h volume were counted for 5 min in a gamma-counter (Selektronik, Horsholm, Denmark). The 24h urinary

excretion of the $^{51}$Cr-EDTA was expressed as a percentage of the dose given orally.

## 13. STATISTICAL METHODS

Results are mostly presented as medians and interquartile range (IQR) (II, III, IV). Data in paper I are presented as mean and standard deviation (SD), except when GSRS data are compared with results from another study population, where mean and 95% confidence intervals are used. All tests were two-tailed and conducted at a 5% significance level.

The *Student's t-test* (I), the *Mann-Whitney U-test* (II,III) or the *Fisher's non-parametric permutation test* (IV) was used for a comparison of continuous and ordinal data in two-sample cases.

The *Kruskal-Wallis test* was used for a comparison of continuous and ordinal data between more than two groups. If $p<0.05$, a post hoc analysis using the Mann-Whitney U test was performed (III).

The *Chi-square test* was used for a comparison of nominal data (I, II, III, IV).

The *Friedman's test* was used to evaluate postprandial glucose and hormone changes. If $p<0.05$ a post-hoc analysis using the *Wilcoxon test* was performed (III).

The *Fisher's permutation test* was used for pair-wise analysis (IV).

Correlations between continuous/ordinal data were analysed by the *Pearson's correlation coefficient* (I), the *Spearman rank order correlation coefficient* (II,III) or the Pitman's Permutation test (IV).

*Multiple stepwise logistic regression* was used to examine the relationship between one dependent variable with one or more independent variables (I,III).

# RESULTS

## 1. GASTROINTESTINAL SYMPTOMS (I)

Patients with liver cirrhosis had increased severity of all GI symptoms except for gastroesophageal reflux (figure 1) and profound impairment in HRQOL compared to healthy controls (figure 2). No gender effect was observed on nutritional status, weight change during the previous 3 or 6 months, GSRS domains, and SF-36 PCS or MCS (data not shown). Age was negatively correlated with GSRS score for abdominal pain in patients (r=-0.26, p=0.003) but not with any other GSRS domain, SF-36 physical or mental component summaries, nutritional status, or weight change (data not shown).

*Figure 1.* GI symptom severity assessed as GSRS scores (means and 95%CI) in patients with liver cirrhosis (continuous line, n=128) and healthy controls (dashed line, n=2162)

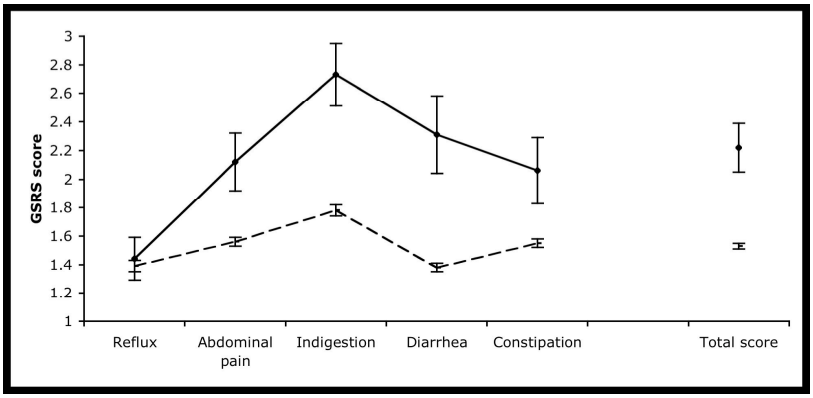
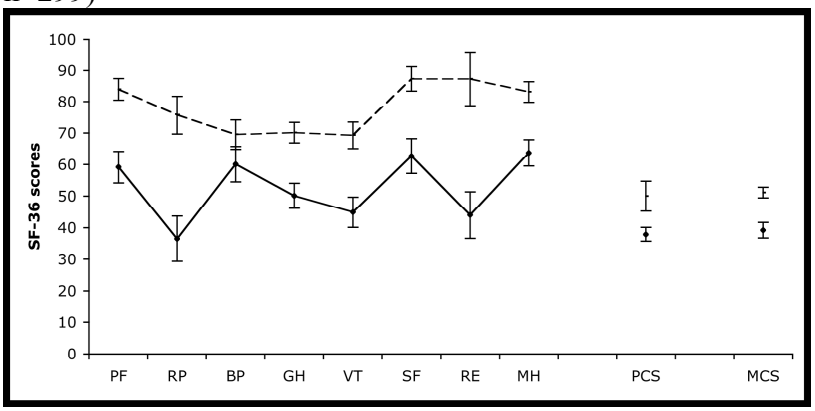



*Figure 2.* HRQOL assessed as SF-36 domain and summary scores (means and 95%CI) in patients with liver cirrhosis (continuous line, n=128) and healthy controls (dashed line, n=299)




PF, physical functioning; RP, role limitations caused by physical health problems; BP, bodily pain; GH, general health perceptions; VT, vitality; SF, social functioning; RE, role limitations caused by emotional problems; MH, mental health; PCS, physical component summary; MCS, mental component summary

## Liver disease characteristics (I)

GI symptom severity was not influenced by the etiology of liver cirrhosis but was associated with hospital admission upon inclusion in the study, the severity of cirrhosis (worsening with increasing impairment of liver disease), hepatic encephalopathy, and ascites (table 5). The presence of varices, previous variceal bleeding, hepatocellular cancer, or active drinking were not significantly correlated with any of the GSRS domains or the SF-36 component summaries (data not shown). Total GSRS score was negatively correlated with all SF-36 domains as well as the PCS (r=-0.42, p<0.001) and MCS (r=-0.31, p<0.001). The Child-Pugh score demonstrated stronger correlations than the MELD score with the severity of GI symptoms and weight loss (table 5).

*Table 5.* Association between clinical variables and gastrointestinal symptoms, weight change, and health-related quality of life in patients with liver cirrhosis (n=128)

| | Hospital admission | Alcoholic etiology | Ascites | Hepatic encephalopathy | Lactulose | Child-Pugh score | | MELD score | |
|---|---|---|---|---|---|---|---|---|---|
| **GSRS** | p | P | p | p | p | r | p | r | p |
| Reflux | >0.1 | >0.1 | 0.054 | **0.049** | >0.1 | 0.28 | **0.001** | 0.26 | **0.003** |
| Abdominal pain | **0.001** | >0.1 | **0.001** | 0.084 | **<0.001** | 0.37 | **<0.001** | 0.28 | **0.001** |
| Indigestion | **0.047** | >0.1 | **0.033** | **0.008** | **0.001** | 0.32 | **<0.001** | 0.29 | **0.001** |
| Diarrhea | 0.056 | >0.1 | >0.1 | **0.022** | **0.04** | 0.24 | **0.008** | 0.11 | >0.1 |
| Constipation | **0.001** | >0.1 | **0.001** | >0.1 | **<0.001** | 0.27 | **0.002** | 0.1 | >0.1 |
| Eating dysfunction | **0.029** | >0.1 | **0.021** | 0.054 | **0.015** | 0.3 | **0.001** | 0.32 | **<0.001** |
| Total score | **0.003** | >0.1 | **0.001** | **0.008** | **<0.001** | 0.39 | **<0.001** | 0.26 | **0.003** |
| **Weight change** | | | | | | | | | |
| in 3 months | 0.05 | **0.028** | **0.001** | **0.006** | **0.011** | -0.3 | **0.001** | -0.24 | **0.01** |
| in 6 months | >0.1 | 0.058 | 0.073 | >0.1 | **0.008** | -0.18 | 0.061 | -0.2 | **0.04** |
| **SF-36** | | | | | | | | | |
| PCS | 0.07 | >0.1 | **0.001** | **0.002** | **0.003** | -0.36 | **<0.001** | -0.28 | **0.002** |
| MCS | >0.1 | >0.1 | >0.1 | 0.044 | 0.061 | -0.1 | >0.1 | -0.05 | >0.1 |

All clinical variables were independently tested. They were considered as binary variables except for Child-Pugh and MELD scores as well as weight change, GSRS and SF-36 scores which were considered as continuous variables. p values of student's t-test or Pearson correlation coefficients (r) and p values are reported as appropriate. Significant results (p<0.05) are indicated in bold. All binary variables were related to negative weight change, i.e. weight loss, increased symptom severity and worse health-related quality of life scores.

GSRS, gastrointestinal symptoms rating scale; SF-36, Short-Form 36, PCS, Physical component summary, MCS mental component summary

## Malnutrition (I)

Patients with vs. without malnutrition did not differ in etiology or severity of cirrhosis nor in any GSRS or SF-36 domain (data not shown). However, the prevalence of malnutrition was higher in cirrhotics with active alcohol drinking compared to abstinent alcoholic patients with cirrhosis (53% vs. 19%; p=0.013). Regarding weight change during the previous 3 or 6 months as continuous variables (with negative values representing weight loss), they were found to be

positively correlated with BMI (r=0.27, p=0.004 and r=0.21,p=0.002, respectively) and with body fat stores as assessed by triceps skinfold thickness (r=0.27, p=0.004 and r=0.24, p=0.016, respectively). Weight change in the previous 3 months was inversely correlated with constipation (r=-0.27, p=0.004), diarrhea (r=-0.19, p=0.044), indigestion (r=-0.25, p=0.008), eating dysfunction (r=-0.19, p=0.034), and total GSRS score (r=-0.24, p=0.011), as well as positively with physical SF-36 domains (data not shown), the SF-36 PCS (r=0.28, p=0.002) and social functioning (r=0.21, p=0.022) but not with any other GSRS or mental-health-related SF-36 domain (data not shown). Negative weight change during the last 3 months was associated with alcoholic etiology and severity of cirrhosis as well as hospital admission upon inclusion in the study, ascites, encephalopathy, and daily lactulose consumption (table 5) but not with the presence of varices, previous variceal bleeding, hepatocellular cancer, or active drinking. Differences were observed between the impact of certain clinical variables on weight change during the previous 3 or 6 months as shown in table 5.

## Comorbidities (I)

The prevalence of comorbidities potentially compromising nutritional status is shown in table 6. Malnutrition was encountered more frequently in patients with GI comorbidities (64.7% vs. 25.2%; p=0.001), but not in those with respiratory or other comorbidities (p>0.1). The presence of any particular comorbidity was not associated with negative weight change, GSRS symptoms or HRQOL (data not shown), except for higher abdominal pain scores (2.7 (1.2) vs. 2.0 (1.1);p=0.036) and a tendency for higher total GSRS scores (2.6 (0.8) vs. 2.1 (1);p=0.051) in patients with GI comorbidities.

*Table 6.* Comorbidities potentially compromising nutritional status in patients with liver cirrhosis (n=128)

| | n (%) |
|---|---|
| Comorbidities | 28 (22%) |
| Gastrointestinal[a] | 17 (13%) |
| Respiratory[b] | 9 (7%) |
| Other[c] | 4 (3%) |

[a] Gastrointestinal comorbidities: 8 (7%)- Ulcerative colitis, 6 (5%)- Crohn's disease, 2 (1%)- pancreatic insufficiency, 1 (1%)- untreated celiac disease

[b]Respiratory comorbidities: 8 (6%)- chronic obstructive pulmonary disease (requiring daily therapy), 1 (1%)- pulmonary fibrosis

[c]Other: 2 (1.5%)- extrahepatic malignancy, 2 (1.5%)- congestive heart failure

Two patients with chronic obstructive pulmonary disease had also other comorbidities: 1- congestive heart failure, 1-extrahepatic malignancy

## Consumption of gastrointestinal drugs (I)

Seventy-three out of 128 (57%) patients with cirrhosis were taking one or more GI drugs daily (table 7). The severity of GI symptoms was higher in patients receiving lactulose (table 3) and in patients consuming proton-pump inhibitors (data not shown).

*Table 7.* Use of drugs for GI disorders in cirrhotics (n=128)

| | n (%) |
|---|---|
| Proton-pump inhibitors | 51 (40%) |
| Lactulose | 37 (29%) |
| Other | 17 (13%) |

Other: 4- mesalazin; 3- salazopyrin; 3- laxantia other than lactulose; 3- cholestyramine, 1- loperamide, 1- oral aluminium and magnesium, 1- dimetikon , 1-metoclopramide

## Regression analyses (I)

Variables univariately correlated ($p<0.1$) with the total GSRS score, weight change in the previous 3 months, and the SF-36 PCS or MCS were entered into stepwise multiple linear regression analyses (table 8).

*Table 8.* Factors independently correlated to total GSRS score, weight change during the previous 3 months, and SF-36 physical and mental component summaries after multivariate analysis in patients with liver cirrhosis (n=128)

| | Adjusted $R^2$ (%) | Beta | p-value |
|---|---|---|---|
| **Total GSRS score** | | | |
| Child-Pugh score | 14.2 | 0.24 | 0.008 |
| Lactulose | 19.1 | 0.31 | 0.001 |
| Gastrointestinal comorbidities | 21.7 | 0.18 | 0.026 |
| **Weight change in 3 months** | | | |
| Ascites | 9.7 | -0.26 | 0.005 |
| GSRS indigestion score | 12.3 | -0.21 | 0.02 |
| Alcoholic etiology | 15.1 | -0.19 | 0.035 |
| **SF-36 physical component summary** | | | |
| Total GSRS score | 16.7 | -0.33 | <0.001 |
| Child-Pugh score | 20.6 | -0.23 | 0.013 |
| **SF-36 mental component summary** | | | |
| Total GSRS score | 9 | -0.31 | <0.001 |

Factors associated with total GSRS score, weight change, SF-36 PCS or MCS are reported in the order they enter the linear stepwise regression analysis.
GSRS, gastrointestinal symptoms rating scale; SF-36, Short-form-36

# 2. GASTRIC SENSORIMOTOR FUNCTION IN LIVER CIRRHOSIS (II)

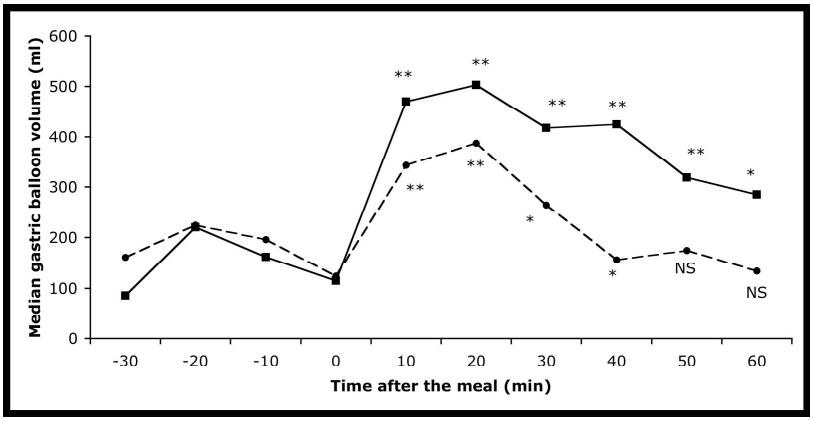
Five out of 16 patients had clinically relevant eating dysfunction and 11/16 clinically relevant indigestion (GSRS score > 2). Five out of 16 patients had a

total GSRS score > 2 and 4/16 patients were malnourished. Two out of 16 had significant weight loss, one of whom was malnourished. Cirrhotics had lower energy intake/kg compared to healthy controls [21 kcal·kg$^{-1}$·day$^{-1}$ (15-29) vs. 26.5 kcal·kg$^{-1}$·day$^{-1}$ (24.5-37.5); p = 0.048]. Ten out of 16 patients had lower energy intake/kg than recommended by the European Society of Parenteral and Enteral Nutrition (recommended energy intake/kg > 25-30 kcal·kg$^{-1}$·day$^{-1}$).

## Gastric accommodation (II)

In both cirrhotics and healthy controls, ingestion of the meal caused relaxation of the proximal stomach (significant increase in the gastric balloon volume) (figure 3).

*Figure 3.* Median gastric balloon volume (ml) (IQR) calculated at 10-min intervals as measured by the electronic barostat in cirrhotics (n=16, continuous line) and healthy controls (n=15, dashed line) before and after meal administration.




Meal administration occurred at time 0

Postprandially median gastric balloon volumes changed significantly both in cirrhotics (p=0.001) and in healthy controls (p<0.001)

** p < 0.005, * p < 0.02, NS non-significant vs. baseline (time 0)

The balloon volumes before the meal (AUC) and gastric compliance did not differ between cirrhotics and controls but the accommodation to the meal and the maximal gastric balloon volume were significantly higher in cirrhotics than in controls (table 9). There was a positive correlation between gastric accommodation to the meal and energy intake/kg in controls (r=0.66, p=0.039) but not in cirrhotics (r=0.2, p=0.47). Patients with vs. without malnutrition and/or significant weight loss demonstrated a more pronounced gastric accommodation (2776 ml·min (2197-3605) vs. 1813 ml·min (692-2451);p=0.037). This was also true for patients with low vs. normal energy intake (according to the guidelines of the European Society of Parenteral and

Enteral Nutrition) [2184 ml·min (1534-2912) vs. 1817 ml·min (713-2668);p=0.04].

*Table 9.* Barostat data in cirrhotics and healthy controls (II)

| | Cirrhotics (n=16) | Healthy controls (n=15) | p-value[a] |
|---|---|---|---|
| MDP (mmHg) | 10 (8.25-11) | 9 (7-10) | 0.096 |
| Balloon volume before the meal (AUC, ml·min) | 516 (278-804) | 474 (395-682) | 0.72 |
| Balloon volumes after the meal (AUC, ml·min) | 2644 (1940-3459) | 1519 (800-2736) | 0.07 |
| Gastric accommodation (AUC, ml·min) | 1985 (1337-2776) | 1025 (353-2137) | 0.045* |
| Vmax (ml) | 781 (618-865) | 507 (299-670) | 0.023* |
| Time to Vmax (min) | 16 (10-32) | 13.5 (10.3-19.3) | 0.52 |
| Compliance (ml·mmHg$^{-1}$) | 27.61 (16.16-36.4) | 22.76 (16.2-27.83) | 0.45 |
| Perception threshold | | | |
| Pressure (mmHg) | 6 (2.5-8) | 4 (2-6) | 0.22 |
| Volume (ml) | 237 (114-630) | 210 (140-564) | 0.75 |
| Discomfort threshold | | | |
| Pressure (mmHg) | 12 (10-18) | 10 (8-12) | 0.07 |
| Volume (ml) | 1000 (842-1000) | 1000 (793-1000) | 0.83 |

Data expressed as median (IQR)
[a]p-value for significance calculation between cirrhotics and healthy controls
Sensory pressure thresholds are expressed relative to MDP
*statistically significant difference
MDP, minimal distending pressure; AUC, area under the volume-time curve; Vmax, maximal gastric balloon volume after administration of the meal

Neither the etiology of liver cirrhosis nor the presence of portal hypertensive gastropathy influenced the gastric balloon volumes or gastric compliance (data not shown). However, the GSRS indigestion score tended to be correlated to gastric accommodation in cirrhotics (r=0.46, p=0.08). The mean postmeal gastric balloon volumes were related to the severity of liver cirrhosis expressed as the MELD score (r=0.53, p=0.041).

## Sensitivity to gastric distension (II)

No significant differences were observed in perception or discomfort thresholds between cirrhotics and healthy controls (table 9). The discomfort pressure threshold was lower (p=0.001) in Child-Pugh B/C [10mmHg (9-12)] than in Child-Pugh A patients [18mmHg (16-20);p=0.001] but liver disease etiology did not affect the sensory thresholds (data not shown). In liver cirrhosis, several GSRS symptom scores were negatively correlated to perception and/or discomfort volume thresholds (table 10). Some GSRS symptom scores were negatively correlated to pressure thresholds as well but results did not reach statistical significance (data not shown). The discomfort pressure threshold was

negatively correlated to the Child-Pugh (r=-0.65, p=0.008) and MELD (r=-0.54, p=0.032) scores.

*Table10.* Correlations between GSRS symptom scores and sensory volume thresholds in cirrhotics

| GI symptom | Perception volume threshold | | Discomfort volume threshold | |
|---|---|---|---|---|
| (GSRS) | r | p | r | p |
| Total score | -0.55 | 0.027* | -0.63 | 0.01* |
| Abdominal pain | -0.64 | 0.007* | -0.33 | 0.22 |
| Constipation | -0.62 | 0.011* | -0.55 | 0.029* |
| Indigestion | -0.58 | 0.018* | -0.63 | 0.008* |
| Diarrhea | -0.3 | 0.26 | -0.63 | 0.009* |
| Eating dysfunction | -0.55 | 0.027* | -0.63 | 0.01* |

*significant results

### Satiety drinking test (II)

In cirrhotics, the amount of calories ingested until the occurrence of maximum satiety was 1694 kcal (1213-2018) and was negatively correlated to the balloon volumes (AUC) before (r=-0.7, p=0.004) and after the meal (r=-0.55, p=0.034). However, a correlation with gastric accommodation failed to reach statistical significance (r=-0.44, p=0.1).

### Gastric emptying studies

Delayed gastric emptying was found in 8/16 (50%) cirrhotic patients and only in 6/131 (5%) controls (p<0.001). Patients with vs. without delayed gastric emptying did not differ in fasting or postprandial balloon volumes or in gastric accommodation (data not shown).

## 3. METABOLIC DISTURBANCES IN LIVER CIRRHOSIS (III)

The basic characteristics of patients and healthy controls participating in study III are shown in Table 11. A total of 5/31 patients were malnourished. Cirrhotics had higher insulin resistance, leptin, and REE/body weight ratio as well as lower energy intake compared to healthy controls (table 12). Patients with alcoholic vs. non-alcoholic cirrhosis, Child-Pugh class A vs. Child-Pugh class B or C, with vs. without malnutrition, and with vs. without hepatic encephalopathy did not differ in any of the variables of table 12 (data not shown).

*Table 11.* Basic characteristics in all subjects (III)

| | All Cirrhotic patients (n=31) | p[6] | Group A[1] | | | | Healthy Controls (n=10) |
|---|---|---|---|---|---|---|---|
| | | | All cirrhotics in group A[1] (n=18) | p[6] | Group B[2] (n=13) | p[6] | |
| Age (years) | 57 (51-63) | 0.41 | 57 (52-63) | 0.62 | 56 (48-62) | 0.93 | 54 (49-63) |
| Gender (M/F) | 18/13 | 0.91 | 11/7 | 0.95 | 11/2 | 0.18 | 6/4 |
| Weight | 77 (70-88) | 0.92 | 83 (70-89) | 0.69 | 86 (76-91) | 0.17 | 77 (72-84) |
| BMI | 26.3 (24.3-29.3) | 0.6 | 26.5 (24.7-29.3) | 0.52 | 26.5 (24.4-29.7) | 0.61 | 25.7 (24.1-27.2) |
| Fat (%)[3] | 36.2 (31.2-40.3) | 0.11 | 38.2 (34.8-46.9) | 0.04 | 38.0 (28.3-49.6) | 0.11 | 31.1 (26.8-37.9) |
| Fat (kg)[3] | 26.9 (23-35) | 0.23 | 29.4 (23.5-41.2) | 0.1 | 33.8 (23.5-43.1) | 0.07 | 25.2 (20.2-29.1) |
| Diabetes (n) | 2 | 0.41 | 1 | 0.45 | 1 | 0.37 | 0 |
| Weight change (%) | 0 (-1.3 - 0) | | 0 (0-5) | | 0 (0 - 5.8) | | |
| Malnutrition (n)[4] | 5 | | 2 | | 2 | | |
| Etiology | | | | | | | |
| Alcoholic | 13 | | 6 | | 5 | | |
| Viral | 5 | | 2 | | 1 | | |
| PBC | 4 | | 4 | | 1 | | |
| Cryptogenic | 6 | | 4 | | 4 | | |
| Other[5] | 3 | | 2 | | 2 | | |
| Ascites (n) | 6 | | 3 | | 3 | | |
| MELD score | 11 (9-14) | | 9.5 (9-14) | | 10.5 (9-14) | | |
| Child-Pugh score | 8 (6-9) | | 7 (6-9) | | 8 (6-10) | | |
| Child-Pugh A/B/C(n) | 11/15/5 | | 8/7/3 | | 5/5/3 | | |
| Encephalopathy grade I(n) | 3 | | 0 | | 0 | | |

Weight change (%), dry weight change expressed as a percentage of actual body weight during the previous 6 months (negative values represent weight loss);
[1] Group A, subgroup of all cirrhotics in which postprandial glucose and insulin were measured
[2] Group B, subgroup of group A in which postprandial leptin and ghrelin were measured
[3] Fat (expressed as percentage of body weight or in kg) calculated from anthropometric data
[4] Number of malnourished patients (triceps skinfold thickness and/or mid arm muscle circumference below the 5$^{th}$ percentile and/or BMI < 18.5 kg/m$^2$)
[5] Other: In all cirrhotics:1- autoimmune hepatitis, 1- autoimmune hepatitis and primary sclerosing cholangitis, 1- non-alcoholic steatohepatitis (NASH); in group A and B: 1- autoimmune hepatitis and 1- NASH
[6] p value compared to healthy controls
Data expressed as median (IQR)

Fasting leptin was positively correlated to BMI in patients with cirrhosis (r=0.48, p=0.007) and to body fat in kg in healthy controls (r=0.78, p=0.008). After controlling for BMI, in cirrhotics fasting leptin was positively correlated to HOMA-IR (r=0.4, p=0.034) and negatively to REE (r=-0.38, p=0.042) but not to energy intake (r=-0.04, p=0.8). After controlling for BMI, in controls fasting leptin was negatively correlated to energy intake (r=-0.72, p=0.029) but not to HOMA-IR (r=-0.48, p=0.2) or REE (r=-0.49, p=0.2).

*Table 12.* Metabolic and dietary data in cirrhotics (n=31) and healthy controls (n=10)

| | Cirrhotics | Healthy Controls | p |
|---|---|---|---|
| Fasting plasma glucose (mmol/l) | 5.4 (4.8-6.6) | 4.7 (4.4-5) | 0.005 |
| Fasting serum insulin (mu/l) | 19 (11-30) | 7.3 (5.3-8.9) | <0.001 |
| Fasting leptin (pg/ml)[1] | 25500 (15950-34525) | 9995 (6528-28525) | 0.039 |
| HOMA-IR | 6 (2-7) | 1 (1-1) | <0.001 |
| REE (kcal·24h$^{-1}$) | 1500 (1400-1790) | 1430 (1320-1477.5) | ns |
| REE/body weight (kcal·24h$^{-1}$·kg$^{-1}$) | 19.6 (18.4-21.3) | 18 (16.3-19.5) | 0.031 |
| Energy intake (kcal·24h$^{-1}$) | 1798 (1537.3-1985.8) | 2271 (1768.8-2932.3) | 0.011 |
| Energy intake/body weight (kcal·24h$^{-1}$·kg$^{-1}$) | 22.1 (17.5-27.8) | 26.7 (24.4-37.7) | 0.028 |
| Energy intake/REE | 1.17 (0.96-1.4) | 1.6 (1.27-2.04) | 0.001 |

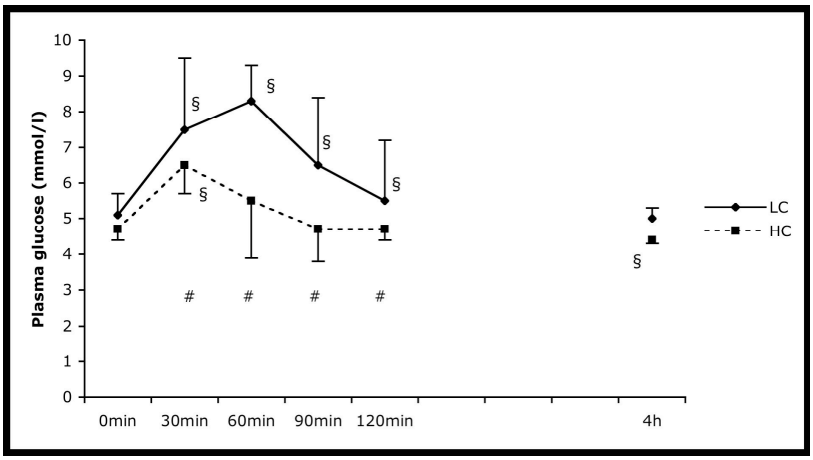
[1] Similar results were obtained when leptin/BMI, leptin/body weight, or leptin/fat in kg were used (data not shown)
Data expressed as median (IQR)

HOMA-IR was negatively correlated to non-protein respiratory quotient in cirrhotics (r=-0.39, p=0.03) but not in controls (r=0.43, p=0.2). Leptin was not correlated to non-respiratory quotient in cirrhotics or in controls (data not shown). HOMA-IR was not correlated to REE, and leptin or HOMA-IR were not correlated to the REE/Predicted REE ratio even after controlling for anthropometric parameters in cirrhotics or in controls (data not shown).

## 3.1 Postprandial glucose (III)

At 30 min postprandially plasma glucose had risen in both the cirrhosis and the control group but subsequently remained elevated only in the former (Figure 4). The area under the glucose curve (AUC) and the increase of glucose from baseline to 60min postprandially were higher in cirrhotics than in controls (13.7 $mmol \cdot l^{-1} \cdot h^{-1}$ (11.9-15) vs. 10.9 $mmol \cdot l^{-1} \cdot h^{-1}$ (8.8-11.2); $p<0.001$ and 54.8% (22.1-79.6) vs. 20% (-21.3 – 31.9); p=0.002, respectively). The increase of glucose from baseline to 60min postprandially was negatively correlated with the energy intake/body weight ratio in patients with liver cirrhosis (r=-0.53, p=0.023) but not in healthy controls (r=0.37, p=0.3).

*Figure 4*. Postprandial plasma glucose response in cirrhotics (n=18) and controls (n=10)

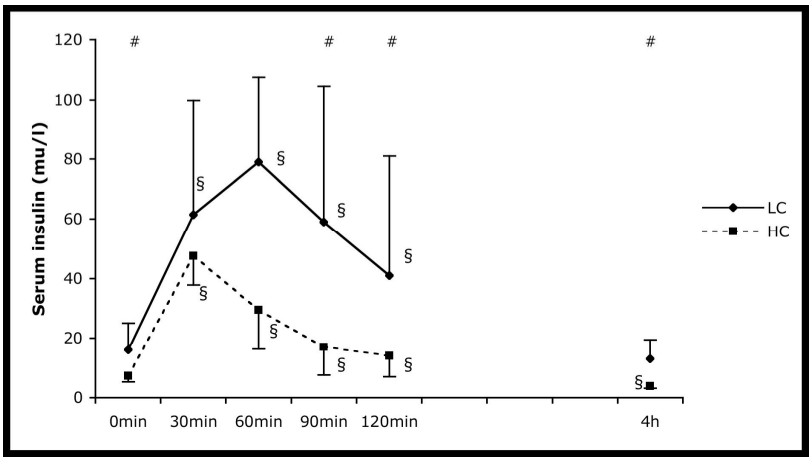



Posprandially plasma glucose changed significantly in patients with liver cirrhosis (LC) ($p<0.001$) and in healthy controls (HC) (p=0.001)
§ significant ($p<0.05$) vs. baseline
# Significant ($p<0.05$) difference between patients with liver cirrhosis and healthy controls
Data presented as median and half IQR

## 3.2 Postprandial insulin (III)

At 30 min serum insulin had risen in both the cirrhotics and controls and remained elevated until 2h postmeal in both groups (Figure 5). The AUC of insulin was higher in cirrhotics than in controls (104.6 $mu \cdot l^{-1} \cdot h^{-1}$ (92-159.6) vs. 44.6 $mu \cdot l^{-1} \cdot h^{-1}$ (27.6-93.5), p=0.015).

*Figure 5.* Postprandial serum insulin response in cirrhotics (n=18) and healthy controls (n=10)

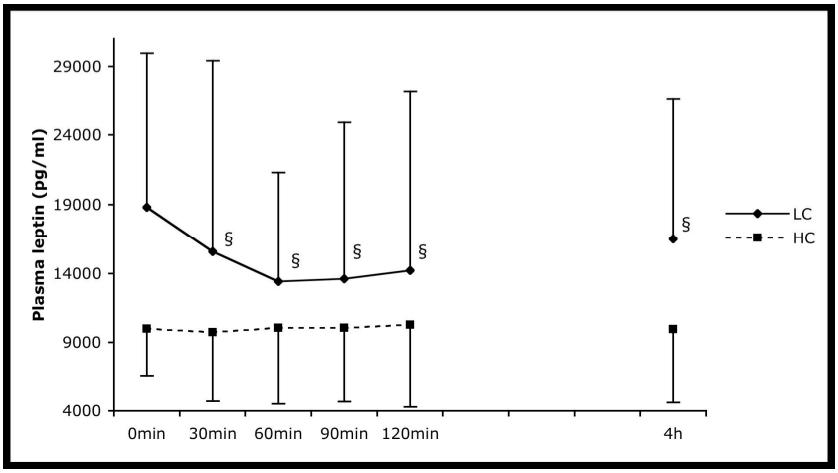



Postprandially serum insulin changed significantly in both patients with liver cirrhosis (LC) and in healthy controls (HC) ($p<0.001$ for both)
§ significant ($p<0.01$) vs. baseline
#Significant ($p<0.01$) difference between patients with liver cirrhosis and healthy controls
Data presented as median and half IQR

## 3.3 Postprandial leptin (III)

Postprandial leptin was lower than baseline levels at all timepoints in patients with liver cirrhosis but not in healthy controls (Figure 6). Leptin levels at all postprandial timepoints and the AUC of leptin were not significantly different between cirrhotics and healthy controls (Figure 6 and AUC of leptin: 28600 $pg \cdot ml^{-1} \cdot h^{-1}$ (19547-52088) vs. 20009 $pg \cdot ml^{-1} \cdot h^{-1}$ (10122-36144), p=0.2). The reduction of leptin 60min postprandially was more pronounced in patients with cirrhosis than in controls (22.4% (18.3-41.5) vs. 12.8% (3.8-29.7), p=0.042) and was negatively correlated to energy intake/body weight ratio in cirrhotics (r=-0.64, p=0.019).

*Figure 6.* Postprandial plasma leptin response in cirrhotics (n=13) and healthy controls (n=10)



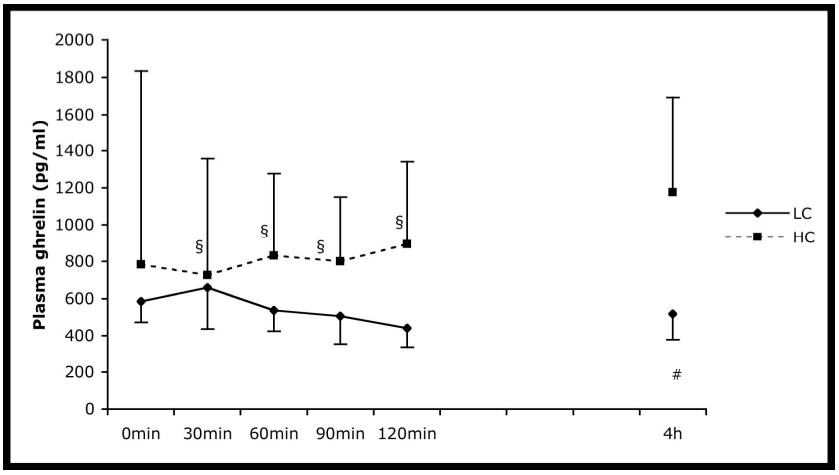

Postprandially plasma leptin changed significantly in patients with liver cirrhosis (LC) ($p<0.001$) but not in healthy controls (HC) (p=0.5)
§ significant ($p<0.05$) vs. baseline
Data presented as median and half IQR

Reductions of leptin were not correlated with increases of glucose or insulin in cirrhotics or controls (data not shown). Similar results were obtained when leptin/BMI, leptin/body weight, or leptin/fat in kg were used (data not shown).

## 3.4 Postprandial ghrelin (III)

Postprandial ghrelin changed significantly compared to baseline only in healthy controls (Figure 7). At 4h ghrelin was higher in healthy controls than in patients with liver cirrhosis (1176 pg/ml (679.3-1692) vs. 519 pg/ml (379.5-607), p=0.021). The increase of ghrelin from its minimal postmeal value to 4h postmeal was higher in healthy controls than in cirrhotics (39% (33.1-48.2) vs. 14.2% (12.8-33.4), p=0.005) and it was positively correlated with weight change in the previous 6 months in cirrhotics (r=0.66, p=0.014).

*Figure 7.* Postprandial plasma ghrelin response in cirrhotics (n=13) and controls (n=10)




Postprandially plasma ghrelin changed significantly only in healthy controls (HC) (p<0.001) but not in patients with liver cirrhosis (LC) (p=0.13)
§ significant (p<0.01) vs. levels at 4h
# Significant (p=0.021) difference between patients with liver cirrhosis and healthy controls
Data presented as median and half IQR

The AUC of ghrelin did not differ significantly between patients with cirrhosis and healthy controls (data not shown). Postprandial ghrelin levels were negatively correlated with glucose and insulin in both patients with liver cirrhosis and healthy controls (table 13). Postprandial ghrelin decrease was positively correlated to leptin decrease in healthy controls and negatively in patients with liver cirrhosis (table 14).

*Table 13.* Correlations of postprandial ghrelin with postprandial glucose and insulin parameters in patients with liver cirrhosis (n=13) and healthy controls (n=10)

| | Ghrelin at 30 minutes postmeal | | | | Ghrelin at 90 minutes postmeal | | | |
|---|---|---|---|---|---|---|---|---|
| | Cirrhotics | | Controls | | Cirrhotics | | Controls | |
| | r | p | R | p | r | p | r | p |
| Glucose increase at 90min postmeal | -0.63 | 0.022 | -0.64 | 0.048 | -0.66 | 0.014 | -0.65 | 0.043 |
| Insulin increase at 2h postmeal | -0.48 | ns | -0.7 | 0.036 | -0.74 | 0.006 | -0.47 | ns |
| Insulin increase at 4h postmeal | 0.25 | ns | -0.71 | 0.019 | 0.16 | ns | -0.61 | ns |

ns, non significant (p>0.05)

*Table 14.* Correlations of postprandial ghrelin with postprandial leptin parameters in cirrhotics (n=13) and in controls (n=10)

| | Ghrelin decrease at 30 min postmeal | | | | Ghrelin decrease at 90 min postmeal | | | |
|---|---|---|---|---|---|---|---|---|
| | Cirrhotics | | Controls | | Cirrhotics | | Controls | |
| | r | P | r | p | r | p | r | p |
| Leptin decrease at 30 min postmeal | -0.54 | 0.058 | 0.04 | ns | -0.12 | ns | 0.29 | ns |
| Leptin decrease at 90 min postmeal | -0.59 | 0.035 | 0.75 | 0.013 | -0.04 | ns | 0.75 | 0.013 |

ns, non significant (p>0.05)

## Regression analysis (III)

Stepwise linear regression analysis was performed with energy intake/body weight ratio as the dependent variable. Child-Pugh score, increase in glucose 60 min postprandially, decrease in leptin 60 min postprandially, increase in ghrelin from its minimal postmeal value to 4h postmeal were used as independent variables. Decrease in leptin 60 min postprandially (Beta=–0.8, p=0.001), increase in glucose 60 min postprandially (Beta=–0.91, p=0.003), and increase in ghrelin from its minimal postmeal value to 4h postmeal (Beta=0.55, p=0.035) were independently correlated to energy intake.

# 4. INTESTINAL PERMEABILITY (IV)

The median percentage of ingested dose of $^{51}$Cr-EDTA excreted in the 24h urine collection was significantly higher in patients with liver cirrhosis [1.94% (1.21-2.70%] compared to controls [1.40% (1.09-1.99%); $p < 0.05$]. However only the A+ patients [2.05% (1.50-3.46%); $p < 0.05$] and not the A- patients [1.94% (1.13-2.53%); $p > 0.1$] had a significantly higher $^{51}$Cr-EDTA excretion in the urine than controls (figure 8). Only 1 of the A- and 4 of the A+ patients had increased intestinal permeability above the upper limit (95% confidence) of normal for controls ($p > 0.1$). Six patients in the A+ group underwent sequential study with the $^{51}$Cr-EDTA test before and after therapeutic paracentesis in which a median of 4850ml (2300-6000ml) of ascites was removed. Figure 9 shows that the urinary excretion of $^{51}$Cr-EDTA did not change significantly [1.69% (1.16-

2.86%) *vs.* 1.30% (1.08-1.79%); p > 0.05]. The volume of ascites evacuated was not statistically correlated to $^{51}$Cr-EDTA excretion before or after paracentesis or their difference (data not shown).

*Figure 8.* 24h urinary $^{51}$Cr-EDTA excretion (%) in cirrhotics with ascites (A+), cirrhotics without ascites (A-), and healthy controls (HC)

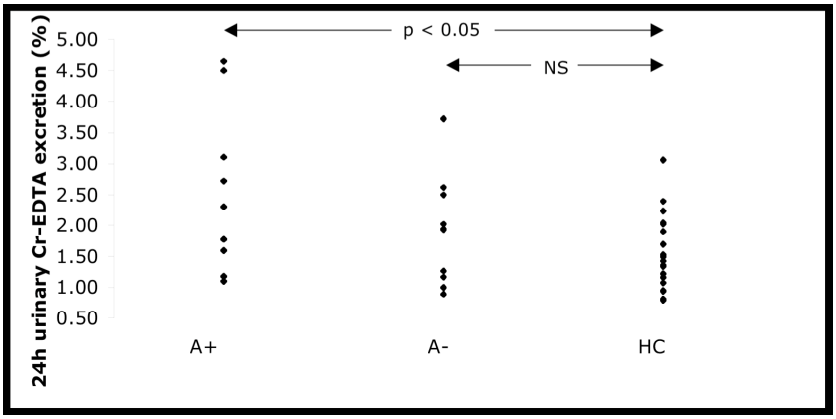
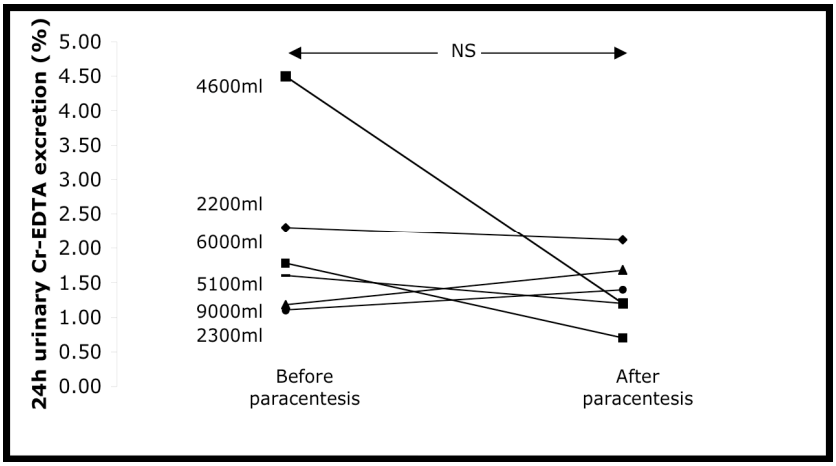



*Figure 9.* 24h urinary $^{51}$Cr-EDTA excretion (%) before and after therapeutic paracentesis in 6 patients [ascites volume obtained at every paracentesis is indicated (ml)]




Child-Pugh class B and C [2.05% (1.29-3.01%); p < 0.05] but not class A [1.94% (1.20-2.47%); p > 0.1] cirrhotics had significantly higher $^{51}$Cr-EDTA excretion than healthy controls. No significant correlation was found between the MELD or the Child-Pugh scores and $^{51}$Cr-EDTA excretion (data not shown). Patients with alcoholic [1.78% (1.09-3.56%)] vs. non-alcoholic cirrhosis [1.94% (1.60-2.72%)] did not differ significantly (p > 0.1) in their mean urinary $^{51}$Cr-EDTA excretions.

# DISCUSSION

The main results in the present studies will be combined and discussed briefly below and compared with other findings in the literature.

## 1. GASTROINTESTINAL SYMPTOMS (I)

Patients with cirrhosis were found to have increased severity of GI symptoms correlating with the severity but not with the etiology of the liver disease. GI symptoms were also associated with recent weight loss and impaired HRQOL.

Negative weight change, i.e. weight loss, was independently correlated with the GSRS indigestion score indicating a possible role of gastrointestinal symptoms in the poor food intake frequently observed in cirrhotics [9, 11, 14]. In accordance with this assumption, we have recently shown that upper gastrointestinal symptoms in patients with cirrhosis are associated with a decreased intake of calories as measured by a caloric satiety drinking test [155]. In the current study (I), weight change during the previous 3 and 6 months was calculated as a percentage of actual weight after subtracting weight 3 and 6 months before inclusion respectively. The latter was deducted from medical records in the majority of cases but some patients were asked to recall their previous weight. Patient-recall weight is frequently used but it is generally recognized that it should be treated with caution [156-158]. In our study, weight 3 and 6 months before inclusion could not be recalled by 11% and 16% of patients, respectively. The observed differences between the impact of different clinical variables on weight change in the previous 3 and 6 months might be the result of increased uncertainty about weight at the more remote time of 6 months. Alternatively, this finding might indicate that such clinical variables as hospital admission, ascites, and encephalopathy are associated with more recent weight loss. Also, actual body weight is notoriously difficult to assess in cirrhotics with fluid retention [9] and although every effort was made to evaluate "dry" weight (by taking into account weight after last paracentesis or before ascites development) some overestimation may not be entirely excluded. However, weight loss in the present study was found to be correlated with lower body mass index and body fat stores in cirrhotics. Ascites and alcoholic etiology of liver cirrhosis were the other two factors, apart from the GSRS indigestion

score, found to be independently related to negative weight change, i.e. weight loss, during the previous 3 months. This is in line with published data showing decreased caloric intake in patients with ascites [14, 62] and data indicating that refractory ascites [11] and alcoholic cirrhosis [13] might be associated with impairment of nutritional status in cirrhotics.

Another interesting finding was the correlation observed between the severity of GI symptoms and impairment of HRQOL in cirrhotics. Previous studies have suggested that subjective symptoms of cirrhosis (such as muscle cramps and pruritus) are important to patients having an impact on HRQOL along with traditional markers of liver disease severity [127]. Similarly, in our study GI symptoms seem to be associated with reduced physical and psychological indices of HRQOL in patients with cirrhosis. However, as psychiatric comorbidities have been suggested to influence patient-reported health status in chronic liver diseases [129], it is possible that impairment of at least mental HRQOL components could be implicated in the development of GI symptoms rather than merely being a consequence of these.

Interestingly, the severity of GI symptoms was independently correlated with the use of lactulose. Lactulose is often used for the treatment of hepatic encephalopathy due to both its laxative effect and its effect in lowering colonic pH whereby decreasing serum ammonia levels [159]. It accelerates colonic transit [160] and, due to a narrow therapeutic window, it can lead to diarrhea. Colonic lactulose fermentation can also induce bloating and abdominal distension [161]. Lactulose is, thus, known to be commonly associated with GI side-effects, the most common being flatulence [162]. In our series, daily use of lactulose was significantly associated with increased severity of almost all GI symptoms including diarrhea. The latter indicates possible lactulose overuse in our series. Therefore, improved physician and patient awareness about the actions of lactulose could potentially help decrease its side-effects. Alternatively, lactitol, another non-absorbed disaccharide reported to have the advantage of lower incidence of meteorism and flatulence compared to lactulose [163] may be used.

Furthermore, GI comorbidities, which were associated with compromised nutritional status, influenced the severity of GI symptoms. The most common diagnosis was inflammatory bowel disease (15/18) and all patients were

clinically in remission. Malnutrition and GI symptoms are common in patients with active inflammatory bowel disease but they have also been reported in patients with inactive disease [132, 164]. Prompt recognition of cirrhotics with underlying GI diseases at risk for malnutrition seems to be important so that rigorous nutritional intervention can be instituted.

The Child-Pugh score was found to be more closely correlated with GI symptoms, weight loss and HRQOL than the MELD score. This is in agreement with previous studies in cirrhotics undergoing pretransplantation evaluation in whom patient-reported health status has been shown to be correlated with the severity of liver disease expressed as the Child-Pugh but not as the MELD score [128]. Ascites and hepatic encephalopathy have been shown to be important factors influencing physical domains of HRQOL [127] and seem to be related to recent weight loss and the severity of GI symptoms (at least in a univariate fashion) according to findings of the current study. The fact that Child-Pugh but not the MELD score includes ascites and encephalopathy may explain, at least in part, the better correlation with GI symptoms, weight loss, and physical HRQOL.

The use of proton-pump inhibitors was very common in cirrhotics (40%) in the present study, particularly in those with severe gastrointestinal symptoms. Compared to healthy controls, patients with liver cirrhosis have been reported to have increased plasma gastrin [39] and higher prevalence of peptic ulcers [40, 41]. This may partly explain the consumption of acid secretion inhibitors, but it is possible that there is a certain degree of overuse in these patients in view of the fact that peptic ulcers are asymptomatic in as many as 70% of cases [42]. Further studies are needed to evaluate the indications for the use of acid secretion inhibitors in cirrhotics.

## 2. GASTRIC SENSORIMOTOR DYSFUNCTION (II)

In paper II, we have demonstrated increased proximal stomach relaxation in cirrhotics compared to healthy controls especially in those with compromised nutritional status and low energy intake. In patients with liver cirrhosis the relaxation of the proximal stomach was not correlated to energy intake although a positive correlation was observed in healthy controls. A relationship between gastric sensitivity to balloon distension and GI symptoms was observed in these patients.

In a previous ultrasonographic study, reduced relaxation of the proximal stomach after a meal was reported in patients with alcoholic liver cirrhosis compared to healthy controls [61]. However, ongoing alcohol overconsumption was not mentioned as an exclusion criterion in this study [61]. Acute and chronic alcohol consumption have been associated with altered motility in the esophagus, stomach, and the small intestine [165, 166]. To date, there are no published studies on the effects of alcohol on gastric accommodation, but it is possible that the results of Izbéki et al. might be due to alcohol *per se*, if patients had not been abstinent, and, in any case, they may not be generalized for liver cirrhosis of any cause [61]. In the current study, only 7/16 patients had alcoholic liver cirrhosis and they had been abstinent for at least 6 months before inclusion. Aprile et al. have recently reported reduced gastric accommodation to gaseous distension in patients with portal hypertension due to hepatosplenic mansonic schistosomiasis [167]. All patients included in this study were classified as Child-Pugh A and had esophageal varices [167]. Patient selection and/or methodological differences might account for discrepancies from results presented in the current study in which only patients with established liver cirrhosis of different severity were enrolled. In the study of Aprile et al, no information was given on whether medications potentially influencing gastric motility were interrupted before the experiments [167]. Furthermore, the method of gaseous distension used by Aprile et al. permits air escape through the esophagus and the pylorus and its results on gastric accommodation are not necessarily comparable with those yielded by assessment after a meal using the method employed in the current study. In a recent study postprandial gastric volumes and accommodation measured by means of SPECT in cirrhotic patients with tense ascites were found to be lower compared to healthy controls [62]. Again, patient selection and/or methodological differences might account for discrepancies from results presented in the current study in which 3 patients had mild and none had tense ascites. SPECT is a non-invasive method of evaluating gastric accommodation in which total gastric volumes are measured as opposed to proximal stomach volumes assessed by a gastric barostat [51, 168]. The latter is considered to be the gold standard for assessing gastric postprandial accommodation [51, 168]. A study comparing SPECT and barostat found SPECT less suitable in detecting gastric tone changes [168]. To our knowledge, a gastric barostat has not been used previously for evaluation of gastric accommodation in cirrhotics. Thus, there are several reasonable explanations

why the present new data reflect gastric pathophysiological factors not previously described in cirrhotics.

Previous studies have shown increased gastric wall thickness in patients with liver cirrhosis [38] and non-cirrhotic portal hypertension [167] especially in those with portal hypertensive gastropathy [10]. It has been proposed that loss of elasticity of the thickened stomach wall in portal hypertension might result in reduced wall compliance, thus possibly compromising proximal stomach relaxation after a meal [61, 167]. However, in the current study (II) gastric wall compliance did not differ between patients with liver cirrhosis and healthy controls and it was not affected by the presence of portal hypertensive gastropathy. Thus, gastric compliance does not seem to be involved in the gastric accommodation response in liver cirrhosis.

Transit studies in patients with liver cirrhosis have shown contradictory results for gastric emptying [74-81]. In our series 50% of patients with cirrhosis were found to have delayed gastric emptying which is in line with results previously presented from our group [74] and others [75-77]. However, the increased proximal stomach relaxation observed in our patients does not seem to be associated with delayed gastric emptying.

Although no conclusions can be drawn from the current study as to the mechanisms leading to increased gastric accommodation in cirrhotics, certain pathophysiologic disturbances observed in liver cirrhosis might contribute to increased proximal stomach relaxation after a meal. Nitric oxide has been shown to be increased in both the systemic and portal circulation in cirrhotics [169-171] and it has been proposed to be involved in the pathogenesis of the hemodynamic circulatory changes in liver cirrhosis [170]. Nitric oxide has also been reported to be involved in maintaining basal fundic tone and in meal-induced gastric accommodation in humans [49, 50]. Glyceril trinitrate, an exogenous nitric oxide donor, has been shown to improve accommodation of the proximal stomach to a meal in patients with functional dyspepsia [172]. Thus, it is possible that nitric oxide might be involved in the increased accommodation in cirrhotics found in the present study. Furthermore, increased levels of cholecystokinin [173, 174] have been reported in liver cirrhosis. Cholecystokinin-A receptors have been demonstrated to be involved in the gastric relaxation response to intraduodenal lipid [175, 176] and exogenous

cholecystokinin decreases food intake [175] and basal gastric tone [177] and inhibits gastric emptying [178]. Therefore, cholecystokinin may possibly be of importance in increasing gastric accommodation in cirrhotics. Thirdly, cirrhotics have been shown to have higher fasting and postprandial glucagon-like peptide 1 (GLP 1) levels compared to healthy controls after oral glucose [179]. GLP 1 is a gut peptide shown to induce proximal stomach relaxation [180, 181], inhibit gastric emptying [178] and decrease spontaneous food intake [178] in healthy individuals. Therefore, presumed increased GLP 1 in our patients could be the common underlying mechanism of the observed increased proximal stomach relaxation after a meal and its dissociation from energy intake which was found to be poor in these patients. Lastly, as we found enhanced gastric accommodation in patients with compromised nutritional status and energy intake, it may be speculated that improved postprandial stomach relaxation might be due to an unknown compensatory, albeit ineffective, mechanism in order to improve food intake.

The maximum amount of calories ingested at the satiety drinking test was found to be inversely correlated to fasting and postmeal balloon volumes and a negative trend with accommodation was also seen in cirrhotics. The endpoint of the satiety drinking test has been proposed as a surrogate marker for the magnitude of the gastric accommodation response in healthy and dyspeptic subjects [60]. It was also recently shown to be positively correlated to fasting and postmeal gastric volumes assessed by SPECT in healthy subjects [182]. In our patient group, the dissociation of gastric accommodation from energy intake and the inverse correlation between the endpoint of satiety drinking test and the balloon volumes before and after the meal suggests that mechanisms other than the response of the proximal stomach to a meal may be involved in the poor dietary intake observed. Therefore, the satiety drinking test does not seem to be a good surrogate marker of accommodation in patients with liver cirrhosis.

## 3. METABOLIC DISTURBANCES IN LIVER CIRRHOSIS (III)

In the current study (III), we observed altered postprandial responses of glucose, insulin, leptin, and ghrelin in patients with liver cirrhosis associated with reduced energy intake and weight loss.

Cirrhotics exhibited insulin resistance with significantly higher baseline and postprandial insulin and glucose levels compared to healthy controls, in

agreement with previous studies [9, 23, 101]. Hyperinsulinemia has been shown to influence GI motility in healthy subjects [183] and it has been proposed as a mediating factor of reduced food intake in liver cirrhosis [101]. However, in the current study the postprandial increase of glucose was found to contribute independently to the reduced energy intake in cirrhotics. Hyperglycemia has been reported to reduce motility in the stomach, duodenum, and jejunum [184]. Decreased hunger and slower gastric emptying have been observed in healthy volunteers during induced hyperglycemia [185]. Postprandial hyperglycemia has been reported to be associated with tachygastria and increased postprandial upper gastrointestinal symptoms [186, 187] compared to euglycemia in healthy volunteers. It is therefore possible that postprandial hyperglycemia results in reduced energy intake by contributing to early satiety and other GI symptoms, which were shown to be increased in cirrhotics (I).

Baseline leptin in cirrhotics was found to be elevated as previously reported [112-116] and leptin effects on energy intake and resting energy expenditure were disturbed in these patients. Leptin has been shown to increase REE [103] but in a recent study performed in non-cirrhotic individuals total and free leptin were reported to be negatively and bound leptin positively associated with REE [104]. We observed a negative association between total leptin and REE in patients with cirrhosis. It might therefore be hypothesized that the resistance to the effects of leptin in cirrhotics observed in the current study may be mediated by a proportional increase of free leptin. However, we did not measure free and bound leptin fractions in our series which is mandatory to show this.

The postprandial leptin reaction was also found to be disturbed in patients with liver cirrhosis. Although no conclusions can be drawn from the current study as to the mechanisms leading to the postprandial leptin reduction in cirrhotics, the insulin resistance or low energy intake observed in these patients might contribute to decreased leptin levels after a meal. Physiological insulinemia has been shown to acutely increase leptin in healthy individuals [107]. In our study, baseline leptin was positively related to insulin resistance in cirrhotics. However, postprandial reductions of leptin were not correlated with increases of glucose or insulin in either the cirrhosis or the control group, thus indicating possible resistance to the effect of insulin in the regulation of leptin levels in patients with liver cirrhosis. Leptin has been shown to increase [108, 188] or remain unchanged postprandially depending on meal size and composition in

non-cirrhotic individuals [108-110, 188]. Fasting has been shown to be associated with an acute leptin reduction [107, 108, 189] which is thought to represent a signal to the brain stimulating energy intake [189]. Similarly in cirrhotics the postprandial leptin decline could represent a compensatory, albeit ineffective, mechanism due to low energy intake.

Ghrelin levels following a meal have not been investigated previously in patients with liver cirrhosis. Cirrhotics had a clearly altered postprandial pattern of ghrelin compared to controls, with an attenuated ghrelin increase at 4 hours postmeal. Ghrelin enhances appetite and food intake, and its level rises preprandially thus playing a role in meal initiation [103]. Therefore, the low ghrelin observed in cirrhotics at 4h postmeal (i.e. before expected lunch in our experiment setting) could be directly involved in the reduced energy intake in these patients. In a recent study, fasting ghrelin was found to be elevated in patients with liver disease compared to healthy controls and especially in Child-Pugh C patients as well as in patients with complications of liver disease [120]. Marchesini et al, reported that fasting ghrelin was comparable in patients with cirrhosis and controls but increased levels identified a group of patients with decreased energy intake and malnutrition [121]. In our study, we were also unable to confirm generally increased fasting ghrelin in cirrhotics. These discrepancies could, at least in part, be explained by different patient and/or control selection. Patients in the former study [120] were transplantation candidates, some had malignancies, and were not BMI-matched with controls whereas in the current study no patients with malignancies were included and BMI-matched controls were chosen.

The mechanisms of altered postprandial ghrelin response might involve glucose, insulin, and/or leptin. Postprandial ghrelin was negatively related to glucose and insulin in both healthy controls and cirrhotics as previously reported [122-124]. According to these studies insulinemia is essential for postprandial ghrelin suppression with glucose having an additional effect [122-124]. In our series postprandial ghrelin decrease was positively related to leptin reduction in healthy controls and negatively in cirrhotics. Although the former observation is of unclear significance since in controls leptin remained unchanged postprandially, the latter is in agreement with earlier data suggesting an inverse relationship between leptin and ghrelin and that leptin could be important for suppression of ghrelin [109]. Therefore insulin resistance resulting in high

postprandial glucose and insulin might be involved in the low ghrelin observed 4h postmeal and the postprandial reduction of leptin might represent a compensatory, albeit ineffective, mechanism. Thus, it is conceivable that treatment of insulin resistance might reduce the hypoghrelinemia prior to a meal in cirrhotics, possibly stimulating appetite. Although this is probably not the single most important reason for reduced energy intake in liver cirrhosis, it certainly warrants further studies.

Insulin resistance was found to be correlated with reduced non-protein respiratory quotient in cirrhotics confirming earlier reports of the importance of insulin resistance in the starvation-type metabolism of cirrhosis [190]. However, insulin resistance was not related to REE. In a previous single study in which diabetic and non-diabetic cirrhotics were studied before and after liver transplantation a weak negative correlation was found between the REE/predicted REE ratio and insulin sensitivity [102]. However, patients with hepatocellular cancer were also included in this study [102] which might have affected the results as REE is commonly increased in cancer patients [191].

Certain methodological aspects should be taken into consideration when interpreting the results of the current study (III). Food intake was assessed by means of food diaries. This is an established method of food intake assessment [104, 108, 191, 192] which has been previously utilized in patients with liver cirrhosis [16, 101, 121]. However, it is known that both normal-weight and obese subjects may underestimate their dietary intake [192] and it is conceivable that patients with hepatic encephalopathy might also be prone to underreporting when filling in detailed food diaries. In the current study, no patients with encephalopathy grade II or higher were included and food intake was not statistically different between patients with vs. without hepatic encephalopathy grade I. Furthermore, our findings confirm previous studies showing reduced energy intake in cirrhotics [9, 16, 121] and reports of a negative correlation between leptin and food intake in healthy subjects [192]. Second, in the current study fasting data are obtained from all subjects but postprandial data are obtained from a smaller subgroup of the main patient population. Although cirrhotics were carefully matched with the group of healthy controls, a type-II error in the assessment of the postprandial responses cannot be ruled out. Lastly, the current study is a cross-sectional one. Thus, statistical correlations between

hormonal disturbances and energy intake or REE in cirrhosis do not necessarily implicate a cause-effect relationship.

# 4. PATHOPHYSIOLOGY OF GI SYMPTOMS IN CIRRHOSIS (II, III)

GI sensorimotor disturbances have previously been reported in patients with cirrhosis and might be involved in the pathogenesis of GI symptoms in these patients. Manometry studies have described altered gut motility [88, 193] in cirrhotics, whereas gastric emptying has been found to be delayed [74-77], normal [78-80] or accelerated [81]. Gastric accommodation has been reported to be impaired in patients with alcoholic cirrhosis [61] and cirrhotics with ascites [62] compared to healthy controls. Delayed gastric emptying [70], hypersensitivity to gastric distension [65, 66], and impaired gastric accommodation to a meal [56] have all been implicated in the development of meal-induced symptoms in patients with functional dyspepsia. Thus, altered gut motility may be involved in the pathogenesis of gastrointestinal symptoms in cirrhotics.

In study II, gastric accommodation was found to be increased in patients with liver cirrhosis compared to healthy controls. Although there was a trend for subjective indigestion severity to be positively correlated with proximal stomach relaxation in these patients, it failed to reach statistical significance. Thus, the accommodation reflex does not seem to be relevant in the pathogenesis of GI symptoms in these patients.

Although no significant differences in sensory thresholds were observed between cirrhotics and healthy controls, the discomfort pressure threshold was related to the severity of liver disease (II). Furthermore, within the liver cirrhosis group, significant correlations were found between the severity of several gastrointestinal symptoms and volume sensory thresholds (II). Both pressure and volume contribute to the generation of gastric wall tension which has been shown to determine perception of gastric distension [64]. Hypersensitivity to gastric distension has been shown to be present in a subset of functional dyspepsia patients [63-66] and to be associated with postprandial epigastric pain, belching, and weight loss [66]. Gastric tension mechanoreceptors have been suggested to be involved in symptom generation in patients with functional

dyspepsia with gastric hypersensitivity [194]. Thus, it seems reasonable to hypothesize that gastric sensitivity might be involved in GI symptom generation in cirrhotics.

In addition, the postprandial increase of glucose was found to contribute independently to the reduced energy intake in cirrhotics (III). As discussed above, postprandial hyperglycemia and euglycemic hyperinsulinemia has been shown to be associated with reduced motility in the stomach, duodenum, and jejunum [184], tachygastria [186], delayed gastric emptying, and decreased hunger [185] as well as postprandial GI symptoms in healthy volunteers [187]. Further studies are therefore needed to delineate the role of postprandial hyperglycemia in the pathogenesis of altered gut motility and GI symptoms in cirrhotics.

## 5. INTESTINAL PERMEABILITY (IV)

Patients with liver cirrhosis and ascites had significantly higher intestinal permeability compared to healthy controls. However therapeutic paracentesis did not seem to improve intestinal permeability in these patients and there were no statistical differences between the patients without ascites and healthy controls.

A number of investigators have observed normal intestinal permeability in patients with liver cirrhosis [29, 95, 97], whereas others have found intestinal permeability to be increased [28, 93, 94, 96]. Limited data exists on the influence of ascites *per se* on intestinal permeability in liver cirrhosis and the impact of paracentesis has not been previously explored.

Apparent discrepancies in results between the current and previous studies (in many of which patients with ongoing infections such as spontaneous bacterial peritonitis were included) may partly be explained by differences in patient selection [28, 29, 93-98]. However, one of the most informative studies by Zuckerman et al. evaluated intestinal permeability in liver cirrhosis with and without ascites with a differential four-sugar intestinal permeability-absorption test [98]. Patients without ascites had normal intestinal permeability and those with ascites had increased permeability, which is in line with the results of the current study. Over a third of the patients in the ascites group in the Zukerman

study had concurrent infections [98], but our study would suggest that this did not by itself affect the results significantly as our results are very similar in cirrhotics without concomitant infections. Campillo et al. have also reported increased intestinal permeability in patients with liver cirrhosis, particularly in those with septic complications [28].

Some previous studies have shown an association between increased intestinal permeability and severity of LC assessed according to the Child-Pugh classification [28, 93] but others have failed to reproduce these results [94, 96, 97]. Methodological and/or patient selection differences may account for these discrepancies. We observed significantly higher intestinal permeability in Child-Pugh class B and C but not in class A patients compared to controls. Child-Pugh and MELD scores were not statistically correlated to intestinal permeability. However, definite conclusions cannot be drawn due to the fact that all patients with Child-Pugh class C had ascites and that only 2 patients with class B (without ascites) were included in the study.

There are certain methodological considerations that should be taken into account when interpreting the results of the current study. We used a single-isotope ($^{51}$Cr-EDTA) probe test with the possible disadvantage that recovery in the urine is affected by pre- and post-mucosal factors [82, 84]. Although $^{51}$Cr-EDTA is not susceptible to bacterial degradation or digestion hydrolysis [84], other premucosal factors, mainly gastric emptying and intestinal transit, which have been reported to be slower in LC [84], could influence results of intestinal permeability studies in LC. $^{51}$Cr-EDTA is not metabolised or produced endogenously but postmucosal factors, such as renal function and tissue distribution, could affect its urinary excretion [84]. However, serum creatinine levels were normal in all patients included in the current study and did not differ between cirrhotics with and without ascites.

Distribution of $^{51}$Cr-EDTA in ascites might have caused a lower urinary excretion rate and thus underestimated possible permeability changes in patients with ascites. However Bac et al. assessing intestinal permeability in 9 cirrhotics (6 with ascites) with $^{51}$Cr-EDTA, with urine collections every 3h for the first 12h followed by a further 12h collection, concluded that a loss of $^{51}$Cr-EDTA into the ascites compartment was unlikely [95]. Furthermore paracentesis in the current study had had no significant effect on the urinary $^{51}$Cr-EDTA excretion

suggesting that ascites by itself does not unduly affect the test results [82, 84, 98]. However, ascitic fluid was not tested for $^{51}$Cr-EDTA in the current study and therefore the possibility of a lower urinary excretion rates due to distribution of $^{51}$Cr-EDTA in ascites can not be fully excluded.

# SUMMARY AND CONCLUSIONS

1. The severity of gastrointestinal symptoms is high among patients with liver cirrhosis and associated with recent weight loss, severity of liver cirrhosis, and impaired HRQOL.

2. Increased proximal stomach relaxation is observed after a meal in cirrhotics in comparison with healthy controls as assessed by a gastric barostat. The relation between gastric accommodation and energy intake seems to be disturbed in these patients.

3. The endpoint of the satiety drinking test does not seem to be a good surrogate marker of gastric accommodation in this patient group.

4. Gastric sensitivity seems to be a relevant factor for gastrointestinal symptom severity in patients with liver cirrhosis.

5. Altered postprandial glucose, leptin, and ghrelin levels correlate with reduced energy intake and weight loss in liver cirrhosis.

6. Effects of leptin on energy expenditure and energy intake seem to be altered in patients with cirrhosis.

7. Insulin resistance and/or leptin resistance might be involved in the altered postprandial glucose, insulin, leptin and ghrelin responses.

8. Only few patients with cirrhosis had increased intestinal permeability, as assessed by a $^{51}$Cr-EDTA test. which was not influenced to a major extent by ascites. Intestinal permeability is probably of limited importance in the pathophysiology of bacterial infections in patients with liver cirrhosis.

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