Fatigue and associated psychosocial symptoms in patients with liver cirrhosis - prevalence, independent predictors and effects of an intensified nutritional intervention: A secondary analysis within the EnErGie study

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List of Abbreviations

ACE	Angiotensin converting enzyme inhibitors
ALAT	Alanine aminotransferase
AP	Alkaline phosphate,
ASAT	Aspartate aminotransferase
AT1	Angiotensin receptor antagonists
BBB	Blood brain barrier
BDR	Bile duct resection
BIA	Bioelectrical impedance analysis
CCL2	CC-chemoligand 2
CCR-2	Chemokine receptor type 2
CECs	Cerebral endothelial cells
CFS	Chronic fatigue syndrome
CI	Confidence interval
CKD-EPI-GFR	Chronic kidney disease epidemiology glomerular filtration rate
CLQD	Chronic liver disease questionnaire fatigue scale
CLQD-NASH	Chronic liver disease questionnaire – non-alcoholic steatohepatitis
CON	Healthy subjects
CON-Pat	Control patients
CP	Chronic pancreatitis
CPS	Child-Pugh score
CRP	C-reactive protein
CT	Sonography or computer tomography
CVOs	Circumventricular organs
DALYs	Disability associated life years
DEGS	German Health Examination Survey for Adults
ECW	Extracellular water
ECW/TBW	Extracellular water/ total body water
EnErGie	Enteral nutrition in malnutrition due to diseases of the gastrointestinal tract: from basic understanding to innovative treatment concept,
ESPEN	European Society for Parenteral and Enteral Nutrition
EWGSOP2	European working group on sarcopenia in older people
F	Fatigued
F-00	Only fatigue symptoms
F-AD	Fatigue with anxiety and/or depression symptoms,
FA	Fatigue scale

FACIT-F	Functional assessment of chronic illness therapy fatigue scale
FBN	Institute of Nutritional Physiology 'Oskar Kellner', Leibniz Institute for Farm Animal Biology
FFM	Fat free mass
FFQ	Food frequency questionnaire
FIS	Fatigue impact scale
FIS	Fatigue inventory scale
FM	Fat mass
FSS	Fatigue severity scale
FV	Frequency value
g	Gram
G-NCP	German nutrition care process
GGT	Gamma-glutamyl transferase
GLIM	Global leadership initiative on malnutrition
h/d	Hours per day
HADS-D	Hospital anxiety and depression scale - German version
HCV	Hepatitis C virus
HCV	Hepatitis C virus
HE	Hepatic encephalopathy
HGS	Handgrip strength
HOMA	Homeostatic model assessment
HRQoL	Health related quality of life
HSNB	University of Applied Sciences Neubrandenburg
IGF-1	Insulin-like growth factor 1
IL-1/6/b	Interleukins 1 and 6
INR	International normalized ratio
IPAQ	International physical activity questionnaire
IR	Insulin resistance
kcal/d	Kilocalories per day
KDIGO	Kidney disease improving global outcomes
Kg	Kilogram
kHz	Kilohertz
KiGGS	German Health Interview and Examination Survey for Children and Adolescents
LC	Liver cirrhosis
LPS	Lipopolysaccharide

mBCA	Medical Body Composition Analyzer
MCP-1	Monocyte chemoattractant protein 1
MCV	Mean corpuscular volume
MELD	Model of End Stage Liver Disease score
METs	Metabolic Equivalent of task
MFI	Multidimensional fatigue inventory
MMSE	Mini-mental status examination
MRI	Magnetic resonance imaging
NASH	Non-alcoholic steatohepatitis
NF-kB	Nuclear factor kappa B cells
NIED	Neubrandenburg Institute of Evidence-Based Dietetics,
NM	Not mentioned
NMR	Nuclear magnetic resonance spectroscopy
Non-F	Non-fatigued
NRS-2002	Nutritional risk screening tool
ONS	Oral nutrition supplement
PBC	Primary biliary cirrhosis
PBC-40	Quality of life for primary biliary cirrhosis
PESR	Problem, etiology, symptoms/signs, resouces/barrieres
POMS	Profile of mood states scale
R	Resistance
R (d)	Rhythm (days)
RPM	Rotation per minute
RR	Risk ratio
rs/rp	Correlation coefficient (spearman/perason)
rsFC	Resting-state functional connectivity
SaNT	Supportive ambulant nutrition therapy
SBS	Short bowel syndrome
SD	Standard deviation
Sf-36	36-Item short form health survey
SMART	Smart, measurable, achievable, relevant
SMI	Skeletal muscle index
STAT	Signal transducers and activators of transcription
TBW	Total body water
TIPS	Transjugular intrahepatic portosystemic shunt
TLR4	Toll like receptor 4

TNFa	Tumour necrosis factor alpha
UAC	Upper arm circumference
UMG	University medicine greifswald
UMR	University medical center rostock
V-CAM-1	Vascular cell adhesion molecule-1
VAS	Visual analogue scale
vs.	Versus
WHO	World health organization
WHR	Waist-to-hip ratio
Xc	Reactance
µA	Microampere
0-00	No symptoms of fatigue, anxiety or depression
0-AD	Only anxiety and/or depression symptoms,
5-HT	5-hydroxytryptamine
°C	Degree celsius
♀	Female
♂	Male

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"*By examining fatigue, two sets of phenomena call our attention. The first is the decrease in muscle strength. The second is fatigue as an internal sensation. In other words, we have a physical fact that we can measure and compare, and a psychic that escapes measurement and comparison*"

Angelo Mosso (1846-1910): La Fatica, 1891

1 Introduction

Chronic liver disease is an important global public health issue impacting over more than 1.5 billion people across the world [1, 2]. Alcohol-induced liver disease, chronic hepatitis B and C virus as well as non-alcoholic fatty liver disease associated with obesity, insulin resistance and dyslipidemia are the most common etiologies [1]. Alcohol-related etiology is common, given an approximate 75 million people worldwide are affected by harmful alcohol use, which places them further in danger of developing alcohol-related liver disease [3]. In Germany, more than 50 % of liver cirrhosis-associated hospital admissions are due to alcohol abuse [4]. Alcohol related cirrhosis contributes about 20 times more often to hospital admissions in Germany, than any other etiology of cirrhosis, such as chronic hepatitis C virus infection or primary biliary cholangitis [4]. In cirrhosis, healthy liver parenchyma is replaced by fibrotic tissue resulting in portal hypertension, systemic inflammation and liver failure [7]. This underlying pathology leaves patients vulnerable to disease-related complications, leading eventually to decreased longevity [5]. The most common complication of liver cirrhosis resulting in admission to hospital is ascites [4, 6, 7].

Worldwide, liver cirrhosis and its complications are a major cause of increasing mortality, morbidity as well as health care costs [1]. According to recent data from the global burden of disease study, in over two decades, the mortality of liver cirrhosis has increased by almost 50 % [8]. Patients with alcoholic cirrhosis have a 20 % 1-year mortality rate, when transitioned to decompensated state compared to only 5 % in compensated patients [6]. The 5-year survival rate of liver cirrhosis patients with ascites is 57 %, compared to 80 % in patients without ascites [7]. In Germany, liver cirrhosis has the highest hospital mortality rate of all chronic diseases at 9.5 %, followed by respiratory diseases at about 8 % [4]. Patients admitted with cirrhosis have a 6.2-fold increased risk of dying during hospitalisation, compared to about 4-fold increased risk for malignancy or about a 1.2-fold increased risk of ischemic heart disease [4].

In terms of cirrhosis morbidity, global rates of disability associated life years (DALYs) have remained fairly constant in almost 30 years, representing 1.3 versus 1.8 percentage of total DALYs in 1990 and 2019, respectively [9]. In Germany, the total number of DALYs related to liver cirrhosis were approximately 450 000 per year between 2005 and 2018 [4]. Liver cirrhosis is therefore, a major global health burden and has a strong impact on the German health care system [4, 10], resulting in annual healthcare cost of between € 10,291 and € 22,561 per patient in compensated and decompensated cirrhosis, respectively [11].

In summary, cirrhosis of the liver is a relevant problem in Germany and worldwide, which affects many patients and places a significant burden on the health care system. Additionally, malnutrition and sarcopenia are also highly prevalent in cirrhotic patients [12, 13].

The management of liver cirrhosis naturally, focuses on treating the patient's symptoms as well as preventing or treating complications and, if necessary, liver transplantation [14]. However, there is a growing recognition that liver cirrhosis as a disease has the potential to concurrently present with, multifactorial psychosocial symptoms during the disease process. These psychosocial symptoms may also need appropriate management since, they have a considerable effect on patients quality of life [15] and influence prognosis [16]. These symptoms include fatigue, anxiety and depression [17, 18]. They may occur at any phase of liver disease and may not be ameliorated by treatment of the primary disease condition [19].

The most common psychosocial signs in patients with chronic liver disease are fatigue symptoms, which makes them a a crucial factor [18-22]. Fatigue symptoms include partly non-specific symptoms that are considered secondary to the disease process itself. These non-specific symptoms include malaise, listlessness, lethargy, concentration inability/problems, loss of interst in social interactions and anorexia [23]. Fatigue symptoms in chronic liver disease are well studied in cholestatic liver disease, chronic hepatitis C and non-alcoholic fatty liver disease but not so much in patients with cirrhosis [24]. Therefore, for liver cirrhosis, there is little published data on the association and significance of fatigue symptoms.

1.1 Fatigue in general

1.1.1 Fatigue – definition

Fatigue as a term is of Latin origin 'fatigare' or 'fatigo' meaning to be weary, "to tire out" or "to exhaust" [25]. Fatigue in clinical settings is currently defined as "*a subjective state of overwhelming, sustained exhaustion and decreased capacity for physical and mental work that is not relieved by rest*" [26].

1.1.2 Comparison: Fatigue symptoms and chronic fatigue syndrome (CFS)

It is important at this juncture to differentiate between fatigue symptoms and chronic fatigue syndrome (CFS). Fatigue symptoms are experienced by many people at some point in their lives. It is characterized by a feeling of tiredness, weakness, or exhaustion that can be physical or mental. Fatigue can be caused by a wide range of factors, including lack of sleep, physical activity, and illness [18]. During illness, fatigue symptoms can affect several areas of daily life,

with patients unable to compensate for these deficits through sleep and/or physical rest. When fatigue symptoms are not resolved, this can then lead to chronic fatigue syndrome (CFS) [27].

The main difference between fatigue symptoms during an illness and CFS, is that fatigue symptoms can be explained by prevailing circumstances of the underlying disease [27]. While CFS, is a chronic and disabling condition with wide ranging clinical features that is characterized by severe, persistent fatigue that is not relieved by rest and is often worsened by physical or mental activity, and significantly impacts a person's quality of life with no traceable medical reason [28, 29]. The core feature of CFS is, persistent fatigue which must last at least 50 % of the time and for at least 6 months [30]. Although the exact cause of CFS is not fully understood, it is believed to be related to abnormalities in the immune system, central nervous system, and/or hormonal regulation [31].

Fatigue symptoms during an illness are diagnosed by relatively simple validated questionnaires (see Table 1) [32]. Here fatigue symptoms are a concomitant sign of the disease that patients and caretakers are often unaware of, which often leads to underdiagnosis and/or misdiagnosis [17]. In contrast to CFS, fatigue symptoms during an illness can be transient, which means they can be alleviated with appropriate management strategies and therapies [18, 33].

In CFS, fatigue is disabling as well as chronic. Individuals with CFS are often unable to work [34]. Furthermore, fatigue associated with CFS is accompanied by a range of other symptoms, including muscle and joint pain, headaches, sleep disturbances, and cognitive impairment (see Table 1). Unlike CFS, individuals with fatigue symptoms are usually partially able to work, but with certain restrictions including, lowered on-task motivation, prolongation of response time, decreased alertness, poor focus, deteriorated overall psychometric coherence, difficulty with recall and with information processing, as well as altered reasoning ability [35]. Despite these differences, fatigue symptoms and CFS are similar in that they are not relieved by rest [36].

Table 1: Diagnosis: CFS compared to illness related fatigue symptoms

Chronic fatigue syndrome	**Fatigue Symptoms during an illness**
Required symptoms 1. Significant decrease or limitation in the performance of normal activities (vocational, intellectual, recreational or personal) accompanied by new onset fatigue of at least 6 months' duration, not explained by prolonged or unusual strain and not relieved significantly by rest. 2. Post-exertional discomfort 3. Lack of refreshing sleep	Diagnosis using: 1. Validated questionnaires for the screening and diagnosis of fatigue symptoms designed for the respective patient group Additionally/optional 2. Blood profile* and urin tests

Any one or morelof the following:
4. Cognitive impairments
5. Orthostatic-related symptoms

Summary on the criteria for the diagnosis of chronic fatigue syndrome of the Institute of Medicine [30, 37], compared to the diagnosis of fatigue symptoms [38]. * Blood profile tests include; complete blood count, liver, kidney and inflammation markers, electrolytes, thyroid hormones, iron and vitamin D.

1.1.3 Fatigue – the concept

As early as 1985, Gibson and Edwards [39], differentiated fatigue into central and peripheral models. This distinction into two separate entities is applicable to both CFS and fatigue symptoms and is still considered useful today [17, 18, 21].

Central fatigue is the result of altered neurotransmission within the brain [21]. It is defined as the inability to start and/or maintain mental tasks that require concentration or physical tasks that demand for self-motivation [21]. The lack of detectable failure of cognition or motor impairment further emphasizes the cognitive component of central fatigue [40, 41].

Peripheral fatigue is classically manifested by neuromuscular dysfunction outside the central nervous system and is typically presented clinically as muscle weakness [21]. Peripheral fatigue is associated with altered metabolism of the muscle [21], changed neuromuscular transmission or a disturbance in peripheral circulation [40] as detailed in section 1.2.2.2. Objective measures that have been established for peripheral fatigue include, muscle testing and aerobic capacity measures [42]. This further emphasizes its physical association.

1.1.4 Screening of fatigue symptoms in the clinical and research setting

Fatigue sreening should be done using brief and validated tools with established cut-off values for severity [43]. Despite their validity, they do not adequately address all aspects, dimensionality and complexity of fatigue [38, 44]. Therefore, in clinical settings or research, when choosing a particular fatigue scale for measurement, it is recommended to consider [45]:

- the aspect of fatigue to be evaluated and reason for evaluation
- the dimensionality of the scale to be used i.e., unidimensional whereby only one dimension of fatigue is evaluated or multidimensional in which more than one dimension of fatigue is evaluated
- its suitability for the patient population (ideally should be validated)

The most commonly used fatigue questionnaires are detailed in Table 2. To date, only a single liver cirrhosis specific questionnaire has been designed [46].

Table 2: Summary of mostly used fatigue scales: Properties and characteristics

Instrument	**Reference**	**Target Population**	**Number of items Likert scale type Time required**	**Dimensionality**	**Internal consistency Test–retest reliability Concurrent validity**
Fatigue severity scale (FSS)	Krupp LB, et al. 1989 [47]	- Chronic disease in general - Multiple sclerosis - Systemic lupus erythematosus - Validated also in liver cirrhosis [48]	9 Items 7-point 5 minutes	Unidimensional – fatigue affecting daily living activities	- 0.88 - 0.84 - Visual analogue scale assessment of fatigue
Functional assessment of chronic illness therapy fatigue scale (FACIT-F)	Webster K, et al. 2003 [49]	- Cancer	13 Items 5-point 10–15 minutes	Unidimensional – fatigue affecting emotional/physical functioning and social well being	- 0.93 - 0.90 - POMS-vigour, and -fatigue, persistent symptoms of fatigue
36-Item short form health survey (Sf-36)	Ware JE, et al. 1993 [50] (for validity [51])	- General population - Wide range of chronic diseases	4 Items 5-point 5-10 minutes	Multidimensional (vitality exclusively fatigue in addition to aspects regarding general health, physical role/functioning, mental/emotional health, social functioning)	- 0.96 - 0.87 - Nottingham questionnaire
Chronic liver disease questionnaire fatigue scale (CLDQ)	Younossi ZM, et al. 1999 [46]	- Chronic liver disease - Versions CLDQ-HCV [52] and CLDQ-NASH [53]	5 Items 7-point 10 minutes	Multidimensional (fatigue together with activity, worry, symptoms both abdominal and systemic)	- 0.84 to 0.94 - 0.84 to 0.93 - Sf-36

Instrument	Reference	Target Population	Number of items Likert scale type Time required	Dimensionality	Internal consistency Test–retest reliability Concurrent validity
Fatigue impact scale (FIS)	Fisk JD, et al. 1994 [54]	- Multiple sclerosis	40 Items 5-point 5-10 minutes	Multidimensional – fatigue impact functioning as well as psychosocial, cognitive and physical components	- 0.87 - 0.72–0.83 - Sickness impact profile
Multidimensional fatigue inventory (MFI)	Smets EM, et al. 1995 [55]	- General population - Chronic disease - Cancer - Chronic fatigue syndrom	20 Items 5-point 5-10 minutes	Multidimensional - fatigue aspects in terms of general, physical, mental/central together with reduced motivation and activity	- 0.84 (0.53 to 0.93) - 0.76 (total), 0.60 to 0.72 (subscales) - VAS-Fatigue
Fatigue scale (FA)	Chalder T, et al. 1993 [56]	- General population	11 Items 4-point 5 minutes	Multidimensional – physical and mental aspects of fatigue	- 0.88 – 0.90 - NM - Fatigue question on the revised Clinical Interview Schedule (CIS-R)
Primary billiary cirrhosis (PBC-40)	Jacoby A, et al.2005 [57]	- Primary biliary cirrhosis	11 Items (fatigue domain) 5-point 5 minutes	Multidimensional – fatigue domain among others	- 0.94 - 0.96 - Sf-36 (Energy/vitality)

HCV: hepatitis C virus, NASH: non-alcoholic steatohepatitis, NM: not mentioned, POMS: profile of mood states scale, VAS: visual analogue scale.

Table 3: Prevalence of fatigue symptoms in patients with liver cirrhosis

Study details	**Patient Population**	**Method**	**Results**	
			Fatigue Prevalence	*Additional results*
Phaw NA, et al. 2021 [58] Cross-sectional cohort study, UK	PBC, n = 2353 (completed PBC-40 n = 2002) Female: 91 %, age: 64 ± 11 years	PBC-40	60 %	Correlation: Fatigue severity and cognitive symptoms (r^2 = 0.22 p < 0.0001) Association between autonomic dysfunction symptoms with fatigue plus cognitive symptoms No difference in daytime somnolence between patients with fatigue but without cognitive symptoms compared to those with mild or no fatigue
Younossi ZM, et al. 2020 [59] 5 continents, 27 countries (Americas, Australia, Asia, Europe)	NASH, n =1669, 52 % cirrhosis Female: 60 %, age: 58 ± 9 years	CLDQ-NASH	33 %	Predictors of fatigue; pruritus (increased risk of fatigue by 3-fold), demographics (female, younger in age, non-Asian), depression history, diabetes and lower serum albumin as well as platelet count Factors associated with less probability of fatigue: male, younger age, Asian ethnicity, higher levels of serum albumin and platelets as well as lower non-invasive tests of fibrosis
Salama ZA, et al. 2016 [60], Prospective cross-sectional study, Egypt	HCV n = 500, cirrhosis n = 400 Female: 46 %, ages: 51 ± 9 years	FIS and FSS	All: 52 % Child-Pugh A/B/C: 60/45/50 %	Significant associations with fatigue: sex (female) and anemia No association with: liver profiles, varices, ascites, HCV load
Mells GF, et al. 2013 [61] Cross-sectional cohort study, UK	PBC, n = 2,353 Female 91 %, age: 65 [21 - 91] years	PBC-40	45 %	Association between fatigue, anxiety and depression Correlation fatigue severity and depression symptoms (HADS-D, r = 0.49, p < 0.0001)
Huet PM, et al. 2000 [62], Cross-sectional study, Canada	PBC, n = 116 Female 89 %, age: 53 [30 - 79] years	FIS	85 %	No association between fatigue with severity of disease
Cauch-Dudek K, et al. 1998 [63], Cross-sectional study, Canada	PBC, n = 88, Female 86 %, age: 57 ± 11 years	FA	68 %	Somnipathy and depression more prevalent in fatigued patients No association between fatigue and age, disease duration, serum bilirubin, prognosis (mayo risk score) and usage of ursodeoxycholic acids

CLDQ-NASH: chronic liver disease questionnaire – non-alcoholic steatohepatitis, FA: fatigue scale, FIS: fatigue inventory scale, FSS: fatigue severity scale, HADS-D: hospital anxiety and depression scale, HCV: hepatitis C virus, NASH: non-alcoholic steatohepatitis, PBC: primary biliary cirrhosis, PBC-40: quality of life for primary biliary cirrhosis.

1.2 Fatigue symptoms in liver cirrhosis

1.2.1 Prevalence of fatigue symptoms in liver cirrhosis

The prevalence of fatigue symptoms in liver cirrhosis differs across studies [20], and ranges from 33-85 % [16, 58-61, 63-67] (Table 3). Fatigue symptoms are most often described in patients with cholestatic liver diseases [23]. In an earlier study in 1998, a Canadian group found that 68 % of 88 patients with primary biliary cirrhosis (PBC) experienced fatigue symptoms [63]. Similar results were obtained in a more recent study with data from the UK PBC cohort, in which 60 % of the patients presented with significant fatigue symptoms [58].

1.2.2 Mechanisms and pathophysiology of fatigue symptoms in liver cirrhosis

The mechanisms involved in the pathogenesis of fatigue symptoms in liver cirrhosis are related in a complex way to features of the central and peripheral nervous systems [68, 69]. Whereby, the activation of the peripheral nervous system can lead to changes in neurotransmission in the brain and consequently fatigue, altered mood (anxiety/ depression), somnipathy or even cognitive defects [21, 22]. Figure 1 shows, a summarized description of the mechanisms and pathophysiology of fatigue symptoms in patients with liver cirrhosis [15, 18, 21, 22, 70, 71].

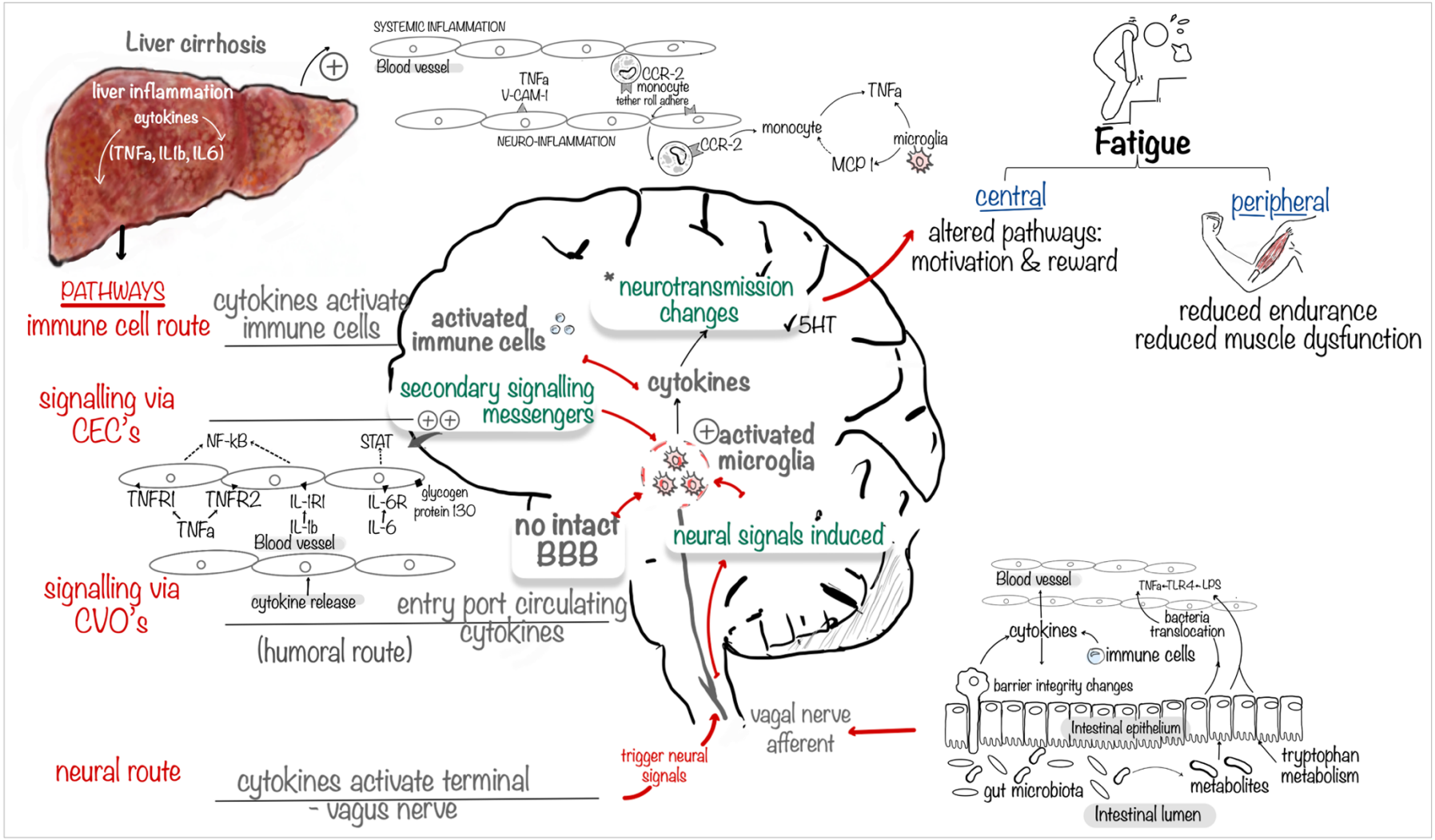


Figure 1: Summary of the pathophysiology and complexity of fatigue in liver cirrhosis

BBB: blood brain barrier, CCR-2: chemokine receptor type 2, CECs: circumventricular organs, CVOs: cerebral endothelial cells, IL1b: Interleukin 1 beta, IL6: interleukin 6, LPS: lipopolysaccharide, MCP-1: monocyte chemoattractant protein 1, NF-kB: nuclear factor kappa B cells, STAT: signal transducers and activators of transcription, TLR4: toll-like receptor 4, TNFa: tumour necrosis factor alpha, V-CAM-1: vascular cell adhesion molecule-1, 5HT: 5-hydroxytryptamine.

1.2.2.1 Central fatigue

The central cause of fatigue is attributed to changes in central neurotransmitter systems involved in mediating fatigue consequent to systemic inflammation [15]. Liver cirrhosis is characterized by the presence of systemic inflammation i.e., increased steady-state immune cell activation as well as circulating inflammatory mediators [72]. In liver inflammation, activation of macrophages - Kupffer cells – that are largely resident in the liver – releases peripheral cytokines such as interleukins 1 (IL-1) and 6 (IL-6) as well as tumour necrosis factor alpha (TNFα) [21, 22].

The production of these cytokines through 4 main **peripheral pathways** can then lead to changes in neurotransmission in the central nervous system and consequently fatigue [15, 17, 18, 21, 73].

The peripheral pathways include:

- neural routes
- immune cell routes
- signalling via the circumventricular organs (CVOs)
- signalling via cerebral endothelial cells (CECs)

Neural routes: The visceral organs, e.g., the liver, are connected to vagus nerve afferents that trigger cytokine receptors and macrophages sensitive to cytokines, which may be the early stimulus for the detection of inflammation. The cytokines produced by the liver can activate the terminals of the vagus nerve, triggering neural signals that are transmitted to the brain and released there. Once in the brain, these signals lead to changes in neurotransmission either, directly or indirectly via the microglia.

Immune cell routes: Liver inflammation can also activate immune cells that are transported in the bloodstream to the brain. These then enter the brain parenchyma by attaching to cerebral endothelial cells. Once inside, pro-inflammatory messengers e.g., TNFα, are also released either directly or indirectly via microglia by the activated immune cells.

Signalling via the circumventricular organs (CVOs): CVOs are specialized structures located in the brain that have a unique characteristic compared to other areas of the central nervous system. These organs are regions of the brain that lack a typical blood–brain barrier. The capillaries in these areas are fenestrated meaning that, their blood vessels have small openings or pores. These openings can serve as an entry port for circulating cytokines to enter the brain and trigger downstream signalling events that alter brain function.

Signalling via cerebral endothelial cells (CECs). The cytokines in the circulation can also stimulate CECs. Both TNFα and IL-1β receptors are located on the surface of CECs glial and neuronal cells in the brain. The binding of TNFα and IL-1β to these receptors sensitizes *nuclear factor kappa B* cells (NF-kB) in the CECs. This then triggers the production of secondary messengers such as nitric oxide, which subsequently enhances brain modifications. Thus, further activating the microglia.

When engaged, the activated microglia are able to generate neuromodulators (TNFα) which can, modulate neurological activity and in turn behavior. These primed microglia, trigger the expression of monocyte chemoattractants (e.g. CC-chemoligand 2 - CCL2) which facilitate the migration of monocytes into the brain [22, 70]. In the mouse model, liver inflammation increased cerebral monocyte chemoattractant protein 1 (MCP-1) concentration and increased the number of CC-receptor 2 expressing circulating monocytes [74].

Alterations in the gut microbiom including dybiosis and gut inflammation have also the potential to influence and affect sentiment/mood, cognition and/or behavior i.e. fatigue [21, 75]. Alterations in the gut microbiota are well established as modulators of brain function, whereby **neural and humoral pathways** of communication are involved in the microbiota-gut-brain axis [21], as shown in Figure 1. In liver cirrhosis, alterations in the gut microbiome occur due to the impaired immune system, making the body susceptible to the influences of both, dysbiosis and complications caused by bacteria as well as systemic dysfunction of the body's immune system with upregulation of systemic inflammation [76]. Changes in the gut microbiome, for example with probiotics and/or prebiotics, have been used to alter brain function and symptoms in liver disease [21]. VSL#3, a probiotic mixture, decreased proinflammatory cytokine plasma levels and sickness behaviour in mice with liver inflammation due cholestasis induced by bile duct ligation [71]. The authors report changes in systemic immunity/peripheral immune cell-brain signalling, reduced brain microglial activation and increased social engagement ability, motivation and interest, however, without measurable changes in the gut microbiome after VSL#3 administration [71]. The same research team using the same mouse model [73], found toll like receptor 4 (TLR4) activation on aggregates of monocytes and platelets regulates microglial activation and the development of sickness behavior. The TLR4 activation may be related to the increased systemic availability of endotoxin, a TLR4 ligand, in liver disease. Endotoxins trigger TLR4, which stimulates signalling molecules for example NF-κB, which in turn induce the production of inflammatory cytokines [77]. Taken together, for patients with liver disease, these findings suggest, the possibility of a treatment approach for fatigue that targets the gut microbiome [21].

During systemic inflammation, neurotransmitter systems are also implicated in mediating behavioral changes [73]. The most involved neurotransmitters related to changes in the brain leading fatigue are [78]:

- serotonin specifically 5-hydroxytryptamine (5-HT) and
- noradrenaline (norepinephrine) [17].

Newsholme et al., [79] suggest that an increase in serotonin concentrations influence the perception of effort and thereby fatigue. Serotonergic cell bodies are primarily situated in the midbrain dorsal raphe nuclei and are projected to multiple brain regions, comprising the hypothalamus, amygdala and hippocampus. Implicated in fatigue, in patients with liver disease are the two receptors 5-HT_{1A} and 5-HT_3 [18, 22, 80]. In experimental settings of cholestasis, 5-HT_3 receptors have been previously implicated in the genesis of central fatigue [81]. In this study, administration of tropisetron (5-HT_3 receptor antagonist) to cholestatic rodents led to an improvement in fatigue-like behaviour, evidenced by an increase in locomotor/physical activity [81]. While in an earlier study, evaluating the effects of enhancement of central serotonergic neurotransmission on fatigue associated with cholestatic liver disease, by Swain and colleagues [82], bile duct resection (BDR) (cholestatic) and sham (controls – non cholestatic) rats were treated with a 5HT1A receptor agonist (LY293284). This resulted in a significantly increased overall activity levels in LY293284-treated BDR rats, but not in sham rats. Evident in unaffected struggling duration from basal levels in the two models, as well as significant reduction in float times relative to basal values, thereby resulting in comparable values between the models. Thus, further implicating the involvement of modified serotoninergic neurotransmission in cholestatic fatigue pathogenesis [82]. In clinical settings, results from a randomized, placebo controlled trial in chronic hepatitis C patients evaluating the effect of the 5-HT receptor type 3 antagonist ondansetron confirmed these findings [83]. Ondansetron significantly reduced fatigue symptoms by 32 % and 38 % starting on day 15 and continuing until the end of the intervention period on day 60. In contrast, the reduction in fatigue in the placebo group always remained far lower, between 6 % and 20 % [83]. These results underline the importance of the serotonin signalling pathway in the pathogenesis of fatigue.

The role of noradrenaline with fatigue in liver disease, especially inflammatory is still poorly understood both experimentally and clinically [15]. It is a classical neurotransmitter involved in behavioural activation, especially acute stress [18]. Nonetheless, noradrenalin could contribute to the central fatigue genesis with pharmalogical results based on beta-noradrenergic antagonist reversing the response suppressing effect of corticotrophin

releasing factor, pointing towards a link between noradrenaline, which is involved in mediating behavioral responses toward stress, and corticotropin-releasing hormone [84].

Additional evidence for a central mechanism of fatigue due to liver cirrhosis is known from studies with primary biliary cirrhosis (PBC) patients in which transcranial magnetic stimulation was used as an assessment tool [15]. The rationale for this approach stems from the fact that central fatigue can also be perceived as decreased neural drive of muscles, consequently leading to a decreased ability to sustain physical activities [20, 22]. Unlike the normal response i.e., an increase in the motor evoked response elicited by transcranial magnetic stimulation during fatigue exercise in healthy control subjects, no decrease in motor evoked response is exhibited in fatigued PBC patients [85]. Neuronal changes in the basal ganglia provide further evidence for a central mechanism of fatigue [21, 22]. Such changes can be observed with modern imaging techniques such as, proton magnetic resonance spectroscopy or cerebral magnetization contrast imaging [86]. Implicitly, the relationship between resting-state functional connectivity (rsFC) and fatigue severity in PBC patients was investigated in an exploratory analysis. In this study, fatigued PBC patients exhibited stronger rsFC between primodial ganglia and premotor cortices compared to non-fatigued patients [87]. This increased rsFC suggests a physiological response of the brain attempting to compensate for this dysfunction [87].

Autonomic dysfunction, particularly vasomotor function impairment, is an addtional mechanisms possibly related to central fatigue in PBC patients [21, 88]. Consistent with these findings, a large cross-sectional British study of 2353 PBC patients, found significant correlations between vasomotor autonomic symptoms with fatigue (r = 0.51), cognitive symptoms (r = 0.48) and sleep disturbance (r = 0.36) [89]. However, the etiology of autonomic dysfunction in PBC patients is rather unclear and may pertain both central and peripheral aspects.

1.2.2.2 Peripheral fatigue

Fatigue in liver cirrhosis is believed to be mainly central in origin but, it can also be peripheral or a coexistence of both [15]. Peripheral fatigue in patients with liver disease appears unlikely an important factor unless decompensated cirrhosis or even liver failure exists [90]. There is limited data on the mechansims behind peripheral fatigue in patients with liver cirrhosis. Despite this, there are some studies indicating that a peripheral component of fatigue does exist [91-93]. A recent study by Corrêa and colleagues [93], showed that patients with liver cirrhosis compared to healthy controls have a significantly lower

peripheral muscle endurance which correlated positively with fatigue symptoms ($p = 0.030$, $r = 0.378$). Muscle dysfunction is another possible peripheral component to fatigue [21]. In fatigued PBC patients, recovery of acidosis is negatively associated with fatigue severity [91, 92]. Intracellular acidosis, primarily attributable to the accumulation of lactic acid due to anaerobic glycolysis, is regarded as the principal cause of skeletal muscle fatigue [94]. Acidosis hampers the function of mitochondria, thus reducing energy production and oxidative phosphorylation [95]. It negatively affects muscle contraction by slowing down the attachment of myosin and detachment speed of actin [96].

In summary, the pathogenesis of fatigue in patients with liver cirrhosis, remains unclear and poorly understood. Central fatigue seems to be the most important clue to the pathophysiology of fatigue in liver cirrhosis. To this end, the following mechanisms have been postulated, key role of inflammation, i.e., cytokine production via peripheral pathways, changes in the immune system with possible effects of cytokines, alterrations in gut microbiota as well as changes in neurotransmitter and autonomic dysfunction. However, some of these theories have not been robustly investigated and there are some findings that suggest a peripheral mechanism is also involved.

1.2.3 Consequences of fatigue in liver cirrhosis

Fatigue in patients with liver cirrhosis is associated with worse clinical outcomes, morbidity and mortality and is an important determinant of impaired health related quality of life (HRQoL) [62, 97-99]. In concordance with this, results from a German crosssectional study investigating HRQoL and fatigue in chronic hepatitis C patients found impaired HRQoL and higher fatigue scores to be associated with degree of fibrosis [100]. Blackburn et al., [97] in their study found PBC patients with high fatigue had more depression and anxiety ($p < 0.001$) as well as worry ($p < 0.05$) compared to patients with low fatigue, and consequently reporting fatigue impairing their quality of life.

In terms of prognosis, a 4 year follow-up in PBC patients conducted in the United Kingdom found that fatigue was independently associated with a 91 % increased risk of death [98]. More than 2/3 of the patients who died during the follow-up had high fatigue scores [98]. Confirming these results, a 5-year follow-up in the same patient sample found high fatigue at baseline to be a risk indicator of liver related death and transplantation urgency [99].

2 Aims and Objectives

Patients with liver cirrhosis (LC) are often affected by fatigue symptoms. They are also malnourished and sarcopenic, both of which are associated with poorer clinical outcomes and increased mortality. Fatigue symptoms are also associated with adverse clinical outcomes and poorer prognosis. The understanding of fatigue and its mechanisms in LC patients is complicated, not only because of its complex subjective nature, but also by its interactions with psychosocial symptoms including symptoms of anxiety and depression. There is also a lack of understanding of the associated factors, while there is limited current evidence on the management of symptoms. It is therefore important, to integrate fatigue with psychosocial fators in the overall view of liver cirrhosis, in order to optimise therapy and recovery.

As a secondary analysis within the research project "*Enteral nutrition in malnutrition caused by diseases of the gastrointestinal tract: from basic understanding to innovative treatment concept*" (EnErGie), the approach of this work was exploratory and hypothesis-generating.

2.1 Primary Aim

The primary aim of this study was to evaluate the prevalence and severity of fatigue symptoms in LC patients of mixed etiology attending a tertiary care center compared to healthy controls (CON).

2.1.1 Primary Endpoint

The primary endpoint was fatigue severity scale (FSS), categorial and metric.

2.2 Secondary aims and endpoints

The secondary aims and associated endpoints included the evaluation of:

- Prevalence of anxiety and depression symptoms in LC patients compared to CON.
  - Endpoint: Hospital anxiety and depression scale (HADS), categorial and metric.
- Correlation of fatigue symptoms with anxiety and/or depression symptoms in LC patients.
  - Endpoint: Binary linear correlation and correlation coefficient.

- Comparison of fatigued LC patients with and without anxiety or depression symptoms.
  - Endpoint: Categorial, metric and ratio measures.
- Associations of fatigue severity with malnutrition and sarcopenia.
  - Endpoint: Global leadership initiative on malnutrition (GLIM), European working group on sarcopenia in older people (EWGSOP2) - categorial, metric and ratio measures.
- Independent indicators of fatigue in LC patients.
  - Endpoint: Correlation coefficient and multiple linear regression analysis (backward stepwise regression).
- Impact of an intensified nutrition intervention on the fatigue, anxiety and depression trajectories in fatigued, malnourished LC patients.
  - Endpoint: Pre/post comparison of fatigue progression at 3 months (end of an intensified nutritional therapy) and follow-up at 6 months.
- Impact of an intensified nutrition intervention on the predictors of fatigue in fatigued, malnourished LC patients.
  - Endpoint: Pre/post comparison at 3 months (end of an intensified nutritional therapy) and follow-up at 6 months.

2.3 Perspective

In perspective, the present work, based on the knowledge and experience gained from the project and other related research, aims to achieve a better understanding of the relevance and interrelations of fatigue symptoms in LC patients and the possible beneficial effects of nutrition interventions.

3 Materials and Methods

This evaluation was carried out as a secondary analysis within the multicentre cross-sectional and intervention study of the research project *"**Ente**ral nutrition in malnutrition due to diseases of the **g**astro**int**estinal tract: from basic understanding to innovative treatment concept*"(EnErGie). As part of the cross-sectional study, this dissertation evaluated and investigated the prevalence of fatigue symptoms in liver cirrhosis patients of mixed etiology in comparison to healthy subjects and described the associations to psychosocial factors (anxiety/ depression symptoms), malnutrition and sarcopenia as well as independent indicators of fatigue in liver cirrhosis patients. Within the framework of the intervention study, the course of fatigue symptoms, psychosocial factors and fatigue indicators were observed along an intensified intersectoral nutritional therapy over 6 months in a small group of malnourished cirrhotic patients.

3.1 EnErGie Project

The EnErGie project was a joint project funded by the European Social Fund of the Ministry of Education, Science and Culture Mecklenburg-Western Pomerania (Germany) from 10/2018 to 06/2022 (ESF/14-BM-A55-0007-11/18). The main project goal was to broaden the knowledge and the mechanistic understanding of disease-related malnutrition in gastrointestinal diseases, specifically in liver cirrhosis (LC), short bowel syndrome (SBS), and chronic pancreatitis (CP). Patients with LC, SBS and CP represent a major group of patients that are affected by malnutrition and sarcopenia [101-104]. The EnErGie project consortium consisted of 5 groups of researchers from clinical nutrition, nutritional sciences and experimental research within Mecklenburg-Western Pomerania. For the purpose of this dissertation only partners involved in human research are summarized in Table 4.

Table 4: The human research partners of the EnErGie project

Partner	***Institution***	*Main study recruits*
UMR	Department of Gastroenterology, Endocrinology and Metabolic Diseases, University Medical Center Rostock - *Project Coordination*	Liver cirrhosis, Short bowel syndrome
UMG	Policlinic for Internal Medicine A, University Medicine Greifswald	Chronic pancreatitis
NIED	Neubrandenburg Institute of Evidence-Based Dietetics, University of Applied Sciences Neubrandenburg	Healthy subjects

The EnErGie project investigated sarcopenia and malnutrition in animal models and clinical studies. The clinical studies were further divided into cross-sectional and intervention studies.

The cross-sectional study was designed as a multicentre, prospective and controlled study to analyse the prevalence and phenotype of malnutrition and sarcopenia in patients with LC, SBS and CP in comparison with two types of controls groups. Healthy controls (CON) were not malnourished or sarcopenic, while control patients (CON-Pat), had no underlying gastroenterological disease, but an indication for oesophago-gastro-duodenoscopy.

In the present dissertation both human study models will be addressed specifically to investigate fatigue in cirrhosis patients as shown in Figure 2 and detailed in sections 3.2 and 3.3.

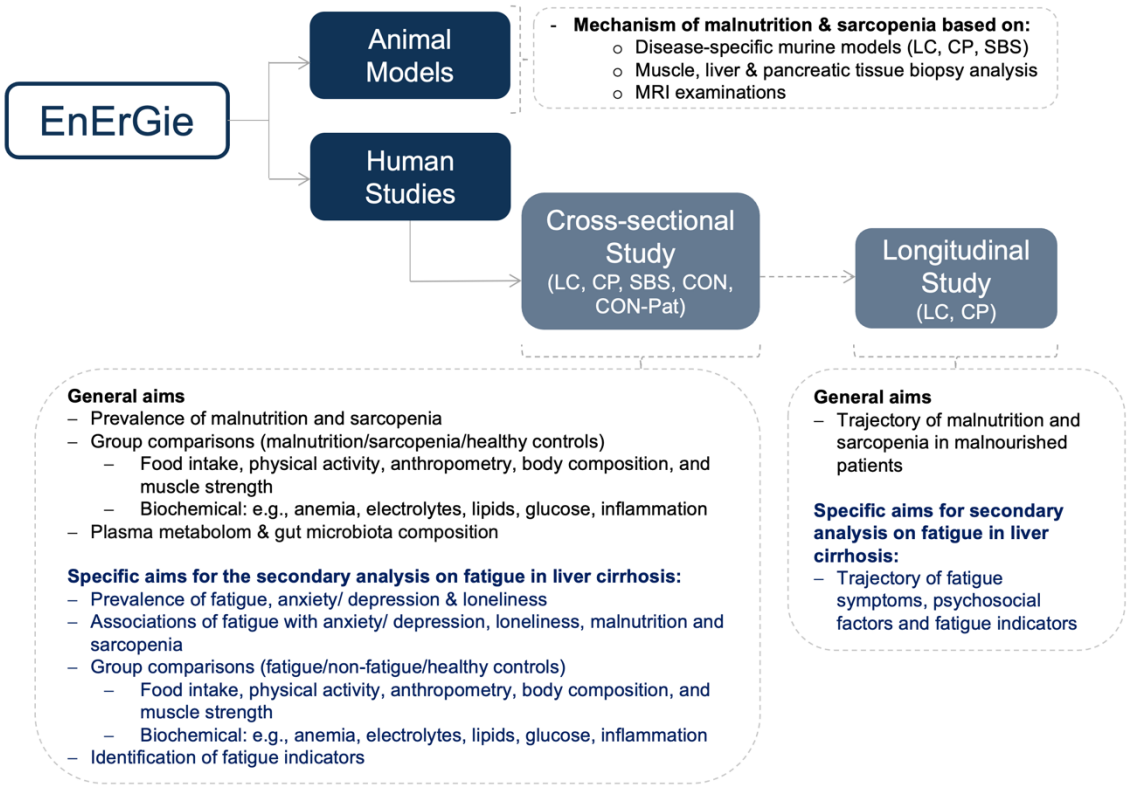


Figure 2: General structure and aims of the joint research project EnErGie

CON: healthy subjects, CP: chronic pancreatitis, CON-Pat: control patients, EnErGie: Enteral nutrition in malnutrition due to diseases of the gastrointestinal tract: from basic understanding to innovative treatment concept, LC: liver cirrhosis, MRI: magnetic resonance imaging, SBS: short bowel syndrome,

3.2 Cross-sectional study

The cross-sectional study of the EnErGie project was conducted at the three study centres. The UMR and UMG, both tertiary referral hospitals located in the north-east of Germany, and at the Neubrandenburg Institute of Evidence-Based Dietetics (NIED), a section of the University of Applied Sciences Neubrandenburg (HSNB). A positive ethics vote was obtained from the Ethics Committee at the UMR on 12.07.2018 (A 2018-0129, amendment approved on 10.08.2020) and UMG on 19.10.2018 (B155/18, amendment 20.01.2021). The follow-up ethic vote from the Ethics Committee at the HSNB was obtained on 13.05.2019 (HSNB/AL/143/18, amendment on 10.10.2019). Additionally, the study was registered in the German Register of Clinical Studies (http://drks.de, DRKS00021124).

3.2.1 Study design

The present work on fatigue and related psychosocial factors only included LC patients and matched healthy controls as shown in Figure 3. LC patients were chosen because of the reported high prevalence of fatigue in liver disease according to literature [16, 20, 58-61, 63-67].

3.2.2 Data collection

Cross-sectional study:	October 2018 to July 2021
Secondary analysis on fatigue:	May 2019 to 10. April 2021

Therefore, 20 LC patients recruited before May 2019 could not be included in the secondary analysis as no evaluations of psychosocial factors (fatigue, anxiety, depression, loneliness) were conducted before this time. An additional 6 LC patients were recruited by UMR after the official end of the cross-sectional study and could also therefore not be taken into account in this evaluation (see Figure 3)

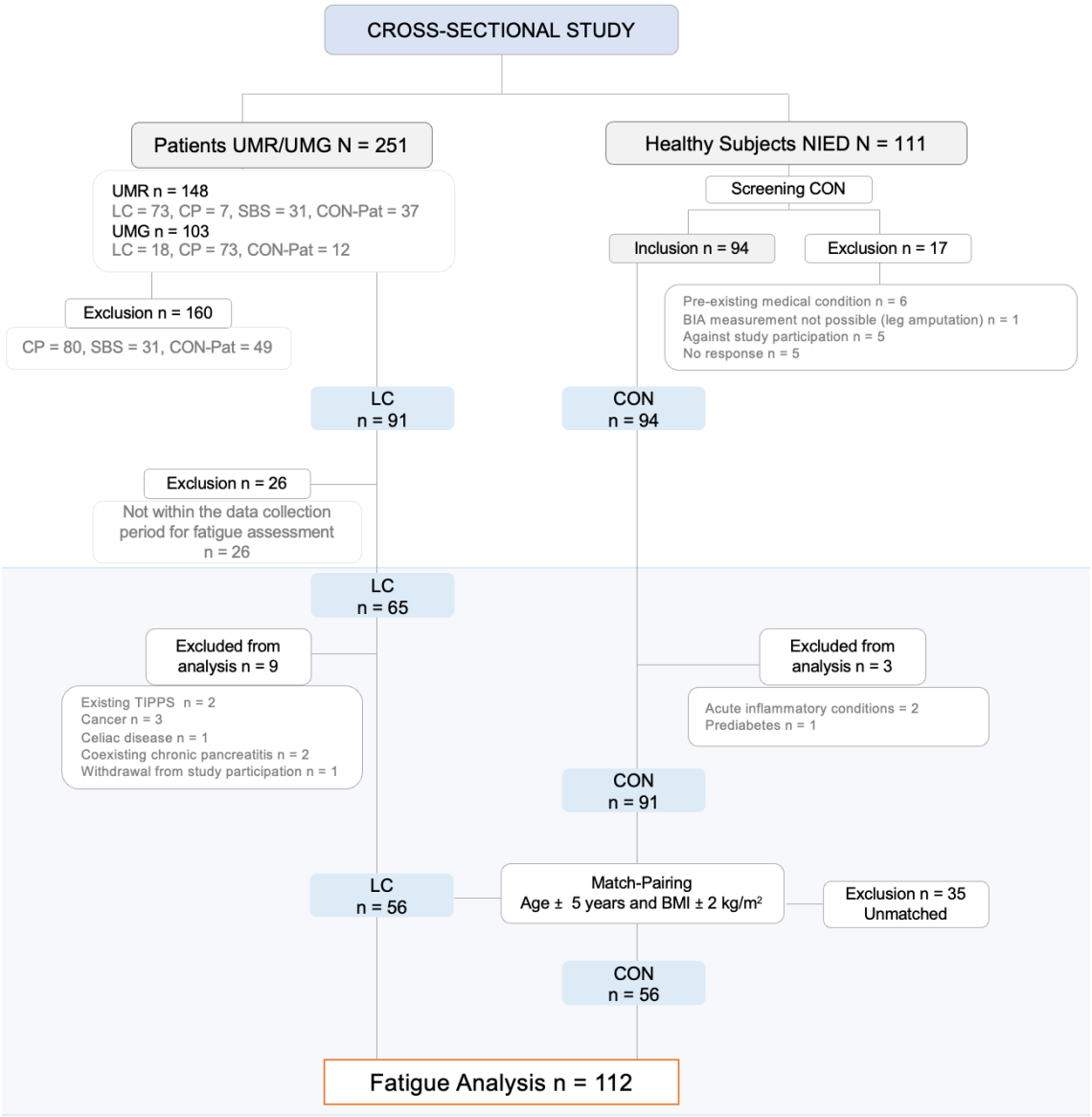


Figure 3: Flow diagram cross-sectional study: Overview study participants

BIA: bioelectrical impedance analysis, CON: healthy subjects, CON-Pat: control patients, CP: chronic pancreatitis, LC: liver cirrhosis, NIED: Neubrandenburg institute of evidence-based dietetics,SBS: short bowel syndrome, TIPS: trans-jugular intrahepatic portosystemic shunt, UMG: University medicine Greifswald, UMR: University medical center Rostock.

3.2.3 Study subjects

3.2.3.1 Recruitment

Liver cirrhosis patients (LC): Adults with medically verified liver cirrhosis based on clinical and imaging criteria (sonography or computer tomography (CT) or nuclear magnetic resonance (NMR) spectroscopy) undergoing either outpatient or inpatient

treatment at UMR and UMG, were recruited. The study participants were screened for eligibility by the clinical study team in cooperation with relevant medical staff.

Healthy controls (CON): Recruitment of healthy controls from the general public within the city radius of Neubrandenburg took place through the existing NIED participant data base (about 600 healthy adults), via the university network's email distribution list, through social networks and a local daily newspaper. Screening for study eligibility was carried out via telephone interviews by the study team. Recruitment began 4 months after the first patient inclusion at UMR/UMG. This delay was strategic in order to accommodate for a match-paired comparison with CON based on the patients' age and BMI (i.e. ± 5 years for age and ± 2 kg/m^2 for BMI).

3.2.3.2 Eligibility criteria

General and disease-specific inclusion and exclusion criteria of the EnErGie cross-sectional study for patients with LC and CON are shown in Table 5. For a negative malnutrition and sarcopenia screening in CON, nutritional risk screening tool (NRS-2002) and SARC-F were used (elaborated in section 3.2.5.13 and 3.2.5.14 respectively).

Table 5: Eligibility criteria: general and specific criteria

Inclusion criteria	LC	CON
General criteria		
Consenting adults, ≥ 18 years	✓	✓
Normal thyroid function*	✓	✓
Specific criteria for LC patients		
Undergoing outpatient or inpatient treatment	✓	-
Liver cirrhosis (Child-Pugh A-C) based on clinical and imaging criteria (sonography or computed tomography or nuclear magnetic resonance spectroscopy) without evidence of hepatocellular carcinoma	✓	-
Specific for CON		
BMI 18.5 - 39.9 kg/m^2	-	✓
Negative malnutrition screening: Nutritional Risk Screening	-	✓
Negative sarcopenia screening (SARC-F)	-	✓
Eastern Cooperative Oncology Group (ECOG) [105] status 0 or 1	-	✓
Subjective absence of physical/mental acute and/or chronic illness/disease	-	✓
No intake of medical drugs except *antihypertensive drugs, lipid lowering agents, antacids and gastric acid reducing drugs (e.g. protonpump inhibitors tyroxine, hormone replacement therapies in post menopausal women, contraceptives*	-	✓

Exclusion Criteria		
General criteria		
Pacemaker or implanted defibrillator	×	×
Pregnancy or breastfeeding	×	×
Non-compliance with protocol expected	×	×
Medications-intake during the past four weeks: *thyroid other than stable thyroid hormone, substitution with euthyroid metabolism, immunomodulators, anti-inflammatory*	×	×
Specific criteria for LC patients		
Parenteral nutrition in the previous 6 months	×	-
Acute alcoholic hepatitis based on clinical/ imaging criteria	×	-
Presence transjugular intrahepatic portosystemic shunt (TIPS)	×	-
Known hepatocellular carcinoma	×	-
Malignant tumors in the last 3 years	×	-
Liver transplantation	×	-
Coexisting chronic pancreatitis	×	-
Intake of the following medications in the last 4 weeks: *Antimetics, non-opioid analgesics (except as needed medication ≤ 1 day/week), steroids*	×	-
Specific criteria for CON		
Diabetes mellitus Typ 1 or 2 (or any other form)	-	×
Malignant neoplasms in the last 5 years or older with being less than 5 years in complete remission	-	×
Other diseases (organ, neurological, cardiovascular, inflammatory…)	-	×
Intake of antibiotics in the last 6 weeks		
Regular intake of antinflammatory or analgesic medication	-	×
Highly atypical or restrictive diets followed voluntarly or due to food intolerances/food allgergies	-	×
Presence of amputations/ paresis including mono-, and diparesis	-	×
Average daily alcohol consumption > 10 g ♀ and > 20 g ♂	-	×
Asian or African descent (both parents)	-	×
Highly above average physical activity (~1-3 h/d, 1000-3000 kcal/d))	-	×
Cognitive impairment (MMSE < 20 points)		
Simultaneous participation in other studies	-	×

* normal thyroid function was clarified by medical history and in case of treated hypothyreodism by recent laboraty results. BMI: body mass index, CON: healthy subjects, EGD: esophago gastro duodenoscopy, LC: liver cirrhosis, MMSE: mini-mental status examination, NRS: nutritional risk screening,

3.2.4 Study implementation

The study implementation included the examination of anthropometrical and body composition measurements (bioelectrical impedance analysis, BIA), malnutrition (Global Leadership Initiative for Malnutrition, GLIM) and sarcopenia diagnostics (European Working Group on Sarcopenia in Older People 2019, EWGSOP 2) comprising of handgrip strength and gait speed (muscle strength and function respectively). Validated questionnaires were also used to assess the measurement of physical activity (International Physical Activity Questionnaire, IPAQ), food intake (German Health

Examination Survey Food Frequency Questionnaire, DEGS FFQ), symptoms of fatigue (Fatigue Severity Scale, FSS), psychosocial factors (Hospital Anxiety and Depression Scale, HADS-D) and loneliness (de Jong Gierveld and van Tilburg scale). Blood samples and stool samples were also collected, but the latter were not considered for in the fatigue analysis. The sequence of study examination steps were different between LC and CON as detailed below.

Liver cirrhosis (LC): General information on socio-demographical characteristics was documented in standardized case report forms, while disease etiology and activity was assessed using disease specific scores (Child-Pugh score-CPS, Model of End Stage Liver Disease score-MELD). The sequence of study examinations was kept flexible in order to take into consideration unavoidable in-hospital circumstances such as pre-occupation of the examination room prior to study examination, time constraint or other patient appointments independent from the project. Based on the data in the electronic patient files, information on medication, concomitant diseases and surgeries as well as weight history were documented.

Healthy controls (CON): Examination of CON took place in person at the NIED using standardized case report forms. Before commencement, all eligibility criteria and examination standards (i.e., fasting period of at least eight hours, abstention from alcohol as well as strenuous physical activity in the past 12 hours and emptying of the urinary bladder) were re-checked, medication intake updated and general information on socio-demographics documented. In case of any violations of the examination standards, for example, non-fasting states, new appointments were scheduled. The participants along with the examiner filled out the questionnaires on physical acitivity (IPAQ) and food intake (DEGS), followed by measurements of handgrip strength, waist and hip circumference, upper arm circumference, weight, height and body composition. While the examiner imputed the BIA data, the study participants filled out the questionnaires on fatigue (FSS), anxiety and depression (HADS) and loneliness (De Jong Gierveld). In conclusion, gait speed was measured followed by the collection of fasting blood samples (details in section 3.2.5.12).

3.2.5 Examination methods

3.2.5.1 General characteristics

Patient data on disease etiology depending upon the clinical conditions/complications and medication were taken from the electronic patient files. In CON, information on

medication intake was obtained from telephone interviews confirmed during the examination. Addiitionally, for CON a healthy status was defined as the subjective absence of physical/mental acute and/or chronic illness/disease. Normal thyroid function was clarified by medical history. For hypothyroidism treated with thyroxine, a recent laboratory result (< 6 months old) was requested and normal thyroid function was verified. Demographic data including age, marital status, employment status, socio-economic status, including use of tobacco products, consumption of alcohol as well as weight loss/progression, food intake and gastrointestinal impairments was collected during the examination through interviews using structured/ standardized questionnaires. All data was documented in a standardised case report form for all the study centres.

3.2.5.2 Disease severity determination (LC only)

Disease severity was determined using the Child Pugh Scoring (CPS) criteria and Model of End Stage Liver Disease score (MELD score) [106, 107]. The CPS also known as Child-Turcotte-Pugh classification is the most popular method and has proven effective in determining the degree of liver dysfunction in clinically relevant situations. [106, 108, 109]. Both CPS and MELD scores are commonly employed in predicting cirrhotic patient outcomes.[109]. A scoring system for predicting operative risk for patients receiving portosystemic shunt surgery for variceal bleeding was first described by Child and Turcotte. Originally it included ascites, hepatic encephalopathy (HE), nutritional status, total bilirubin and albumin. This system was later adapted by Pugh et al., [106, 109], who incorporated prothrombin time or international normalised ratio and dropped nutritional status. The CPS divides patients into low (A), intermediate (B), and high (C) risk classes with a ten-step range between the least sick patient and the most advanced [110, 111]. The problems and variability between investigators associated with the parameters used to classify CPS led to the creation MELD, a score that relies solely on clinical laboratory parameters intended to be more objectively precise than CPS [108].

Initially, the MELD score was created for patients who underwent transjugular intrahepatic portosystemic shunt (TIPS) (TIPS-MELD score) [107]. It has since been adapted for survival prediction in general cirrhosis patients (MELD) [112]. MELD represents the combined effect of creatinine, international normalised ratio (INR) and bilirubin, for the 3-month survival prediction by Cox proportional hazards regression analysis and validation in an independent patient sample [107, 112]. Both CPS and MELD have several shortcomings. For example, the original MELD formula [107], was

adapted by deleting the variable “cause of cirrhosis” [113]. Nonetheless, the formula and the weighting of the regression coefficients were not reassessed. [108, 114]. While with CPS, issues of concern include the requirement of subjective assessment of grading ascites and encephalopathy, non-consideration of renal function and only 10 different point based scores therefore inadequate differentiation based on disease severity. Despite these short comings, the CPS can help predict all-cause mortality risk and development of other complications [115]. CPS and MELD score have comparable predictive value in most cases and distinctive advantages for some specific conditions [109], therefore, we included both scores for the determination of disease severity in LC.

3.2.5.3 Anthropometrical measurements

Anthropometrical measurements are among the most widely usable, cost-effective and non-invasive method for evaluating the human body's composition and dimensions. [116]. Height and body weight were measured while patients wore light clothing and no shoes. Body height was measured using the stadiometer seca 274 (seca GmbH & Co. KG, Hamburg). The measurement was taken in an upright position and with the gaze directed straight ahead on an imaginary horizontal line at the level of the zygomatic arch (Frankfurt horizontal) [117, 118]. Weight was measured using the medical Body Composition Analyzer (mBCA 515,Seca, Hamburg) via the four load cells integrated and calibrated scale with an accuracy of 0.05 kg. The measurements were carried out under standardised conditions, including a fasting period of at least 4 hours and an emptied bladder. Depending on the attire, the measurement result was corrected by - 0.5 to - 1.0 kg. For patients with fluid overload, no standardised reduction of body weight was performed. To calculate the body mass index (BMI), the body weight was divided by the square of the height.

Waist and hip circumferences were measured manually to the nearest 0.1 cm using a non-stretchable tape (seca 201, seca, Hamburg), in accordance with world health organization (WHO) recommendations [119]. Waist circumference was assessed at the midpoint of the lowermost rib and iliac crest at the midaxillary level and at the termination of full exhalation. Hip circumference was determined at the point of the symphysis. (broadest point of the rear), using a measuring tape positioned parallel with the ground. The subjects were asked to stand with feet planted firmly on the ground together, with arms by their sides, with their weight equally balanced and lightly clothed for both measurements.. The waist/hip ratio (WHR) was computed as a proportion of the circumferences. Upper arm circumference (UAC) measurements was taken by

determining the distance between the acromion and the olecranon using the seca measuring tape with the arm flexed at 90°/ palm facing upwards and the midpoint marked. UAC was then determined at an accuracy of 0.1 cm.

Nutritional assessment can be difficult in patients with end stage liver disease, because standard parameters such as anthropometric can be altered by disease severity especially when ascites, edema, and inflammation occur [120, 121]. Therefore, measuring the UAC is ideal since these not affected by the presence of fluid retention [122]. All anthropometric measurements were conducted in accordance with the established standard procedure. This was achieved through investigator training conducted at the NIED, with the objective of ensuring uniformity of measurement throughout the entire study.

3.2.5.4 Body composition: Bioelectrical impedance analysis

Body composition was measured using the bioelectrical impedance analysis (BIA), seca medical Body Composition Analyzer 515 and 525 (mBCA515/525, seca, Hamburg, Germany). As well as being inexpensive, BIA is easy to use, reproducible and suitable for both outpatient and hospitalised patients [116]. This makes it an attractive diagnostic alternative for routine clinical and research use [123]. The prediction equations for BIA are validated for multiple ethnicities with established reference values for adult Caucasians [116, 124]. Its use is limited in cirrhotic patients owing to hypervolemia [125]. The BIA examination was carried out under standardised conditions; adherence to a fasting period of at least four hours, abstention from alcohol as well as strenuous physical activity in the past 12 hours and emptying of the urinary bladder. Deviations from the standardised experimental conditions were documented.

The seca mBCA 515 and 525 uses four pairs of electrodes (a total of eight electrodes). For the mBCA525 these are attached to the hand and foot of each patient. Segmental impedance measurement of the arms and legs is possible with the 8-electrode technique. Impedance is measured at frequencies between 1 and 1,000 kHz using a current of 100 µA. The mBCA 515 octapolar measurement was performed while standing and in a still posture. The feet were positioned with the heels and the toes on the respective foot electrode. For the hands, one of the three possible electrode pairs was chosen so that the arms were not tensed. The finger separators were located between the ring and middle fingers during the measurement. While, the mBCA 525 is designed for supine use with either four adhesive electrodes on each side of a patient's body or eight electrodes on each side of a patient lying on a non-conductive material.

Predictive equations for fat free mass (FFM), total body water (TBW), and extracellular water (ECW) are validated by Bosy-Westphal et al., [126]. While fat mass (FM) was calculated as the difference between body weight and FFM. The prediction equations used resistance (R) and reactance (Xc) values obtained at 5 and 50 kHz for different body segments. The individual cut off values for low muscle mass defined as 5th percentile of a healthy reference population were calculated with the skeletal muscle index (SMI) using a regression formula provided by SECA (www.seca.com/de_de.html). The regression formula included age, gender and BMI.

3.2.5.5 Assessment of fatigue symptoms

One of the most commonly used inventories to measure fatigue in chronic disease is the Fatigue Severity Scale (FSS) [127]. Fatigue was evaluated using the German validated FSS (dFSS) which was validated in patients with relapsing multiple sclerosis and healthy controls [128, 129], but the FSS has also been validated in other patient groups. Due to high validity and reliability (r_s 0.600 $p = 0.01$ and 0.73 $p < 0.001$), and good internal consistence (Cronbach α: patients 0.95, healthy controls 0.86), the FSS can measure fatigue in an economic and rapid fashion for German speaking individuals [128]. Additionally, in cirrhotic patients the FSS has been validated by Rossi et al., [48]. The authors found good psychometric performance of the FSS in this patient group and a reliability of 0,933 (Cronbach α) [48]. Therefore, the FSS can be used in clinical situations in the evaluation of fatigue in German speaking individuals, healthy controls and in patients with cirrhosis of diverse etiologies [48, 128].

The FSS is a nine-item unidimensional questionnaire developed by Krupp and colleagues in 1989 to assess fatigue in patients with multiple sclerosis [47]. Each question comprises a series of items rated on a seven-point Likert scale which ranges from 1 (“strongly disagree”) to 7 (“strongly agree”). The composite score is the average of the 9 item scores, where higher scores indicate severity of fatigue. In their original work, Krupp et al., [47, 130] set the cut-off for severe fatigue as FSS score ≥ 4. Recently an FSS Score ≥ 5 is used for categorizing severe fatigue [47, 129, 130]. Based on data from the literature showing that an FSS score ≥ 4 reliably discriminates subjects with fatigue from controls, we used a cut-off score of ≥ 4 to define fatigue. [131, 132]. A number of studies have also classified the FSS score into three categories: low fatigue (FSS score < 4), medium or borderline fatigue (FSS score ≥ 4 and < 5), and high or severe fatigue (FSS score ≥ 5) [130]. Severe fatigue here means higher limitation in aspects of central fatigue. This is associated with failure to initiate and/or maintain

concentration tasks and physical activities that require self-motivation. As well as, associated with high limitation in aspects of peripheral fatigue, which manifests clinically as a feeling of weakness. This is associated with reduced strength and endurance, prolonged exercise recovery and muscle pain [130]. Based on the differentiation of fatigue in chronic liver disease into central, peripheral and associated features by Swain and Jones [21], we further categorised fatigue items from the FSS as follows; items 1,3,5,7 – central features, items 2,4,6,8 – peripheral features and item 9 – associated features. The evaluation of the FSS scale was carried out centrally for all study centres by the NIED.

3.2.5.6 Assessment of anxiety and depression symptoms (HADS-D)

Anxiety and depression symptoms were assessed by the German validated version of the Hospital Anxiety and Depression Scale (HADS-D) [133, 134]. The HADS was developed by Zigmond and Snaith first published in 1983 [133, 135] and is a self-assessment scale developed to measure commonly occurring mental disorders of depression and anxiety in adults between the ages 16 to 65 years in an outpatient clinical setting (non-psychiatric) [130, 135]. The scale development involved brevity, distinction between depression and anxiety, avoidance of “somatic” symptoms attributed to physical illnesses (e.g., fatigue) and provide clear instructions for score interpretation [130, 133, 136]. There are seven items each in the depression and anxiety subscales. [134]. Whereby, the depression subscale is based to a large extent on questions that relate to the lack of positive affect and not on the presence of negative affect. While, the anxiety subscale includes items relating to worry or cognitive aspects of anxiety, panic and nervousness or tension. It is important to note that the two scales should not be merged into one [130, 133-136].

A 4-point Likert scale is used for each subscale, ranging from 0 (“not at all”) to 3 (“most of the time”). Reverse scoring is used for items with positive phrasing, thus a higher score indicates more severe disorder with scores ranging from 0 to 21 for each subscale. Scores of 7 or less represent non-cases, 8–10 doubtful cases, and 11 or higher indicate definite depression or anxiety [134, 135]. Following further research, it is also recommended that a score of 8–10 be considered mild, 11–15 as moderate, and 16 or more as a severe disorder [130]. The depression subscale relies predominantly on question concerning the lack of positive affect rather than the presence of negative affect. A severe depressive disorder means the presence of; central loss of motivation and enjoyment of life, loss of interest, joylessness and reduction of drive While a severe

disorder in anxiety means the presence of, generalised anxiousness and worry, nervousness, relaxation deficits and the prevalence of panic symptoms [134]. The evaluation of the HADS-D was carried out centrally for all study centres by the NIED.

3.2.5.7 Assessment of loneliness (De Jong Gierveld Scale)

Loneliness was assessed using the 6-item loneliness scale from De Jong Gierveld [137]. This is a shortened version of the De Jong Gierveld 11-item loneliness scale with items formulated with reference to Weiss's 1973 differentiation of loneliness into two states, social and emotional [138-140]. The De Jong Gierveld 6-item version was developed by tripling the original scale, including one general loneliness, three emotional and three social loneliness items [137, 141]. Emotional loneliness stems from not having a close relationship or emotional connection, while social loneliness stems from the absence of a broader social circle or an engaging social outlet [130]. The reliability of the scale is high with good internal consistency between $\alpha = 0.82$ and $\alpha = 0.87$. While congruent validity showed a high correlation between the 6 and 11-item loneliness scale varying between 0.93 and 0.95 [130, 137, 141]. Aspects of loneliness were rated on a 4-point Likert scale with all six statements rating from ‘strongly agree’ to ‘strongly disagree’. The scale formation if based on the mean value over all statements (where necessary recoded) with values ranging from 1 (low loneliness) to 4 (high loneliness) however, by definition an average score of 2.6 points or higher, subjects are rated as 'lonely'. The evaluation of the De Jong Gierveld scale was carried out centrally for all study centres by the NIED.

3.2.5.8 Physical activity evaluation (IPAQ-SF)

The International Physical Activity Questionnaire (IPAQ) was developed for adults aged 18 to 65 years with the purpose of assessing population levels of physical activity across different countries and to facilitate surveillance of physical activity based on a global standard [142]. Physical activity plays a key role in health-related quality of life and better clinical outcomes in patients with chronic disease including those with End-Stage Liver Disease [143]. We used the IPAQ-Short Form (SF) to assess physical activity since it is the most widely used physical activity questionnaire with widely accepted high reliability (correlation coefficients above 0.65 and ranging from 0.88 to 0.32) and reasonable agreement in concurrent validity (short and long form 0.67 95 % CI 0.64 - 0.70) [142, 144, 145]. This questionnaire comprises 7 questions; 6 items related to the number of days and minutes spent in the last week spent in vigorous, moderate and walking

activities with one item on sedentary habits. Physical activity level according to IPAQ-SF were reported as a continuous variable equivalent to minutes per week computed as Metabolic Equivalent of task (METs) or based on the METs in three categories (low, moderate or high activity levels). For the calculations, all activities were initially converted to minutes with activities lasting less than 10 minutes not being considered and activities lasting more than 3 hours truncated. In order to obtain the METs, reported minutes a week were multiplied with the MET value provided for with 3.3 for walking, 4 for moderate activity and 8 for vigorous activity. The mean total METs were summed up and classified in three levels low (< 600 total METs/week), moderate (≥ 600 to < 3000 total METs/week) and high (≥ 3000 total METs/week) based on the IPAQ scoring protocol [146]. The evaluation of the IPAQ-SF scale was carried out centrally for all study centres by the NIED.

3.2.5.9 Food intake assessment (DEGS FFQ)

In the assessment of food intake, we used the food frequency questionnaire (FFQ) of the German Health Examination Survey for Adults (DEGS) [147]. This FFQ is a revised version of the questionnaire used in the German Health Interview and Examination Survey for Children and Adolescents (KiGGS) [148]. The DEGS study included healthy adults aged 18-79 years and was implemented by the Robert Koch Institute since 2008 to survey the overall population health and its determinants in Germany both in a cross-sectional and interventional component *(www.degs-studie.de*) [149]. Based on this survey, the DEGS FFQ was developed and validated against two 24H-recalls with a correlation coefficient ranging between 0.15 and 0.80 with most values at 0.30 or higher. While, the weighted kappa values ranged from 0.09 to 0.54, with 10 of 14 food groups yielding weighted kappa values of 0.29 and above [147]. In its assessment, the DEGS-FFQ retrospectively records the consumption frequencies and usual portion quantities of a total of 53 food groups consumed in the last 4 weeks. Furthermore, the FFQ is composed of 11 frequency categories; i.e., never with a frequency value = 0 (FV = 0), once a month FV =1, 2-3 times a month FV = 2.5, 1 - 2 times a week FV = 6, 3-4 times a week FV = 14, 5 - 6 times a week FV = 22, once a day FV = 28, twice a day FV = 56, thrice day FV = 84, 4 - 5 times per day FV = 126 and more than 5 times per day FV- = 168). Additionally, the study participants indicated the quantities consumed, using illustrations of common portion sizes, specifically illustrated for 33 food items [147].

$$Average\ daily\ intake\ =\ Portion\ size\ *\ Frequency\ value\ */28$$

$*$ *Frequency values are shown above*

According to the calculated average daily intake, macro- and micronutrients were calculated using the German national nutrient table. Similar to the other questionnaires, the evaluation of the DEGS questionnaire were carried out centrally for all study centres by the NIED.

3.2.5.10 Clinical chemistry

At the UMR/UMG, blood samples were collected by the medical staff and sent to the clinic's own laboratories on the same day with duration/variations in fasting states duly noted. At the NIED, fasting blood samples were collected by the staff in the blood donation centre of the German Red Cross in Neubrandenburg. This was possible because of the cooperation between NIED and DRK-NB, as well as the location of the NIED institute located at DRK facilities (blutspendemv.de). Immediately after blood sample collection, each blood sample was centrifuged at the NIED (apart from complete blood count parameters) in order to separate the plasma within 30 min after blood collection in a pre-cooled centrifuge set at 15 min, 4 °C and 3500 rotation per minute (rpm). The plasma was then carefully pipetted and aliquoted in the appropriately labelled cryotubes. For analysis at the laboratory in Neubrandenburg, the aliquots were sent to the Institute for Laboratory Diagnostics, Microbiology and Transfusion Medicine (Head: Prof. Dr. med Egon Werle) at the Dietrich-Bonhoeffer Hospital in Neubrandenburg (dbknb.de/) on the same day.

In addition, all partners stored serum and plasma aliquots of blood samples for further analysis at - 80°C for analysis at the UMR/UMG and the Institute of Nutritional Physiology 'Oskar Kellner', Leibniz Institute for Farm Animal Biology (FBN) in Dummerstorf, Germany (EnErGie Project Partner - Animal model). For data evaluation, complete blood count, liver function tests, coagulation markers, cardiovascular risk factors, inflammation, electrolytes and trace elements were evaluated. All laboratory examinations were conducted according to standard laboratory methods in accordance with the German accreditation standards for medical laboratories (DIN EN ISO 15189) and the European Union regulation directive.

3.2.5.11 Handgrip strength (Jamar dynamometer)

Handgrip strength (HGS) is an indicator of overall muscle strength [150]. HGS in end stage liver disease is the most studied muscle test using a calibrated dynamometer [151]. HGS was measured using the Jamar plus digital hand dynamometer (Patterson Medical, Warrenville, IL USA). This measurement was in accordance with the latest American

Society of Hand Therapists protocol [152]. Participants were asked to sit in a chair without an armrest, with feet fully resting on the floor, hips as far back in the chair as possible, and the hips and knees positioned at approximately 90°. This was done with the dominant arm in adduction and neutral, the elbow at 90°, the forearm in mid-prone (ie., neutral), and the wrist in 15 - 30° dorsiflexion and 0 - 15° ulnar deviation. Three measurements were performed with maximum isometric contraction. Between measurements, there was a rest period of at least 15 seconds. The highest measurement result was documented to the nearest 0.1 kg. The gender-specific thresholds proposed by the EWGSOP2 criteria were used to define reduced hand strength [153, 154]. These thresholds were < 16 kg hand strength for women and < 27 kg for men.

3.2.5.12 Gait speed

Gait speed has been shown to be a predictor of adverse outcomes related to sarcopenia. It is widely used in practice because of its reputation as a safe, quick and highly reliable test for sarcopenia. [153]. Additionally, gait speed has been validated as an indicator of health status and physical decline in a wide range of studies in different populations. [155]. As a measure of muscle function, we selected gait speed on account of its integration in sarcopenia assessment. Gait speed was measured over 4 meters for each participant and calculated using distance and time in meters and seconds respectively. All study centres used the instructions to walk at a normal walking pace and from a standing start position. The time was recorded using a stopwatch and measurement stoped when both feet had crossed the 4-metre line. Nonetheless, the study participants were instructed to cross the finish line at the same speed. A gait speed of < 0,8 m/s was defined as reduced physical function [153].

3.2.5.13 Diagnosis of malnutrition according to GLIM

The Global Leadership Initiative on Malnutrition (GLIM) criteria published in 2019 were used to diagnose malnutrition [156]. The GLIM criteria include both phenotypic criteria (weight loss, low body mass index, reduced muscle mass) and etiologic criteria (reduced food intake or assimilation and inflammation “c-reactive protein (CRP value) ≥ 5 mg/l” [156, 157]. The GLIM criteria was used for the main analysis of malnutrition. For the diagnosis of malnutrition, the GLIM consensus calls for at least one phenotypic criterion (non-volitional weight loss, low body mass index, and reduced muscle mass) and one etiologic criterion (reduced food intake or assimilation, and inflammation or disease burden), after identifying the risk of malnutrition through a screening test [156]. Nutritional

risk screening was performed using the nutritional risk screening tool (NRS-2002). The NRS-2002 is a nutrition screening tool recommended by the European Society for Parenteral and Enteral Nutrition (ESPEN) guidelines in clinical settings to assess malnutrition risk [158]. It has three components; nutrition score (BMI, weight loss, and dietary intake), disease severity and age score (age > 70 years). Mild disease severity was assumed in all included patients. Patients with an NRS-2002 ≥ 3 points were at risk of malnutrition. In healthy controls, the NRS-2002 was used to screen the subjects for inclusion in the study. Specifically, a negative NRS_2002 pre-screening or total score < 3 in the main screening.

3.2.5.14 Diagnosis of sarcopenia according to EWGSOP2

Sarcopenia was diagnosed following the original reviewed criteria of the European Working Group of Sarcopenia in Older People 2019 (EWGSOP2) [153]. In the reviewed criteria, the pre-sarcopenia stage was called probable sarcopenia and was defined with the presence of low muscle strength (detailed in section 3.2.5.11). Diagnostic of sarcopenia was confirmed with low skeletal muscle index with specific-population and instrument specific cut-off points were used for SMI as shown in section 3.2.5.4. Severity grading of sarcopenia after diagnosis was carried out using gait speed (see section 3.2.5.12) [153]. Sarcopenia has been defined as a reduction in both muscle mass and handgrip strength, while severe sarcopenia has been described as a reduction in muscle mass, handgrip strength and slower walking speed [153]. EWGSOP2 recommends that the risk of sarcopenia be identified with a screening assessment (SARC-F or clinical suspicion). SARC-F is advocated as a screening tool for sarcopenia and comprises five assessment components: muscle strength, walking with assistance, getting up from a chair, climbing a flight of stairs and fall-related concerns [153, 159]. Scoring ranges between 0 and 10, with each component scoring between 0 and 2 points. A total score ≥ 4 representing a positive and < 4 points negative screening respectively [159]. A negative sarcopenia screening verification using SARC-F result (< 4) was an inclusion criteria for healthy controls.

3.3 Intervention study

The scope of the intervention study within the EnErGie project was to implement an intensified cross-sectoral nutritional therapy in malnourished LC and CP: This was based on selected patients from the cross sectional study. All patients gave their written consent, including their permission for their medical and personal data to be shared with

the NIED team for nutrition and physical activity training. The EnErGie intervention study was approved by the ethics committees of UMR (DRKS_ID: DRKS00021181, 03.04.2020, registration number: A 2019-0052) and UMG (BB 069/19, 03.06.2019).

3.3.1 Study design

The study design was a single arm proof of principle longitudinal study in malnourished LC and CP patients with an inpatient stay or visit in the university outpatient clinics at the UMR or UMG) and a 3-month supportive ambulant nutrition therapy (SaNT), with follow-up after 6 months. Only LC patients from UMR and only CP patients from UMG were included in the intervention study, as these patients corresponded to the respective foci of the centres. In this thesis, only malnourished LC patients from UMR with a positive fatigue status were taken into account for the analysis. The study design for LC patients is shown in Figure 4 and the evaluation schedule in Table 6.

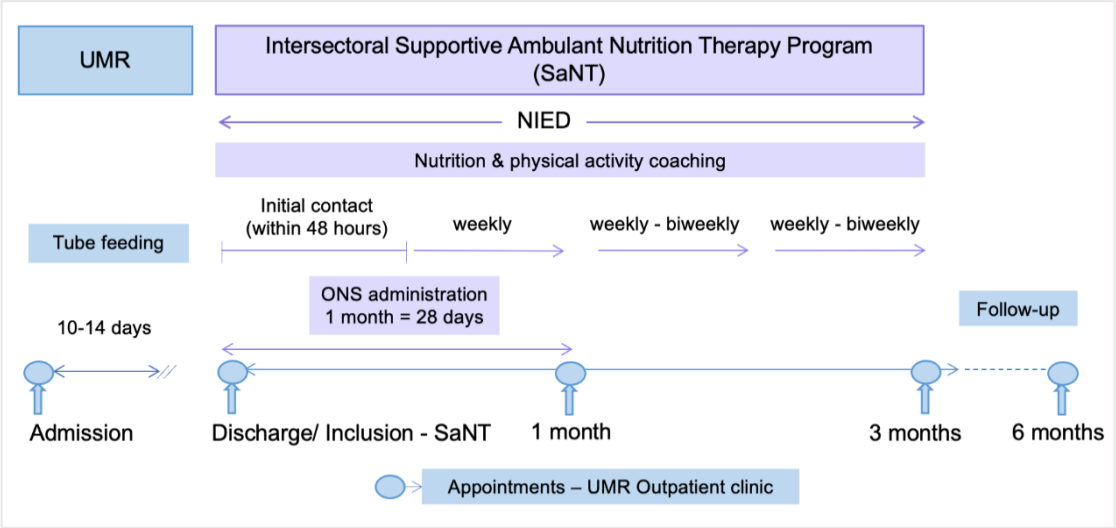


Figure 4: Study design of the intervention study for LC patients

NIED: Neubrandenburg institute of evidence-based, ONS: oral nutrition supplement. UMR: university medical center Rostock

Table 6: Evaluations within the timelines of the intervention study

Analysis/ Timeline	Admission	Discharge/ inclusion SaNT	After 1 month	3 months	6 months
Malnutrition & sarcopenia diagnosis	✓	✓	✓	✓	✓
Anthropometry, body composition and muscle strength	✓	✓	✓	✓	✓
Questionnaires: FSS, HADS-D, De Jong Gierveld, IPAQ, DEGS	✓	-	✓	✓	✓
Blood samples	✓	-	✓	✓	✓

DEGS = German health examination survey for adults, FSS = fatigue severity scale, HADS-D = hospital anxiety and depression scale German version, IPAQ = international physical activity questionnaire,

3.3.2 Data collection

May 2019 to January 2022.

3.3.3 Study subjects

Six malnourished and fatigued patients with LC were eligible for the secondary analysis of fatigue.

3.3.4 Primary aim and primary endpoint

The primary aim of the intervention study in the EnErGie was the attainment of a "normal nutritional status" defined as absence of malnutrition in accordance with the GLIM consensus criteria [156], after 3 months. This was also the 3 months target in the SaNT program since it was predefined by the study design.

In the secondary analysis on fatigue, the primary aim was to observe the trajectory of fatigue symptoms from hospital admission to 6 months after hospital discharge. While, the secondary aims were to observe the trajectory of anxiety, depression, loneliness and identified indicators of fatigue within this time frame.

3.3.5 Intervention (inpatients with liver cirrhosis)

The intervention was conducted in close cooperation between UMR and NIED (see Figure 4). All nutrition counselling and coaching sessions were implemented by trained dietitians and investigators. For a structured and process-driven approach, all sessions were conducted according to the German Nutrition Care Process (G-NCP) [160, 161], using behaviour-modifying communication strategies, such es motivational interviewing [162].

The four additional study examinations at the UMR outpatient clinic during the intervention study took place at hospital discharge, and 1, 3 and 6 months afterwards (see Figure 4). This included the repetition of the examinations in the cross-sectional study; malnutrition diagnostic, sarcopenia diagnostics, collection of fasting blood samples, anthropometrical data, body composition measurements, muscle function and strength. Using validated questionnaires, the assessment of fatigue (FSS) and its associated psychosocial factors (HADS-D) as well as loneliness (de Jong Gierveld),

physical activity (IPAQ), food intake (DEGS) were performed as described in section 3.2.5.

3.3.5.1 Inpatient Nutrition Therapy

Additional to the cross sectional study examinations, all LC patients underwent a standardised nutritional assessment prior to the start of intervention to identify any nutritional problems. Inpatient nutritional therapy for LC patients at UMR was provided by a clinical nutrition team from hospital admission to discharge, and included enteral nutrition via nasoduodenal tube for a maximum of two weeks. One of the six patient rejected the administration of the enteral nutrition but was not excluded from the study and the analysis. The first individual nutrition counselling sessions took place when patients were clinically stabilised, followed by the second session shortly before the end of hospitalisation and transfer to the SaNT program.

3.3.5.2 Outpatient nutritional therapy - The intersectoral SaNT Program

The SaNT program provided an outpatient intervention interface between NIED and the UMR outpatient clinic over 3 months with a follow-up after 6 months. It began with discharge from hospital and comprised three pillars, as shown in Figure 4:
(1) Oral nutrition supplement (ONS) administration over 1 month = 28 days
(2) Nutrition and physical activity coaching - NIED
(3) Appointments at the university outpatient clinic - UMR

1: ONS administration: High-protein- and high-energy ONS (1 - 3 bottles Fresubin Protein Energy (Fresenius Kabi AG, Bad Homburg, Germany), 1.5 kcal/ml, 300 – 900 kcal and 12 - 60 g protein per day) were prescribed for the outpatient setting by the UMR medical staff for the first 28 days. An ONS protocol form developed by NIED was given to the patients by UMR dietitians either on hospital discharge or after the first appointment at the university outpatient clinic. The protocol was to be filled out daily by the patient and served to document the actual ONS intake. At the end of the 28 days, patients either emailed the protocol to the NIED coach or delivered it in person to the UMR study team, who uploaded it to the shared document management system (SharePoint, Microsoft , Redmond, Washington, USA). The daily ONS intake of at least ≥ 400 kcal energy and 20 g protein was set as 100 % achievement of the daily target intake for the evaluation. A patient continued the prescription of ONS due to therapeutical reasons. This was not taken into account in the calculation of ONS compliance.

2: Nutrition and physical activity coaching - NIED: For data protection reasons, the transmission of patients' personal contact details from UMR to NIED, e.g., telephone number, and other personal information required for nutrition and physical activity coaching was done by telephone. Objective biomarkers were retrieved from the shared document management system (SharePoint, Microsoft , Redmond, Washington, USA).

The NIED coaching team contacted patients by telephone within 48 hours after hospital discharge. The initial contact consisted of a personal introduction of the coach and an appointment for the first 4 weekly sessions for the nutrition and physical activity coaching. Following completion of the first 4 and 8 weeks, appointments were made for the respective next 4 weeks, at weekly or fortnightly intervals, depending on the patients' preference. The overall scheme resulted in a minimum of 8 and a maximum of 12 coaching sessions. Each coaching session was scheduled for 10 to 20 minutes.

In preparation for the first coaching session, the NIED coaching team summarized the patient information uploaded by the UMR team on the SharePoint server. This information included medical history, current laboratory parameters, medication use, anthropometry, dietary intake (DEGS-FFQ) and physical activity (IPAQ). The medical information was updated by UMR after every visit to the outpatient clinic.

According to the GNC-P tools, during the first coaching session to identify the nutritional problem and need for action on physical activity, the coach formulated the nutritional diagnosis according to the sub-elements; *Problem, Etiology, Symptoms/Signs, Resouces/Barrieres* (PESR) - statement and *Smart, Measurable, Achievable, Relevant* (SMART) targets. At the end of each coaching session, individually agreed short-term goals were set with the patients to be implemented by the next appointment. New targets were set with the patient after successful implementation, which were further reviewed at each appointment, whilst motivational interviewing techniques were used as needed. The overall goal was to have malnutrition treated after 3 months (negative GLIM diagnosis of malnutrition). The coach monitored the nutrition and physical activity therapy goals and modified them according to the patient's progress.

The coach recorded the use of health services such as doctor visits, re-hospitalisations, changes in medication intake, but also self-disclosure of the patient's weight were used to recognise problems in time. During the first month, the amount of ONS actually taken and amount prescribed were also documentated enabling an immediate intervention in case of intolerances (i.e., ONS compliance check). In the concluding telephone consultation after 3 months, the progress of the program and further goals were

summarised and reviewed with the patient. A standardised outcome-oriented patient report was prepared after the final interview and uploaded on SharePoint.

3: Appointments – UMR outpatient clinic: A total of 4 appointments took place in the UMR out-patient clinics during the SaNT (see Figure 4). The first appointment at admission to the SaNT was not taken into account in the calculation for study adherence. UMR and NIED were in constant communication with theUMR staff being informed of the patient's current status at each visit, which supported targeted and individualised nutritional intervention.

3.4 Data management

For secure data backup and joint data evaluation, the pseudonymized data (e.g. medical history, questionnaires, laboratory samples etc.) were transferred to and stored on the SharePoint server, a web application from Microsoft (SharePoint, Microsoft , Redmond, Washington, USA). Sharepoint offers password-protected access and is hosted in the UMR's data centre. Each recruitment centre (UMR, UMG and NIED) maintained separate pseudonymisation lists of their respective participants. For the intervention study (telephone coaching), patient contact information was only transferred via telephone between UMR and NIED and stored separately in a file. The pseudonymisation lists, patient contact information files and study consent forms were stored in locked cabinets only accessible to the local study investigators (who are bound confidentiality and contractually to the participating institutions). The source documents (filled in paper CRFs and questionnaires, lab results, signed consent forms) were stored on each site in protected cabinets.

3.5 Data clearing

For data clearing, plausibility checks were carried out for all data sets to ensure data quality, and the corresponding study centres were consulted in the event of uncertainties. The data sets were checked/re-checked for input errors, completeness and inconsistencies.

3.6 Statistical analysis

All statistical analyses were performed using SPSS software (version 29.0 IBM, Armonk, New York, USA) and R Project for Statistical Computing (R-Studio Version 1.3.1073 © 2009-2020 R-Studio, PBC). Graphs were created with SPSS, R-Studio and optically

adapted with Inkscape (2020, Version 1.0.2-2 © 2020 Inkscape-Entwicklungsteam). Figures and tables were prepared using Microsoft Office Word (version 16.73, Microsoft, Redmond, Washington, USA).

Data are presented as the mean and standard deviation (± SD) while categorical variables are described as frequency (percentage (%)). From a sample size of n = 30, parametric test procedures (independent T-test) were applied [163]. If the sample size was less than n = 30, the normality of distribution was verified with determination of frequencies and the Shapiro-Wilk test with distribution plots. Normally distributed data were analysed with Student's t test or the Mann-Whitney U test if skewed. Categorical variables were expressed using the Pearson's chi-square test with the Fisher's exact modification if appropriate. Descriptive statistics and frequencies were generated to determine the sample characteristics and prevalence/associations of fatigue. In cases involving more than two dependent samples, Friedman or Friedman two-way analysis of variance and Cochran's Q test where appropriate were used. To assess the linear relationship between fatigue and psychosocial factors as well as loneliness, Pearson correlation coefficient was computed and used. As for the multiple linear regression model, the backward stepwise regression was used to gradually eliminated variable from the regression model to find a reduced model that best explains predictors of fatigue in patients with liver cirrhosis. A significance level of 5 % ($p = < 0.05$, two-sided significance test) was set for the study. Two-step clustering analysis was applied to determine 2 groups of study subjects identified as "low inflammation" and "high inflammation" clusters using all available inflammatory parameters listed in results section 4.1.10.5. Figure 5 shows the cluster quality which was good to proceed with the analysis.

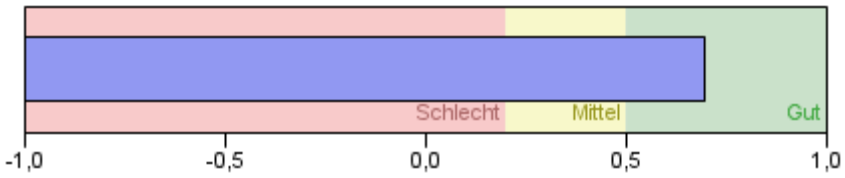


Figure 5: Two-step cluster quality for low and high inflammation LC group clustering: Silhouette measure of cohesion and separation

4 Results

4.1 Cross-sectional Study

4.1.1 Study Workflow

The recruitment workflow for the cross-sectional study is shown in Figure 6. In total, 112 subjects were included in the analysis, thereof, 56 cirrhotic patients (LC) and 56 match paired healthy controls (CON). As detailed in section, 4.1.5, subjects were categorized into fatigued (F) and non-fatigued (Non-F), while LC were further categorized into fatigue without anxiety and/or depression symptoms (F-00) as well as fatigue with anxiety and/or depression symptoms (F-AD).

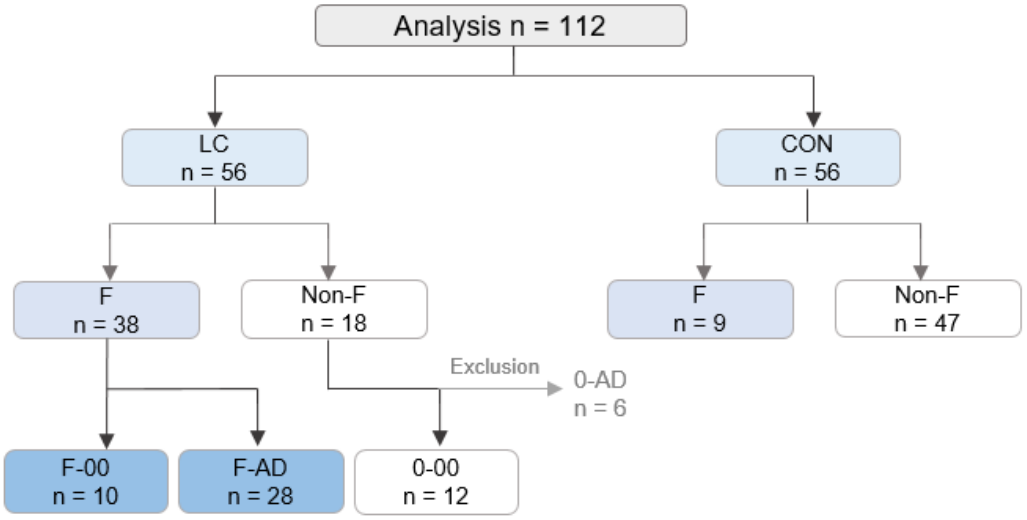


Figure 6: Flow diagram for the recruitment in the cross-sectional study

LC:liver cirrhosis, CON:healthy controls, F: fatigued, Non-F: non-fatigued, F-00: only fatigue symptoms, F-AD: fatigue with anxiety and/or depression symptoms, 0-AD: only anxiety and/or depression symptoms, 0-00: no symptoms of fatigue, anxiety or depression

4.1.2 General subject characteristics

No difference existed between LC and CON in sex, age and body mass index (BMI). The majority of subjects were male, middle-aged on average and overweight (Table 7).

Table 7: General subject characteristics

Parameters % (n), mean ± SD		Total n = 112	CON n = 56	LC n = 56	p Value
Sex					
	male	63 % (70)	63 % (35)	63 % (35)	1.000[a]
	female	38 % (42)	38 % (21)	38 % (21)	
Age (years)		56.2 ± 11.5	55.9 ± 12.4	56.5 ± 10.6	0.801[b]
BMI (kg/m^2)		n = 111 27.5 ± 5.05	27.0 ± 4.05	n = 55 28.0 ± 5.90	0.135[b]

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, BMI: body mass index, (a) chi-2 test, (b) students-t test. Statistical significance for group comparisons was set at p value 0.05 (shown in bold face**)**

4.1.3 Prevalence of fatigue, anxiety and depression symptoms

The prevalence and severity of fatigue, anxiety and depression symptoms according to the fatigue severity scale (FSS) and hospital anxiety and depression scale (HADS) respectively are shown in Table 8, Figure 7 and Figure 8. Moderate to severe fatigue symptoms were present in 68 % LC and 18 % CON (see Figure 7). Both anxiety and depression symptoms were present each in 52 % LC compared to only 25 % CON with anxiety and only 9 % CON with depression symptoms (see Figure 8). Consequently, mean anxiety and depression scores were higher in LC compared to CON especially for depression and much less for anxiety. The evaluation of the HADS-D items also indicated the aspects of anxiety; nervousness (A1), relaxation deficit (A4) and restlessness (A6) were less common in both LC/CON. All items of depression were clearly compromised in LC compared to CON with the exception of activities of relaxation (D7) being similarly good among the groups (LC 88 % vs. CON 96 %, p = 0.162).

Table 8: Prevalence and severity of fatigue, anxiety and depression symptoms

Parameters % (n). Mean ± SD		Total n = 112	CON n = 56	LC n = 56	p Value
Fatigue Severity Score (FSS)		3.72 ± 1.84	2.74 ± 1.25	4.70 ± 1.82	**< 0.001[a]**
Fatigue					
	severe	27 % (30)	7 % (4)	50 % (28)	**< 0.001[b]**
	moderate	15 % (17)	11 % (6)	18 % (10)	
	no fatigue	58 % (65)	82 % (46)	32 % (18)	
Anxiety Score (HADS-D)		6.48 ± 3.98	5.38 ± 3.19	7.59 ± 4.40	**0.003[a]**
Anxiety					
	moderate-severe ((≥11)	16 % (18)	7 % (4)	27 % (15)	**0.007[b]**
	mild (8-10)	21 % (24)	18 % (10)	25 % (14)	
	no anxiety (0-7)	63 % (70)	75 % (42)	48 % (27)	

Depression Score (HADS-D)	5.74 ± 4.63	3.46 ± 2.61	8.02 ± 5.09	**< 0.001**[a]
Depression Symptoms				
moderate-severe ((≥11)	17 % (19)	2 % (1)	32 % (18)	**< 0.001**[b]
mild (8-10)	13 % (14)	7 % (4)	20 % (11)	
no depression (0-7)	71 % (79)	91 % (51)	48 % (27)	

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, HADS-D: Hospital Anxiety and Depression Scale – German version, (a) students-t test, (b) exact-fisher test. Statistical significance for group comparisons was set at p value 0.05 (shown in bold face)

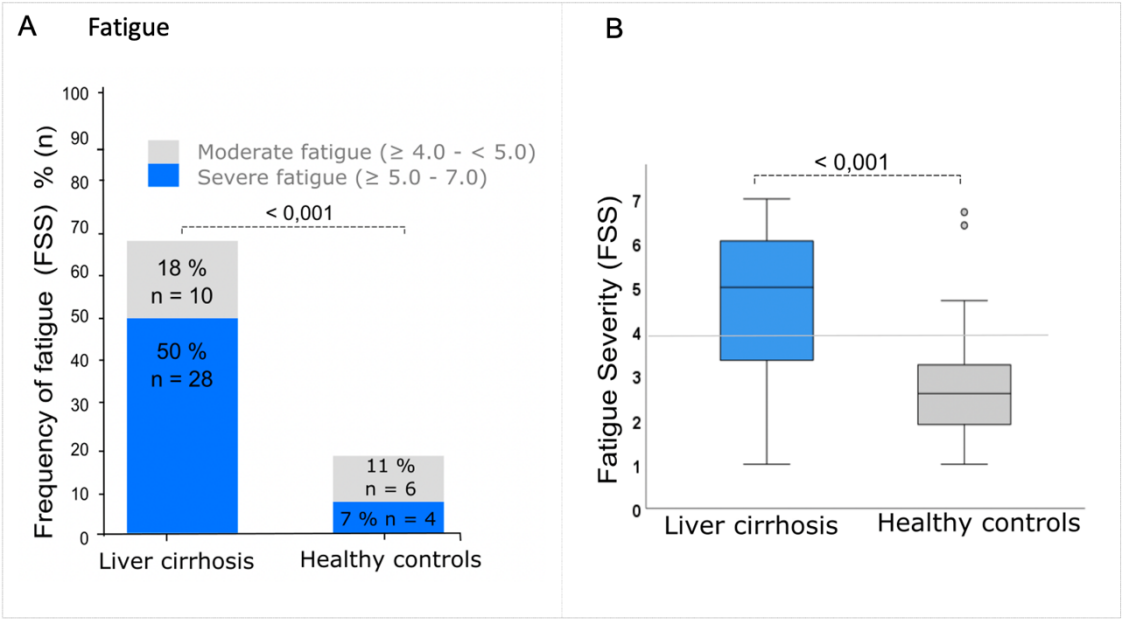


Figure 7: Prevalence and severity of fatigue symptoms

(A) Prevalence of fatigue symptoms in patients with LC vs. CON (B) Fatigue severity in LC patients compared to CON. LC: liver cirrhosis, CON: healthy controls, FSS: Fatigue Severity Scale, Fatigue threshold ≥4 points indicated with the grey line. Statistical significance was set at p value 0.05 (shown in bold face). The data set was complete in all analyses, n = 56 each

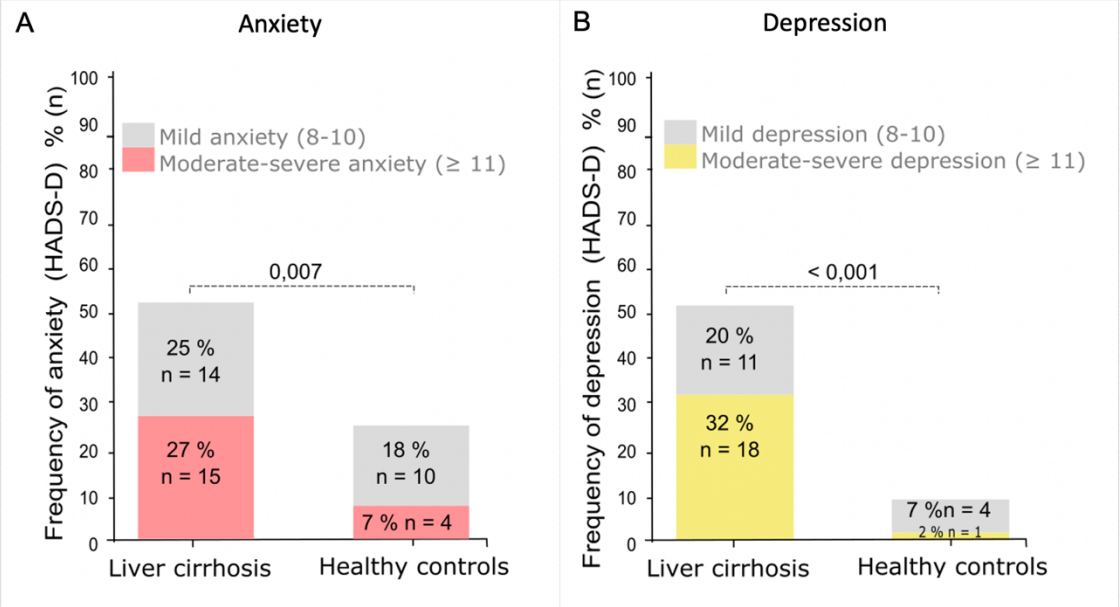


Figure 8: Prevalence of anxiety and depression symptoms

(A) Prevalence of anxiety symptoms in patients with LC vs. CON (B) Prevalence of depression symptoms in LC vs. CON. LC: liver cirrhosis, CON: healthy controls, HADS-D: Hospital Anxiety and Depression Scale – German version. Statistical significance was set at p value 0.05 (shown in bold face). The data set was complete in all analyses, n = 56 each

4.1.3.1 Central and peripheral fatigue

All features and FSS items of fatigue (central, peripheral and associated) scored higher and were more affected in LC compared to CON (see Table 9). However, contrary to literature [15, 20, 22, 164], central and peripheral fatigue were similarly manifested in LC patients. 63 % LC presented with central fatigue while 73 % peripheral fatigue, none had only central fatigue and only 11 % had only peripheral fatigue.

Table 9: Severity of central, peripheral and associated fatigue

Parameters % (n). Mean ± SD	Total n = 112	CON n = 56	LC n = 56	p Value
Central fatigue score	3.78 ± 1.89	2.84 ± 1.40	4.71 ± 1.87	**< 0.001[a]**
Peripheral fatigue score	3.85 ± 1.92	2.79 ± 1.27	4.90 ± 1.89	**< 0.001[a]**
Associated aspect	3.00 ± 2.18	2.13 ± 1.40	3.88 ± 2.47	**< 0.001[a]**

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, (a) students-t test. Statistical significance for group comparisons was set at p value 0.05 (shown in bold face)

4.1.4 Correlation of fatigue, anxiety and depression symptoms

Both LC patients and CON showed a positive correlation between fatigue and anxiety as well as fatigue and depression symptoms as shown in Figure 9.

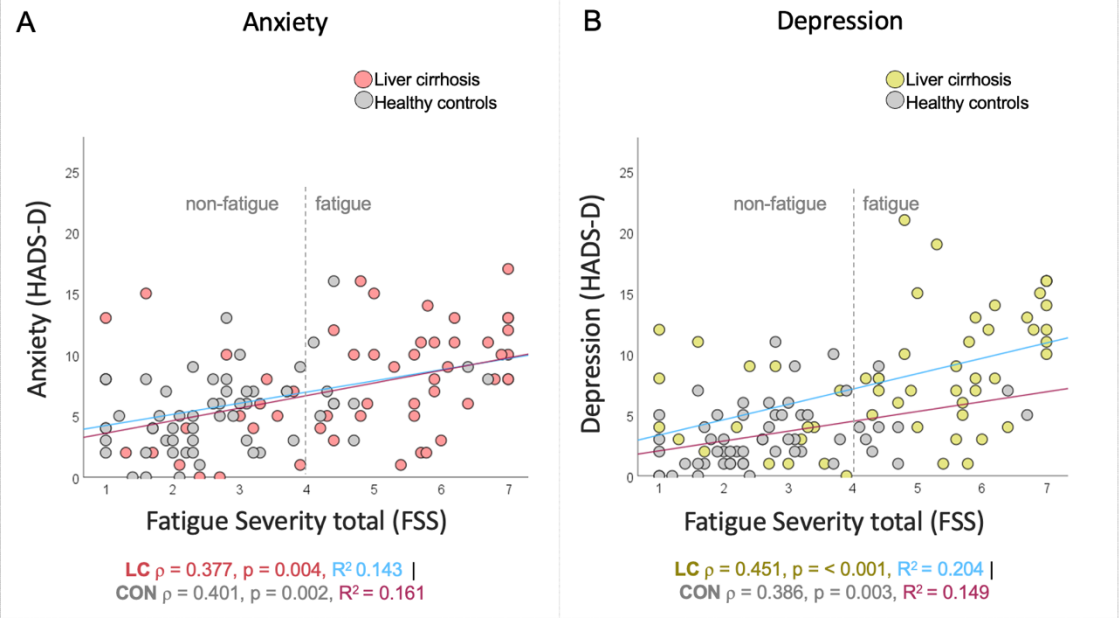


Figure 9: Correlation between fatigue, anxiety and depression symptoms

A) Correlation between fatigue and anxiety in patients with LC and CON, (B) Correlation between fatigue and depression symptoms in patients with LC and CON. LC: liver cirrhosis, CON: healthy controls, HADS-D: Hospital Anxiety and Depression Scale – German version. Statistical significance was set at p value 0,05. The data set was complete in all analyses, n = 56 each, pearson correlation coefficient.

4.1.4.1 Correlations of central/peripheral fatigue

Similar to overall fatigue, central and peripheral fatigue showed a positive correlation to both anxiety and depression symptoms as shown in Figure 10.

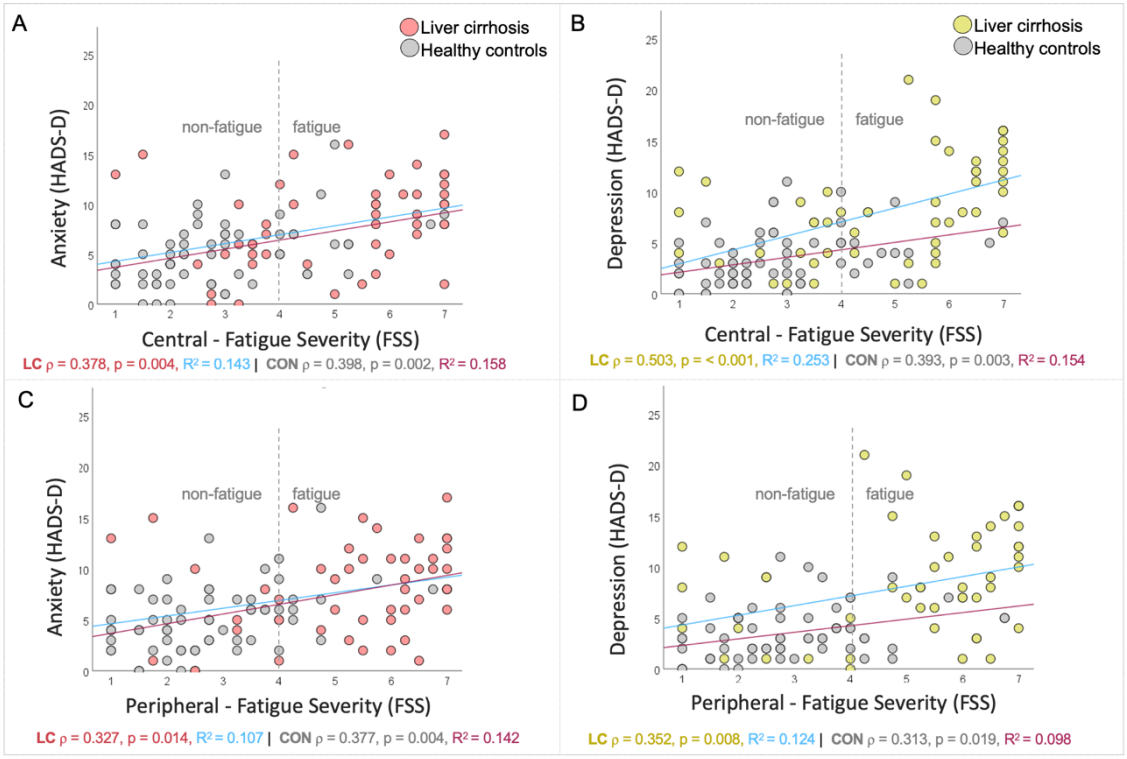


Figure 10: Correlations of cental/peripheral fatigue with anxiety/depression symptoms

Correlation between central fatigue (A-B) and peripheral fatigue (C-D) with anxiety (old rose) and depression symptoms (yellow). The data set was complete in all analyses, n = 56 each.

4.1.5 Clustering fatigue, anxiety and depression symptoms (LC only)

Given the high prevalence of fatigue, anxiety and depression symptoms in LC, we hypothesized that LC who presented with both fatigue symptoms and anxiety and/or depression symptoms will be more compromised than LC with isolated fatigue symptoms. Therefore, further analysis compared fatigued LC with and without psychosocial symptoms . We clustered LC patients according to the presence of fatigue, anxiety and depression symptoms (Figure 11). The most represented cluster was the presence of all three symptoms, followed by fatigue only. Interestingly, the presence of only two symptoms was seen in only 7 %, of each combination. While only anxiety or depression was evident in one patient each. No symptoms were present in only 21 % of patients.

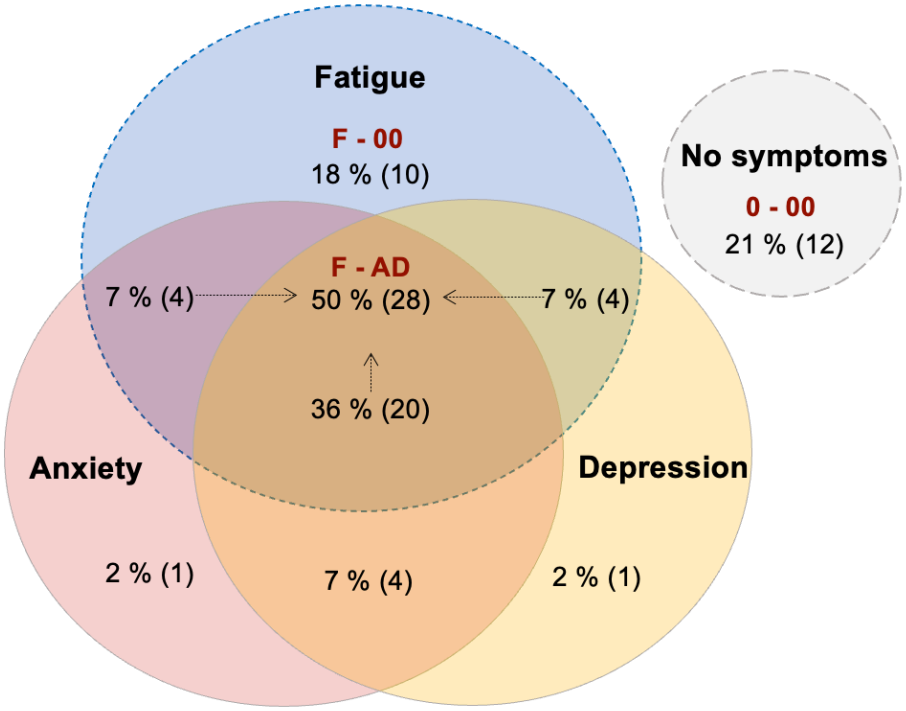


Figure 11: Symptom clustering and overlap of fatigue with anxiety and depression in LC patients

The circles in the venn-diagram represent the numbers of LC patients (n = 56), with only fatigue (blue circle), anxiety (red circle), depression symptoms (yellow circle) and the overlap between the three symptoms (showing the colour mix for corresponding overlap of the blue, red or yellow circles while the presence of no symptoms is represented in the grey ring). F-00: only fatigue symptoms, F-AD: fatigue with anxiety and/or depression symptoms, 0-00: neither fatigue, anxiety nor depression symptoms.

4.1.6 Liver cirrhosis: Disease etiology and severity

The primary etiology of liver cirrhosis was excessive alcohol consumption present in more than 2/3 of the patients, followed by autoimmune hepatitis and other causes. The proportion of patients within Child-Pugh scores A, B and C were 21 % (n = 12), 38 % (n = 21) and 41 % (n = 23), respectively. This was accompanied by a 3-month mortality probability of 6 % based on the mean MELD score of 16.9. As expected, edema and ascites were predominant in LC, affecting 51 % and 77 % of patients, respectively. Furthermore, about half the LC's had both complications (see table 10).

Disease etiology and severity was comparable between F/Non-F and F-00/F-AD. F LC's presented with more cases of edema, ascites & edema or ascites than Non-F. F-00 and F-AD presented with comparable presence of these complications although ascites in F-AD affected almost all patients (93 %).

Table 10: Disease etiology and severity in patients with liver cirrhosis

Parameters % (n). Mean ± SD	only liver cirrhosis				
	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Disease etiology		p 0.072[b]		p 0.419[b]	
Ethyltoxic	68 % (38)	56 % (10)	74 % (28)	80 % (8)	71 % (20)
Autoimmune hepatitis	13 % (7)	28 % (5)	5 % (2)	10 % (1)	4 % (1)
Other	20 % (11)	17 % (3)	21 % (8)	10 % (1)	25 % (7)
Non-alcoholic steatohepatitis	n = 3	-	n = 3	-	n = 3
Primary biliary cholangitis	n = 2	-	n = 2	n = 1	n = 1
Idiopathic	n = 2	-	n = 2	-	n = 2
Budd-Chiari syndrome	n = 1	n = 1	-	-	-
Primary sclerosing cholangitis	n = 1	n = 1	-	-	-
Hepatitis C	n = 1	-	n = 1	-	n = 1
Trauma	n = 1	n = 1	-	-	-
Child Pugh Score		p 0.246[b]		p 0.441[b]	
Child-Pugh A	21 % (12)	28 % (5)	18 % (7)	30 % (3)	14 % (4)
Child-Pugh B	38 % (21)	22 % (4)	45 % (17)	30 % (3)	50 % (14)
Child-Pugh C	41 % (23)	50 % (9)	37 % (14)	40 % (4)	36 % (10)
MELD Score		p 0.563[c]		p 0.231[c]	
	n = 54	n = 17	n = 37		n = 27
MELD	16.9 ± 8.04	16.2 ± 8.84	17.2 ± 7.75	14.4 ± 6.85	16.9 ± 7.41
Complications		**p 0.044[a]**		p 0.709[b]	
	n = 55	n = 17			
Edema	51 % (28)	29 % (5)	61 % (23)	70 % (7)	57 % (16)
		p 0.017[a]		p 0.103[b]	
Ascites	77 % (43)	56 % (10)	87 % (33)	70 % (7)	93 % (26)
		p 0.085[a]		p 0.727[a]	
Edema and ascites	47 % (26)	29 % (5)	55 % (21)	50 % (5)	57 % (16)
		p 0.003[b]		p 1.000[b]	
Edema or ascites	82 % (45)	56 % (10)	92 % (35)	90 % (9)	93 % (26)

SD: standard deviation, LC: liver cirrhosis, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), (a) chi-2 test, (b) exact-fisher test, (c) mann-whitney-u test. Cumulative percentages may deviate from 100% due to rounding of decimal places. Statistical significance set at p value < 0.05 (shown in bold face).

4.1.7 Socioeconomic status

Data on socioeconomics are summarized in Table 11. Patients with LC had significantly shorter duration of education and higher unemployment rates compared with CON. The high rates of early retirement in the LC is mirrored in the average age of 57 years. In terms of fatigue, F/Non-F and F-00/F-AD were comparable in education status. While in terms of employment status, significant differences were only seen in F-00/F-AD. Specifically, most of F-00 patients were unemployed while majority of the F-AD patients were early retired. The prevalence of loneliness in LC patients was 3 times higher compared to CON. Of which, the loneliness score was significantly higher in LC compared to CON. This was attributed to emotional rather than social loneliness. In F LC, loneliness was tendentially more prevalent compared to Non-F. As reflected in F LC

having a significantly higher loneliness scores (emotional loneliness). This was however, was comparable between F-00 and F-AD. Nicotine consumption in LC patients was 3.5 times higher as compared to CON, while no difference existed between F/Non-F and F-00/F-AD.

Table 11: Socioeconomic status

Parameters % (n). Mean ± SD	CON vs. LC		LC n = 56			
	CON n = 56	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Education	**p < 0.001[a]**		p 0.448[b]		p 0.592[b]	
Duration						
≤ 10 years	39 % (22)	84 % (47)	78 % (14)	87 % (33)	80 % (8)	89 % (25)
> 10 years	61 % (34)	16 % (9)	22 % (4)	13 % (5)	20 % (2)	11 % (3)
Employment	**p < 0.001[b]**		p 0.292[b]		**p 0.030[b]**	
Employed	79 % (44)	23 % (13)	33 % (6)	18 % (7)	0 % (0)	25 % (7)
Unemployed	0 % (0)	20 % (11)	11 % (2)	24 % (9)	50 % (5)	14 % (4)
Early Retired	7 % (4)	43 % (24)	33 % (6)	47 % (18)	30 % (3)	54 % (15)
Retired	14 % (8)	14 % (8)	22 % (4)	11 % (4)	20 % (2)	7 % (2)
Loneliness	p 0.056[b]		p 0.079[b]		p 0.225[b]	
STATUS						
Lonely	7 % (4)	21 % (12)	6 % (1)	29 % (11)	10 % (1)	36 % (10)
Not Lonely	93 % (52)	79 % (44)	94 % (17)	71 % (27)	90 % (9)	64 % (18)
Loneliness	**p 0.013[c]**		**p 0.012[d]**		p 0.168[d]	
Score	1.60 ± 0.57	1.94 ± 0.81	1.54 ± 0.53	2.12 ± 0.85	1.81 ± 0.74	2.23 ± 0.86
Social	p 0.159[c]		p 0.096[d]		p 0.193[d]	
loneliness Score	1.73 ± 0.65	1.92 ± 0.76	1.65 ± 0.59	2.04 ± 0.81	1.77 ± 0.70	2.14 ± 0.83
Emotional	**p 0.002[c]**		**p 0.004[d]**		p 0.205[d]	
loneliness Score	1.48 ± 0.60	1.95 ± 0.93	1.43 ± 0.53	2.19 ± 0.98	1.87 ± 0.88	2.31 ± 1.00
Nicotine	**p 0.004[b]**		p 0.699[b]		p 1.000[b]	
CONSUMPTION						
Smoker	11 % (6)	38 % (21)	44 % (8)	34 % (13)	30 % (3)	36 % (10)
Non-Smoker	61 % (34)	43 % (24)	33 % (6)	47 % (18)	50 % (5)	46 % (13)
Ex-Smoker	29 % (16)	20 % (11)	22 % (4)	18 % (7)	20 % (2)	18 % (5)

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), (a) chi-2 test, (b) exact-fisher test, (c) students-t test, (d) mann-whitney-u test. Statistical significance for group comparisons was set at p value 0.05 (shown in bold face).

4.1.8 Medication and micronutrient supplementation

Medication and micronutrient intakes are shown in Table 12. As expected, LC and CON showed a significant difference in drug intake. With LC having a higher intake compared to CON in proton-pump inhibitors, coagulants, beta blocker and cholestering lowering medication. Furthermore in diuretics, antibiotics, albumin and antidiabetic medication compared to none of the CON . In LC most of the medication taken was for the reduction of stomach acid production, followed by diuretic intake. Intake of antihypertensive drugs

and coagulants was also high in LC and one third were on antibiotics. The most supplemented micronutrients in LC were vitamin B complex, folic acid, and zinc. All medication and micronutrient supplementation intake was comparable in F/Non-F and F-00/F-AD with the exception of F/Non-F in the intake of proton-pump inhibitors and antibiotics (82 % vs. 50 %, and 42 % vs. 11 %, respectively).

Table 12: Medication and micronutrient supplementation intake

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON	LC	Non-F	F	F-00	F-AD
	n = 56	n = 56	n = 18	n = 38	n = 10	n = 28
Proton-pump inhibitors	**p < 0.001[b]**		**p 0.025[a]**		p 0.351[b]	
	4 % (2)	71 % (40)	50 % (9)	82 % (31)	70 % (7)	86 % (24)
Diuretics	**p < 0.001[b]**		p 1.000[a]		p 1.000[b]	
	0 % (0)	63 % (35)	61 % (11)	63 % (24)	60 % (6)	64 % (18)
	p 0.005[a]		p 0.859[b]		p 0.444[b]	
*Antihypertensives**	14 % (8)	37 % (21)	39 % (7)	37 % (14)	50 % (5)	32 % (9)
ACE inhibitor	13 % (3)	9 % (5)	11 % (2)	8 % (3)	10 % (1)	7 % (2)
AT1 antagonist	13 % (3)	13 % (3)	13 % (1)	13 % (2)	0 % (0)	7 % (2)
Calcium antagonist	0 % (0)	7 % (4)	11 % (2)	13 % (2)	10 % (1)	13 % (1)
Others	4 % (2)	16 % (9)	11 % (2)	18 % (7)	30 % (3)	14 % (4)
Coagulants	**p < 0.001[b]**		p 0.233[b]		p 0.469[b]	
	4 % (2)	36 % (20)	22 % (4)	42 % (16)	30 % (3)	46 % (13)
Antibiotics	**p < 0.001[b]**		**p 0.031[b]**		p 0.469[b]	
	0 % (0)	32 % (18)	11 % (2)	42 % (16)	30 % (3)	46 % (13)
Albumin	**p < 0.001[b]**		p 0.510[b]		p 1.000[b]	
	0 % (0)	25 % (14)	18 % (3)	28 % (11)	30 % (3)	29 % (8)
*Beta blocker**	**p 0.002[b]**		p 1.000[b]		p 1.000[b]	
	2 % (1)	21 % (12)	22 % (4)	21 % (8)	20 % (2)	11 % (6)
Cholesterol-lowering	p 0.203[b]		p 0.414[b]		p 0.351[b]	
	5 % (3)	14 % (8)	6 % (1)	18 % (7)	30 % (3)	14 % (4)
Antidepressants	p 0.118[b]		p 1.000[b]		p 0.552[b]	
	0 % (0)	7 % (4)	6 % (1)	8 % (3)	0 % (0)	11 % (3)
Antidiabetics	p 0.243[b]		p 0.544[b]		p 1.000[b]	
	0 % (0)	5 % (3)	0 % (0)	8 % (3)	10 % (1)	7 % (2)
Supplements. Vitamins & Minerals	**p < 0.001[a]**		p 0.732[b]		p 0.650[b]	
	32 % (18)	80 % (45)	78 % (14)	82 % (31)	90 % (9)	79 % (22)
Combined Formula						
Vitamin B Complex		95 % (53)				
Multivitamin		13 % (1)				
Single Minerals & vitamins						
Magnesium		33 % (15)				
Zinc		44 % (20)				
Potassium		24 % (11)				
Folic acid		44 % (20)				
Vitamin D		17 % (8)				
others		13 % (7)				

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms). * Beta blocker in congestive heart failure, ACE inhibitors: angiotensin-converting-enzyme inhibitors, AT1: angiotensin II receptor antagonists. Cumulative percentages may deviate from 100% due to rounding of decimal places,

(a) chi-2 test, (b) exact-fisher test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.9 Anthropometry and body composition

Detailed anthropometrical data and body composition are presented in Table 13. Due to match pairing, no difference existed between LC and CON in weight and height. However, as expected, upper arm circumference was markedly lower while, waist circumference (WC) was significantly higher in LC compared with CON, indicating LC-associated ascites and edema.

In line with ascites/edema, body composition showed clearly increased extracellular water (ECW) and increased ECW/total body water (TBW) ratio in LC compared with CON. The fluid shifts in LC may have positively biased the results for fat free mass index and resulted in a trend towards similar values of skeletal muscle mass index between LC and CON. Noticeably, the raw values, reactance and phase angle were clearly compromised in LC whereas resistance was comparable. In LC F/Non-F and F-00/F-AD no difference was seen in anthropometrics as well as in body composition with the exception of; significantly higher WC and ECW/TBW ratio that trended to higher values in F compared to Non-F, while F-AD compared to F-00 showed trending higher waist hip ratio and TBW values. These results are consistent with the presence of ascites in almost all subjects in F-AD (93 %, n = 26/28).

Table 13: Anthropometry and body composition

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON n = 56	LC n = 55*	Non-F n = 18	F n = 37*	F-00 n = 10	F-AD n = 27*
Weight (kg)	p 0.391[a]		p 0.190[a]		p 0.161[b]	
	80.7 ± 15.2	83.6 ± 19.6	79.1 ± 15.0	85.7 ± 21.3	79.0 ± 20.6	88.2 ± 21.4
Height (cm)	p 0.933[a]		p 0.697[a]		p 0.501[a]	
	1.72 ± 0.09	1.73 ± 0.11	1.73 ± 0.11	1.72 ± 0.11	1.70 ± 0.14	1.73 ± 0.10
Upper arm circumference (cm)	**p 0.019[a]**		p 0.114[b]		p 0.354[a]	
	31.6 ± 2.96	29.2 ± 6.81	27.1 ± 3.04	30.2 ± 7.87	28.2 ± 6.71	31.0 ± 8.24
Waist circumference (cm)	**p < 0.001[a]**		**p 0.038[a]**		p 0.166[a]	
	94.1 ± 12.1	106 ± 16.6	99.0 ± 14.0	109 ± 17.0	102 ± 15.6	111 ± 17.2
Waist hip ratio (WHR)	**p < 0.001[a]**		p 0.156[b]		p 0.081[b]	
	0.91 ± 0.08	1.05 ± 0.16	1.03 ± 0.16	1.07 ± 0.16	1.01 ± 0.08	1.09 ± 0.17
Fat Free Mass Index (kg/m^2)	**p 0.029[a]**		p 0.603[b]		p 0.161[b]	
	18.5 ± 2.35	19.9 ± 4.45	19.2 ± 3.55	20.2 ± 4.83	18.0 ± 2.20	21.0 ± 5.29
SMI (kg/m^2)	p 0.091[a]		p 0.806[a]		p 0.171[b]	
	8.77 ± 1.43	8.11 ± 2.48	7.99 ± 2.08	8.17 ± 2.68	6.95 ± 2.33	8.62 ± 2.70

Total Body Water (TBW) l	p 0.268[a]		p 0.724[a]		p 0.091[a]	
	41.0 ± 7.93	43.2 ± 11.8	42.3 ± 9.99	43.6 ± 12.7	37.8 ± 9.57	45.7 ± 13.2
Extracellular water (ECW) l	**p < 0.001[a]**		p 0.415[a]		p 0.114[a]	
	17.9 ± 2.89	21.1 ± 5.45	20.3 ± 4.42	21.5 ± 5.89	19.0 ± 3.71	22.5 ± 6.32
ECW/TBW (%)	**p < 0.001[a]**		p 0.093[b]		p 0.607[b]	
	43.9 ± 2.50	49.4 ± 5.05	48.2 ± 4.84	50.0 ± 5.12	51.5 ± 7.50	49.4 ± 3.93
Resistance (R)	p 0.595[a]		p 0.591[a]		p 0.170[a]	
	561 ± 70.5	550 ± 135	564 ± 130	543 ± 138	579 ± 58.1	529 ± 157
Reactance (Xc)	**p < 0.001[a]**		p 0.221[a]		p 0.763[a]	
	51.9 ± 8.12	36.3 ± 14.7	39.8 ± 14.8	34.6 ± 14.6	35.8 ± 11.1	34.1 ± 15.9
Phase angle °	**p < 0.001[a]**		p 0.104[b]		p 0.921[a]	
	5.27 ± 0.65	3.72 ± 1.01	4.00 ± 1.04	3.58 ± 0.98	3.56 ± 1.08	3.59 ± 0.96

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms),ECW/TBW: extracellular water/ total body water,SMI: skeletal muscle mass index, * 1 missing – declined BIA/ anthropometric examinations, (a) students-t test, (b) mann-whitney-u test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.10 Clinical chemistry

4.1.10.1 Anemia

Lower hemoglobin and hematocrit in LC signaled anemia as shown in Table 14. In LC, 75 % suffered from anemia (4 % microcytic, 29 % macrocytic) compared to none in CON. In F LC, twice as many patients were diagnosed with macrocytic anemia compared to Non-F and none with microcytic anemia. The majority of LC patients, and regardless of fatigue, seemed to have normocytic anemia with the exception of F-00 (1/2 with normocytic and marcocytic) while in both F-00 and F-AD none of the patients had microcytic anemia, as is often seen in anemia of chronic disease [165]. All results of the fatigue comparisons were not statistically significant, except for significantly lower MCV values in F-AD.

Table 14: Anemia

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON n = 56	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Hemoglobin (mmol/l) ♀: 7.5 - 9.9 ♂: 8.4 - 10.9	**p < 0.001[c]**		p 0.160[d]		p 0.345[d]	
	n = 54					
	9.20 ± 0.68	6.64 ± 1.63	7.08 ± 1.62	6.44 ± 1.62	6.86 ± 1.58	6.29 ± 1.65
Anemia	**p < 0.001[b]**		p 0.322[a]		p 0.653[b]	
Anemic	4 % (2)	75 % (42)	67 % (12)	79 % (30)	70 % (7)	82 % (23)
		30 % (17)	28 % (5)	32 % (12)		29 % (8)
mild	4 % (2)	25 % (14)	33 % (6)	21 % (8)	40 % (4)	25 % (7)

moderate	-	18 % (10)	6 % (1)	24 % (9)	10 % (1)	25 % (7)
severe	-	2 % (1)	-	3 % (1)	20 % (2)	4 % (1)
life-threatening	-				-	
Hematocrit (--)	**p < 0.001[c]**		p 0.100[c]		p 0.344[c]	
	n = 54					
	0.44 ± 0.03	0.31 ± 0.07	0.34 ± 0.07	0.30 ± 0.07	0.32 ± 0.07	0.30 ± 0.08
MCV (fl)	**p < 0.001[c]**		p 0.245[c]		**p 0.047[d]**	
	n = 55					
	89.1 ± 3.30	96.3 ± 8.14	94.5 ± 8.87	97.2 ± 7.73	102 ± 8.00	95.6 ± 7.09
	p < 0.001[b]		p 0.069[b]		0.263[a]	
Microcytic	0 % (0)	4 % (2)	11 % (2)	0 % (0)	0 % (0)	0 % (0)
Normocytic	100 % (55)	68 % (38)	72 % (13)	66 % (25)	50 % (5)	71 % (20)
Macrocytic <80. 80-100. > 100	0 % (0)	29 % (16)	17 % (3)	34 % (13)	50 % (5)	29 % (8)

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), , MCV : mean corpuscular volume, (a) chi-2 test, (b) exact-fisher test, (c) students-t test, (d) mann-whitney-u test. Cumulative percentages may deviate from 100% due to rounding of decimal places. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.10.2 Liver and coagulation parameters

As expected, liver and coagulation markers were significantly deteriorated in LC compared to CON. Liver as well as coagulation markers were not distinguishable between F and Non-F LC, apart from gamma-glutamyl transferase (gamma-GT) values that showed tendentially higher values in F compared to Non-F. While all values in F-00 and F-AD were comparable as shown in Table 15.

Table 15: Liver and coagulation parameters

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON n = 56	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
ASAT (U/l)	**p < 0.001[a]**		p 0.833[b]		p 0.765[b]	
♀: < 35 ♂: < 59	24.4 ± 6.14	85.1 ± 67.8	89.5 ± 81.3	83.0 ± 61.4	88.9 ± 60.9	80.9 ± 62.6
ALAT (U/l)	**p < 0.001[a]**		p 0.636[b]		p 0.389[b]	
♀: < 35 ♂: < 50	25.6 ± 12.1	51.8 ± 52.5	74.5 ± 78.9	41.0 ± 29.4	47.3 ± 30.3	38.8 ± 29.3
Gamma-GT (U/l)	**p < 0.001[a]**		p 0.075[b]		p 0.881[b]	
♀: < 40 ♂: < 60	28.3 ± 19.0	189 ± 182	136 ± 128	214 ± 199	196 ± 157	220 ± 215
AP (U/l)	**p < 0.001[a]**		p 0.661[b]		p 0.894[b]	
♀: 35 - 104 ♂: 40 - 129	67.7 ± 16.4	161 ± 76.3	167 ± 88.1	158 ± 71.2	151 ± 55.4	160 ± 76.8
Pseudo-cholinesterase	**p < 0.001[a]**		p 0.419[b]		p 0.945[b]	
(kU/l) 0.001		n = 55		n = 37		n = 27
	8.39 ± 1.82	3.48 ± 3.26	3.78 ± 3.36	3.34 ± 3.24	4.14 ± 3.97	3.04 ± 2.96
	p < 0.001[a]		p 0.895[b]		p 0.715[b]	
Bilirubin. total (µmol/l) < 21	10.2 ± 4.64	132 ± 181	128 ± 188	135 ± 180	113 ± 147	142 ± 192

	p < 0.001[a]		p 0.641[b]		p 0.211[b]	
INR 0.80 – 1.20		n = 54	n = 17	n = 37		n = 27
	1.01 ± 0.06	1.51 ± 0.59	1.45 ± 0.48	1.54 ± 0.64	1.44 ± 0.72	1.58 ± 0.61

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase, gamma-GT: gamma-glutamyl transferase, AP: alkaline phosphate, INR: international normalized ratio, (a) students-t test, (b) mann-whitney-u test. Cumulative percentages may deviate from 100% due to rounding of decimal places. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.10.3 Renal function

The glomerular filtration rate was impaired in 16 % LC compared to 5 % in CON (Table 16). In line, urea was increased in LC compared to CON. Creatinine and uric acid were comparable between LC and CON. Similarly, renal function (creatinine, glomerular filtration rate, urea and uric acid) was comparable between F/Non-F and F-00/F-AD except for trending higher creatinine values in F and F-AD and urea only in F-AD.

Table 16: Renal function

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON	LC	Non-F	F	F-00	F-AD
	n = 56	n = 56	n = 18	n = 38	n = 10	n = 28
Creatinine (µmol/l) ♀: 73-109 ♂:82.127	p 0.200[c]		p 0.078[d]		p 0.079[d]	
	81.8 ± 16.5	95.5 ± 78.0	77.9 ± 47.4	104 ± 88.2	71.6 ± 19.4	115 ± 100
Above OR below reference	**p 0.010[a]**		p 0.042[b]		p 1.000[b]	
	52 % (29)	77 % (43)	94 % (17)	68 % (26)	70 % (7)	68 % (19)
CKD-EPI-GFR	p 0.741[c]		p 0.135[c]		p 0.447[c]	
		n = 45	n = 14	n = 31	n = 7	n = 24
	87.3 ± 8.95	88.9 ± 30.8	99.1 ± 30.9	84.2 ± 30.1	92.0 ± 28.4	82.0 ± 30.8
< 60 ml/min	p 0.105[b]		p 1.000[b]		p 1.000[b]	
	5 % (3)	16 % (7)	11 % (2)	13 % (5)	10 % (1)	18 % (4)
Urea (mmol/l) < 8.3	**p 0.020[c]**		p 0.164[d]		p 0.070[d]	
		n = 55		n = 37		n = 27
	4.76 ± 1.22	7.30 ± 7.76	6.62 ± 7.76	7.63 ± 7.85	4.65 ± 2.09	8.74 ± 8.90
Above reference	**p < 0.001[b]**		p 0.346[b]		p 0.224[b]	
	0 % (0)	26 % (14)	17 % (3)	29 % (11)	10 % (1)	37 % (10)
Uric acid (µmol/l) < 420	p 0.147[c]		p 0.099[d]		p 0.614[c]	
	323 ± 79.8	361 ± 174	313 ± 171	383 ± 173	359 ± 184	392 ± 171
Above reference	**p 0.013[a]**		p 0.241[b]		p 0.709[b]	
	13 % (7)	34 % (19)	22 % (4)	40 % (15)	30 % (3)	43 % (12)

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), CKD-EPI-GFR: chronic kidney disease epidemiology collaboration glomerular filtrate rate, GFR categories according to KDIGO: kidney disease improving global outcomes, mod.: moderately. (a) chi-2 test, (b) exact-fisher test, (c) students-t test, (d) mann-whitney-u test. Cumulative percentages may deviate from 100% due to rounding of decimal places. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.10.4 Cardiovascular risk factors

Mean fasting (> 4 hrs) glucose concentration in LC was 26 % and 21 % in the pre-diabetic and diabetic ranges, respectively (Table 17). In line, fasting insulin was also significantly higher in LC compared to CON, pointing towards insulin resistance as seen in hepatic diabetic mellitus [166]. This is further supported by the homeostatic model assessment index (HOMA), which showed that 33 % of LC`s had an indication/probability of insulin resistance while 42 % were insulin resistant.

F-LC patients showed comparable glucose homeostasis compared to Non-F whereas mean fasting glucose in F-AD was significantly elevated compared to F-00. Only 2 patients in F-00 were pre-diabetic and none were diabetic compared to 36 % versus 28 % diabetic and pre-diabetic in F-AD.

Lipid values showed comparable triglycerides and significantly lower total cholesterol concentration in LC compared to CON. Whereas in the fatigued groups (F/Non-F and F-00/F-AD), both lipid values were comparable except for a trend towards lower total cholesterol values in F.

Table 17: Cardiovascular risk factors

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON n = 56	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Glucose (mmol/l) 3.3 – 5.5	**p 0.004[b]**		p 0.468[c]		**p 0.014[c]**	
	5.63 ± 0.55	n = 55 6.46 ± 1.97	6.20 ± 1.85	n = 37 6.58 ± 2.03	5.54 ± 0.52	n = 27 6.96 ± 2.25
	p < 0.001[a]		p 0.506[a]		**p 0.038[a]**	
Pre-diabetic	7 % (4)	n = 53* 26 % (14)	25 % (5)	n = 35 26 % (9)	20 % (2)	n = 25 28 % (7)
Diabetic	4 % (2)	21 % (11)	11 % (2)	26 % (9)	0 % (0)	36 % (9)
Insulin (µIU/ml) 2.6 – 24.9	**p < 0.001[b]**		p 0.557[c]		p 0.437[c]	
	8.15 ± 5.39	n = 54 17.5 ± 12.6	16.4 ± 13.7	n = 36 18.0 ± 12.2	15.8 ± 13.7	n = 26 18.9 ± 11.8
HOMA IR:	**p < 0.001[a]**		p .828[a]		p 0.375[a]	
		n = 52		n = 34		n = 24
Indication 2.0-2.5	18 % (10)	12 % (6)	11 % (2)	12 % (4)	10 % (1)	13 % (3)
Probable 2.5.5.0	7 % (4)	21 % (11)	17 % (3)	24 % (8)	20 % (2)	25 % (6)
Present > 5.0	11 % (6)	42 % (22)	39 % (7)	44 % (15)	30 % (3)	50 % (12)
Total Cholesterol (mmol/l) < 5.2	**p < 0.001[b]**		p 0.089[c]		p 0.908[c]	
	5.25 ± 0.94	3.44 ± 1.81	3.97 ± 1.94	3.19 ± 1.72	3.54 ± 2.61	3.06 ± 1.32
Triglycerides (mmol/l) < 2.3	p 0.598[b]		p 0.840[c]		p 0.679[c]	
	1.34 ± 0.69	1.26 ± 0.85	1.26 ± 0.85	1.27 ± 0.85	1.61 ± 1.33	1.15 ± 0.60

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), HOMA: homeostatic model assessment. *Only fasting glucose values were considered: glucose: pre-diabetic range: 6.11-

6.98 mmol/l, diabetic range ≥ 6.99 mmol/l (fasting period: LC ≥ 4 hours, CON overnight > 8 -12 hours). (a) exact-fisher test, (b) students-t test, (c) mann-whitney-u test. Cumulative percentages may deviate from 100% due to rounding of decimal places. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.10.5 Inflammation

With respect to inflammation in LC, leucocytes and positive acute phase proteins (c-reactive protein, tumor necrosis factor α (TNF α), and interleukin 6) except for interleukin-1β were elevated, while the negative acute phase proteins, albumin and transthyretin were decreased (Table 18). Acute and chronic inflammation as well as fluid shifts in LC prevented any interpretation of albumin and transthyretin as nutrition markers [167]. Leucocytes and all positive acute phase proteins (c-reactive protein, interleukin-1β, interleukin 6 and TNF-α) were also numerically higher in F compared to Non-F. Similarly, inflammation markers were numerically higher in F-AD compared to F-00 with the exception of TNF α.

We further performed a cluster analysis (see methodology section 3.6), and divided participants in “low inflammation” and “high inflammation” groups. Expectedly, significant differences were seen between LC and CON with 51 % LC having a high inflammation status compared to only one CON subject. F-LC compared to Non-F were comparable. However, in F-00 most patients had a low inflammation cluster compared to F-AD, where most of the patients had a high inflammation status.

Table 18: Inflammation

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON n = 56	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Albumin (g/l) 37 – 53	**p < 0.001[c]**		p 0.371[d]		p 0.908[d]	
	46.3 ± 2.46	30.4 ± 6.37	31.3 ± 7.12	30.0 ± 6.03	29.6 ± 4.00	30.1 ± 6.67
Transthyretin (g/l) ♀: 0.26 – 0.28 ♂: 0.29 - 0.32	**p < 0.001[c]**		p 0.127[d]		p 0.946[d]	
	0.25 ± 0.05	n = 55 0.09 ± 0.08	n = 17 0.10 ± 0.06	0.08 ± 0.09	0.08 ± 0.06	0.08 ± 0.09
Leucocytes (Gpt/l) 3.9 – 10.2	**p 0.018[c]**		p 0.986[d]		**p 0.047[d]**	
	n = 55 6.21 ± 1.76	7.69 ± 4.25	7.43 ± 3.81	7.82 ± 4.48	5.88 ± 2.77	8.51 ± 4.80
Above OR below reference	**p 0.002[b]**		p 1.000[a]		p 1.000[b]	
	6 % (3)	29 % (16)	28 % (5)	29 % (11)	30 % (3)	29 % (8)
C-reactive protein (mg/l) < 5	**p < 0.001[c]**		p 0.127[d]		p 0.507[d]	
	1.39 ± 1.09	27.5 ± 31.3	16.4 ± 16.1	32.8 ± 35.3	26.1 ± 35.4	35.1 ± 35.7
Above reference	**p < 0.001[b]**		p 1.000[b]		p 1.000[b]	
	2 % (1)	77 % (43)	78 % (14)	76 % (29)	80 % (8)	75 % (21)

Interleukin-1β (pg/ml) < 5	p 0.125[c]		p 0.160[d]		p 0.810[d]	
		n = 51	n = 17	n = 34	n = 9	n = 25
	6.16 ± 3.06	9.01 ± 12.8	5.58 ± 2.32	10.7 ± 15.3	5.71 ± 1.52	12.5 ± 17.6
Above reference	p 0.379[a]		p 0.328[b]		p 1.000[b]	
	21 % (12)	29 % (15)	18 % (3)	35 % (12)	33 % (3)	36 % (9)
Interleukin 6 (pg/ml) < 7	**p < 0.001[c]**		p 0.106[d]		p 0.572[d]	
		n = 54		n = 36		n = 26
	2.10 ± 0.96	50.7 ± 64.3	46.6 ± 76.8	52.7 ± 58.1	50.8 ± 60.7	53.4 ± 58.3
Above reference	**p < 0.001[b]**		p 0.461[b]		p 0.119[b]	
	0 % (0)	83 % (45)	78 % (14)	86 % (31)	70 % (7)	92 % (24)
Tumor Necrosis Factor α (pg/ml) < 8.1	**p < 0.001[c]**		p 0.549[d]		p 0.435[d]	
		n = 51	n = 17	n = 34	n = 9	n = 25
	5.92 ± 6.36	13.0 ± 6.54	12.7 ± 7.22	13.2 ± 6.28	11.5 ± 5.44	13.8 ± 6.56
Above reference	**p < 0.001[b]**		p 0.728[b]		p 0.348[b]	
	5 % (3)	76 % (39)	71 % (12)	79 % (27)	67 % (6)	84 % (21)
Inflammation CLUSTER	**p < 0.001[b]**		p 0.382[a]		**p 0.025[b]**	
	n = 55	n = 51	n = 17	n = 34	n = 9	n = 25
Low	98 % (54)	49 % (25)	59 % (10)	44 % (15)	78 % (7)	32 % (8)
High	2 % (1)	51 % (26)	41 % (7)	56 % (19)	22 % (2)	68 % (17)

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms).Reference values indicated below the inflammation markers, (a) chi-2 test, (b) exact-fisher test, (c) students-t test, (d) mann-whitney-u test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.10.6 Insulin-like growth factor (IGF-1)

Patients with LC compared to CON showed significantly lower insulin-like growth factor (IGF-1) values (Table 19), usually seen in LC [168]. Clear but not statistically significant differences in mean IGF-1 concentration were seen, between F and Non-F; suggesting possible underpowering due to the small sample size, while IGF-1 concentration was comparable between F-00/F-AD.

Table 19: Insulin-like growth factor (IGF-1)

Parameters % (n). Mean ± SD	CON vs. LC		LC n = 56			
	CON n = 56	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
IGF 1 (ng/ml)	**p < 0.001[a]**		p 0.166[b]		p 0.672[b]	
		n = 54		n = 36		n = 26
	140 ± 45.3	43.1 ± 41.9	60.0 ± 50.3	34.7 ± 34.8	27.2 ± 14.7	37.6 ± 39.8

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), (a) students-t test, (b) mann-whitney-u test. Statistical significance was set at p value < 0.05 (shown in bold face**)**

4.1.10.7 Others: Electrolytes and trace elements

All measured electrolytes and the trace element zinc, were significantly lower in LC compared to CON (Table 20). In F/Non-F and F-00/F-AD electrolytes and zinc values were comparable.

Table 20: Electrolytes and trace elements

Parameters % (n). Mean ± SD	CON vs. LC		LC n = 56			
	CON n = 56	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Sodium (mmol/l) 133 - 146	**p < 0.001[a]**		p 0.436[b]		p 0.395[b]	
	143 ± 1.99	135 ± 5.14	135 ± 4.00	135 ± 5.63	137 ± 2.98	135 ± 6.30
Calcium (mmol/l) 2.15 – 2.50	**p < 0.001[a]**		p 0.135[a]		p 0.614[a]	
	2.39 ± 0.11	2.18 ± 0.17	2.23 ± 0.15	2.15 ± 0.18	2.13 ± 0.20	2.16 ± 0.17
Magnesium (mmol/l) 0.7 – 1.1	**p < 0.001[a]**		p 0.485[a]		p 0.559[a]	
	0.84 ± 0.05	0.77 ± 0.13	0.78 ± 0.11	0.76 ± 0.14	0.74 ± 0.13	0.75 ± 0.14
Potassium (mmol/l) 3.5-5.1	**p < 0.001[a]**		p 0.384[a]		p 0.285[a]	
	4.39 ± 0.34	3.81 ± 0.50	3.88 ± 0.30	3.78 ± 0.57	3.61 ± 0.63	3.84 ± 0.54
Zinc (µmol/l) 9.18 – 18.3	**p < 0.001[a]**		p 0.064[b]		p 0.426[b]	
	12.6 ± 2.00	7.64 ± 4.06	8.63 ± 3.53	7.17 ± 4.25	6.47 ± 3.69	7.43 ± 4.47

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), , (a) students-t test, (b) mann-whitney-u test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.11 Malnutrition and sarcopenia

The diagnosis of malnutrition and sarcopenia are summarized in Table 21 and Table 22. None of the CON were malnourished or sarcopenic.

Malnutrition was present in 60 % of LC. However, further investigations of the single components of the malnutrition diagnosis revealed significant differences between LC and CON. Specifically, the phenotypic criteria, weight loss as well as reduced fat-free mass muscle index and etiological criteria; food intake reduction and inflammation were more prevalent in LC. Of note, 2/3 of LC showed a reduction in food intake while all CON had regular food intake. The inflammation criteria scored positive in 77 % of LC compared to only one subject in CON. In terms of malnutrition diagnosis, F/Non-F and F-00/F-AD were comparable except for a 2.5 higher prevalence of reduced food intake or malassimilation in F-LC compared to Non-F.

Table 21: Malnutrition diagnosis (GLIM)

Parameters LC n = 56 % (n)		CON vs. LC		LC n = 56			
		CON n = 56	LC n = 55*	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Malnutrition STATUS		**p < 0.001[b]**		p 1.000[a]		p 0.708[a]	
			n = 55		n = 37		n = 27
	malnourished	0 % (0)	60 % (33)	61 % (11)	59 % (22)	50 % (5)	63 % (17)
	not malnourished	100 % (56)	40 % (22)	39 % (7)	41 % (15)	50 % (5)	37 % (10)
GLIM Phenotypic Criteria	Weight loss > 5% 6 Mo OR > 10% beyond 6 Mo	**p < 0.001[b]**		p 0.573[b]		p 0.713[b]	
		0 % (0)	61 % (34)	67 % (12)	58 % (22)	50 % (5)	61 % (17)
	BMI < 20% (<70) OR < 22 (>70)	p 0.364[b]		p 1.000[b]		p 0.164[b]	
		2 % (1)	7 % (4)	6 % (1)	8 % (3)	20 % (2)	4 % (1)
	FFMI < 15 (♀) OR < 17 (♂)	**p 0.013[b]**		p 0.499[a]		p 0.360[b]	
			n = 55		n = 37		n = 27
		5 % (3)	21 % (12)	28 % (5)	19 % (7)	30 % (3)	15 % (4)
GLIM Etiologic Criteria	Food intake < 50% ER > 1Wk. **OR** Food intake reduction > 2wk.	**p < 0.001[b]**		**p < 0.001[b]**		p 0.351[b]	
		0 % (0)	66 % (37)	33 % (6)	82 % (31)	70 % (7)	86 % (24)
	Chronic GI condition that adversely impacts food assimilation or absorption	p 0.243[b]		p 0.544[b]		p 1.000[b]	
		0 % (0)	5 % (3)	0 % (0)	8 % (3)	10 % (1)	7 % (2)
	Inflammation CRP (≥ 5 mg/l)	**p < 0.001[b]**		p 1.000[b]		p 1.000[b]	
		2 % (1)	77 % (43)	78 % (14)	76 % (29)	80 % (8)	75 % (21)

LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), GLIM: global leadership initiative on malnutrition, * 1 missing – declined BIA/ anthropometric examinations, (a) chi-2 test, (b) exact-fisher test. Statistical significance was set at p value < 0.05 (shown in bold face).

Sarcopenia was prevalent in 23 % of LC patients (Table 22). In terms of sarcopenia diagnosis, LC were significantly more likely to have reduced grip strength, decreased SMI and slower gait speed compared to CON. The prevalence for sarcopenia was comparable for F/Non-F and F-00/F-AD According to each European working group on sarcopenia in older people (EWGSOP2) criteria, overall fatigue was associated with reduced gait speed, but not with reduced grip strength or skeletal muscle index. None of the Non-F and 42 % of the fatigued LC had a reduced gait speed.

Table 22: Sarcopenia diagnosis (EWGSOP2)

Parameters LC n 56 % (n)		CON vs. LC		LC n = 56			
		CON n = 56	LC n = 56 n = 55	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Sarcopenia STATUS		p < 0.001[b]		p 0.514[b]		p 1.000[b]	
	sarcopenic	0 % (0)	23 % (13)	17 % (3)	26 % (10)	30 % (3)	25 % (7)
	non-sarcopenic	100 % (56)	77 % (43)	83 % (15)	74 % (28)	70 % (7)	75 % (21)
EWGSOP2 Criteria	↓Handgrip strength (kg) (<27 ♀<16♂)	**p < 0.001[b]**		p 0.514[b]		p 1.000[b]	
		0 % (0)	23 % (13)	17 % (3)	26 % (10)	30 % (3)	25 % (7)
	↓Skeletal Muscle Index (kg/m²)	**p < 0.001[b]**		p 0.249[a]		p 0.481[b]	
		5 % (3)	n = 55 53 % (29)	39 % (7)	n = 37 60 % (22)	70 % (7)	n = 27 56 % (15)
	↓Gait speed (m/s) (≤ 0.8)	**p < 0.001[b]**		**p 0.002[b]**		p 0.694[b]	
		0 % (0)	n = 47 30 % (14)	n = 15 0 % (0)	n = 32 44 % (14)	n = 9 33 % (3)	n = 23 48 % (11)

LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms), (a) chi-2 test, (b) exact-fisher test. Statistical significance was set at p value < 0.05 (shown in bold face).

Given that malnutrition and sarcopenia in chronic diseases often coexists [169], we further analysed the relative risk of malnutrition in the presence of sarcopenia as well as risk of malnutrition and sarcopenia in the presence of fatigue/psychosocial factors (Table 23 and Table 24). In LC patients only, sarcopenia did not increase the risk of malnutrition diagnosis whereas in the total sample sarcopenia increased the risk of malnutrition by 3.3 times.

Table 23: Risk of malnutrition in the presence of sarcopenia

Parameter (n)	Total n = 112		LC n = 56		CON n = 56	
	Malnutrition +	Malnutrition -	Malnutrition +	Malnutrition -	Malnutrition +	Malnutrition -
Sarcopenia +	10	3	10	3	0	0
Sarcopenia -	23	75	23	19	0	56
RR [95% CI]	3.28 [2.06-5.22]		1.41 [0.94-2.11]		-	

LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, GLIM: global leadership initiative for malnutrition, EWGSOP2: European working group on sarcopenia in older people 2019, RR: risk ratio, CI: 95 % confidence interval.

The presence of fatigue symptoms did not affect the risk of malnutrition or sarcopenia in LC patients (Table 24).

Table 24: Risk of malnutrition/sarcopenia in the presence of fatigue/psychosocial factors

Parameter (n)	Malnutrition GLIM		Sarcopenia EWGSOP2	
Fatigue/ Non-fatigue	Malnutrition +	Malnutrition -	Sarcopenia +	Sarcopenia -
Fatigue	22	15	10	28
Non-Fatigue	11	7	3	15
RR [95% CI]	0.97 [0.62-1.53]		1.58 [0.49-5.05]	
Fatigue without/with psychosocial factors	Malnutrition +	Malnutrition -	Sarcopenia +	Sarcopenia -
Fatigue + (FAD)	17	10	7	21
Fatigue only (F00)	5	5	3	7
RR [95% CI]	1.26 [0.64-2.50]		0.83 [0.27-2.62]	

LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, GLIM: global leadership initiative for malnutrition, EWGSOP2: European working group on sarcopenia in older people 2019, RR: risk ratio, CI: 95 % confidence interval.

4.1.12 Physical function and muscle strength

Physical activity based on the international physical activity questionnaire (IPAQ) was significantly lower in LC compared to CON, except for similar walking activity (see Table 25). Almost 70 % of the LC's had low physical activity compared to 20 % CON. By contrast, 80% of LC patients who were fatigued exhibited a markedly lower physical activity level compared to just about 50% of their non-fatigued counterparts. F-LC also had a significantly lower total and walking METs compared to Non-F. Physical activity in F-00 was comparable to F-AD.

Gait speed and handgrip strength was significantly lower in LC compared to CON. F-LC compared to Non-F had a significantly compromised gait speed and comparable handgrip strength. Gait speed and handgrip strength was comparable in F-00 and F-AD.

Table 25: Physical function and muscle strength

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON	LC	Non-F	F	F-00	F-AD
	n = 56	n = 56	n = 18	n = 38	n = 10	n = 28
IPAQ categories	**p < 0.001[a]**		**p 0.021[b]**		p 0.145[b]	
		n = 55	n = 17			
low	20 % (11)	69 % (38)	47 % (8)	79 % (30)	60 % (6)	86 % (24)
moderate	39 % (22)	18 % (10)	24 % (4)	16 % (6)	30 % (3)	11 % (3)
high	41 % (23)	13 % (7)	29 % (5)	5 % (2)	10 % (1)	4 % (1)
Total METs/week	**p 0.002[c]**		**p 0.005[d]**		p 0.484[d]	
		n = 55	n = 17			
	2450 ± 2044	1148 ± 2250	2336 ± 3346	616 ± 1267	994 ± 1553	481 ± 1151
Vigorous METs/week	**p 0.002[c]**		p 0.890[d]		p 0.416[d]	
		n = 55	n = 17			
	1021 ± 1722	157 ± 978	424 ± 1746	37.9 ± 172	96.0 ± 304	17.1 ± 90.7

Moderate METs/week	p 0.076[c]		p 0.107[d]		p 0.295[d]	
		n = 55	n = 17			
	640 ± 778	348 ± 931	607 ± 1310	233 ± 692	520 ± 1073	130 ± 481
Walking METs/week	p 0.437[c]		**p 0.001[d]**		p 0.631[d]	
		n = 55	n = 17			
	789 ± 926	642 ± 1057	1306 ± 1410	345 ± 692	378 ± 641	334 ± 721
Gait speed m/s	**p < 0.001[c]**		**p < 0.001[c]**		p 0.276[d]	
		n = 47	n = 15	n = 32	n = 9	n = 23
	1.37 ± 0.26	1.08 ± 0.49	1.48 ± 0.54	0.90 ± 0.34	1.07 ± 0.48	0.83 ± 0.26
Handgrip strength kg	**p < 0.001[c]**		p 0.161[c]		p 0.727[c]	
	40.9 ± 13.6	28.8 ± 10.1	31.6 ± 11.7	27.5 ± 9.08	28.4 ± 9.75	27.2 ± 8.99

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms). Physical activity was analysed using IPAQ: international physical activity questionnaire, METs: metabolic equivalent of task, (a) chi-2 test, (b) exact-fisher test, (c) students-t test, (d) mann-whitney-u test. Cumulative percentages may deviate from 100% due to rounding of decimal places. Statistical significance was set at p Value < 0,05 (shown in bold face).

4.1.13 Nutrient intake and alcohol consumption

Nutrient intake and alcohol consumption are presented in Table 26. Total calories were comparable between all groups (LC/CON, F/Non-F and F-00/F-AD). Macronutrient intake (protein, carbohydrates, total fats) were comparable between LC/CON and F/Non-F apart from lower dietary fibre intake in LC and F, respectively. The macronutrient intake was comparable between F-00 and F-AD. Mean alcohol intake in LC was insignificantly higher compared to CON. Nevertheless, detailed analysis of alcohol consumption revealed that only 34 % LC consumed alcohol compared to 89 % CON. Almost half of LC were ex-drinkers compared with only 7 % of CON. There was no difference in alcohol consumption between F/Non-F and F-00/F-AD.

Concerning micronutrients, intakes of magnesium, iron, zinc, vitamin E, vitamin B6 and folic acid were significantly lower in LC compared with CON, while intakes of sodium, calcium, vitamin A, vitamin B1, vitamin B2 and vitamin B12 were similar. F-LC presented with significantly lower vitamin E and trendigly lower vitamin B1 compared to Non-F. All other micronutrients were insignificantly lower except for sodium and calcium which were insignificantly higher in F versus Non-F. Sodium and calcium intake was significantly lower in F-00 compared to F-AD. All other differences between the fatigued LC groups were not statistically significant.

Table 26: Nutrient intake and alcohol consumption

Parameters	CON vs. LC		LC n = 56			
% (n). Mean ± SD	CON n = 56	LC n = 56	Non-F n = 18	F n = 38	F-00 n = 10	F-AD n = 28
Total calories (kcal)	p 0.788[b]		p 0.752[c]		p 0.407[c]	
	2099 ± 755	2151 ± 1237	2011 ± 1029	2217 ± 1331	1746 ± 798	2386± 1451
Macronutrients						
Protein (g)	p 0.100[b]		p 0.380[c]		p 0.667[c]	
	83,1 ± 28,4	72,3± 39,3	76.2 ± 32.4	70,4± 42,5	60.1 ± 28.7	74,1 ± 46,3
Total fats (g)	p 0.9950[b]		p 0.726[c]		p 0.573[c]	
	73,5± 26,3	73.5 ± 51.2	71.8 ± 38.0	74.3 ± 56,8	57.6 ± 29.6	80,2± 63,2
Carbohydrates (g)	p 0.718[b]		p 0.623[c]		p 0.446[c]	
	230 ± 108	240± 171	228 ± 121	245 ± 191	203 ± 146	260 ± 205
Dietary fiber (g)	**p < 0.001[b]**		**p 0.014[b]**		p 0.782[b]	
	26,9± 26.4	19.2 ± 10,1	24,0± 10,4	16,9± 9,34	16.2 ± 10.5	17,2± 9,07
Alcohol						
Alcohol DEGS (g)	p 0.156[b]		p 0.125[c]		p 0.900[c]	
	9.69 ± 9.94	21.2 ± 59.0	6.86 ± 20.4	28.0 ± 69.5	12.5 ± 24.3	33.5 ± 79.4
Alcohol consumption	**p < 0.001[a]**		p 0.182[a]		p 0.836[a]	
regular	7 % (4)	21 % (12)	17 % (3)	24 % (9)	20 % (2)	25 % (7)
occasionally	82 % (46)	13 % (7)	22 % (4)	8 % (3)	10 % (1)	7 % (2)
never	4 % (2)	18 % (10)	28 % (5)	13 % (5)	20 % (2)	11 % (3)
not anymore	7 % (4)	48 % (27)	33 % (6)	55 % (21)	50 % (5)	57 % (16)
Minerals and Trace Elements						
Sodium (mg)	p 0.274[b]		p 0.599[c]		p 0.091[c]	
	2263 ± 779	2055 ± 1181	2051 ± 802	2057 ± 1333	1452 ± 650	2273 ±1453
Calcium (mg)	p 0.534[b]		p 0.861[c]		p 0.921[c]	
	1122 ± 480	1055 ± 644	1041 ± 610	1062 ± 668	933 ± 419	1108 ± 738
Magnesium (mg)	**p 0.001[b]**		p 0.713[b]		**p 0.030[b]**	
	449 ± 180	352 ± 127	361 ± 114	347 ± 134	270 ± 107	375± 133
Iron (mg)	**p 0.002[b]**		p 0.902[c]		**p 0.033[b]**	
	16,3± 6,49	12,8± 5,08	13,3 ± 5,14	12,6± 5,10	9.66 ± 4.21	13,6± 5,05
Zinc (mg)	**p 0.023[b]**		p 0.371[c]		p 0.921[c]	
	14,0± 4,73	11,7± 5,79	12,8± 5,91	11,1± 5.91	10.2 ± 4.68	11.5 ± 6,11
Vitamins						
Vitamin A (µg)	p 0.786[b]		p 0.806[c]		p 0.486[c]	
	2.13 ± 2.28	2.26 ± 2.61	2.37 ± 2.96	2.21 ± 2.47	2.16 ± 2.95	2.22 ± 2.34
Vitamin E (mg)	**p 0.015[b]**		**p 0.030[b]**		p 0.629[b]	
	10,9± 4,76	8,79± 4,32	10,6 ± 4,86	7,93± 3,82	7.42 ± 2.92	8,11 ± 4,13
Vitamin B1 (mg)	p 0.274[b]		p 0.099[c]		p 0.185[c]	
	1,69± 0.79	1.52 ± 0.88	1,64± 0.697	1,46± 0.97	1.04 ± 0.42	1,61± 1,07
Vitamin B2 (mg)	p 0.582[b]		p 0.739[c]		p 0.691[c]	
	1,95 ± 0.69	1.85 ± 1.09	1.83 ± 0.87	1.86 ± 1.19	1.64 ± 0.91	1,94 ± 1,28
Vitamin B6 (mg)	**p 0.018[b]**		p 0.103[c]		p 0.974[c]	
	1,98± 0.77	1.63 ± 0.79	1.85 ± 0.79	1.52 ± 0.78	1.40 ± 0.60	1.57 ± 0.84
Vitamin B12 (µg)	p 0.640[b]		p 0.472[c]		p 0.619[c]	
	7.41 ± 3.49	7.05 ± 4.64	7.23 ± 4.04	6.96 ± 4.95	6.52 ± 4.74	7.12 ± 5.10

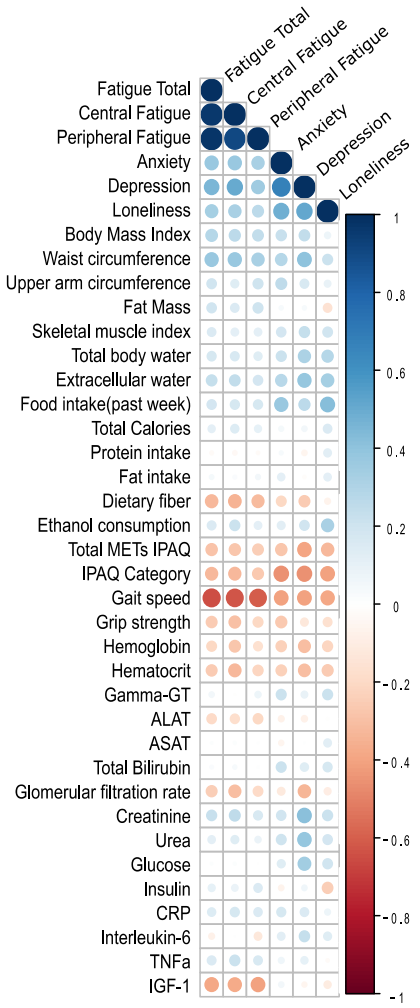
Folic acid (µg)	**p 0.049[b]**		p 0.309[b]		p 0.487[b]	
	284 ± 140	237 ± 111	259 ± 116	226 ± 108	205 ± 89.6	234 ± 115

SD: standard deviation, LC: liver cirrhosis, CON: healthy controls, F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms).Food intake measured using the DEGS FFQ: German health examination survey food frequency questionnaire, (a) exact-fisher test, (b) students-t test, (c) mann-whitney-u test. Cumulative percentages may deviate from 100% due to rounding of decimal places. Statistical significance was set at p value < 0.05 (shown in bold face).

4.1.14 Correlations of fatigue and psychosocial symptoms in LC patients

Based on the aforementioned results, we carried out partial correlation analysis between fatigue, features of fatigue (central and peripheral), anxiety/depression symptoms and loneliness with specific parameters of; anthropometrics, body composition, nutrient/alcohol intake, physical function/strength and laboratory markers as shown in Figue 12. Significant positive correlations were found between fatigue, features of fatigue, anxiety/depression symptoms, loneliness and waist circumference. Interestingly, central and peripheral fatigue manifested similar correlations as in overall fatigue.

Gait speed and physical activity were negatively correlated to all psychosocial conditions, while biomarkers of anemia risk showed only weak trending negative correlations. Handgrip strength trended to associate with fatigue and anxiety, but not with depression and loneliness. Only fatigue correlated with IGF-1 and dietary fiber intake while only loneliness correlated with ethanol intake and only depression with biomarkers of kidney function (glomerular filtration rate, creatinine, urea) and diabetes risk (fasting glucose).



	Fatigue		Anxiety		Depression		Loneliness	
	R_p	p Value	R_p	p Value	R_p	p Value	R_p	p Value
Fatigue			0.377	0.004	0.451	<0.001	0.331	0.013
Anxiety	0.377	0.004			0.675	<0.001	0.487	<0.001
Depression	0.451	<0.001	0.675	<0.001			0.515	<0.001
Loneliness	0.331	0.013	0.487	<0.001	0.515	<0.001		
Body Mass Index	0.289	0.031	0.227	0.092	0.241	0.074	0.080	0.560
Waist circumference	0.377	0.004	0.284	0.034	0.404	0.002	0.211	0.119
Upper arm circumference	0.196	0.148	0.255	0.058	0.162	0.233	0.092	0.501
Fat mass	0.196	0.148	0.035	0.798	0.034	0.802	-0.154	0.258
Skeletal muscle index	0.151	0.267	0.186	0.170	0.233	0.083	0.199	0.140
Total body water	0.175	0.198	0.214	0.114	0.318	0.017	0.281	0.036
Extracellular water	0.238	0.077	0.274	0.041	0.381	0.004	0.336	0.011
Food intake (past week)	0.183	0.177	0.377	0.004	0.266	0.048	0.425	0.001
Total calories	0.111	0.416	0.049	0.722	0.057	0.677	0.154	0.256
Protein intake	-0.028	0.836	0.031	0.822	-0.057	0.679	0.126	0.354
Fat intake	0.041	0.765	0.111	0.417	-0.019	0.888	0.115	0.397
Dietary fiber	-0.329	0.013	-0.207	0.126	-0.252	0.062	-0.070	0.609
Ethanol	0.159	0.243	0.129	0.345	0.192	0.155	0.321	0.016
Total METs IPAQ	-0.283	0.035	-0.279	0.038	-0.398	0.002	-0.323	0.015
IPAQ Category	-0.321	0.016	-0.458	<0.001	-0.454	<0.001	-0.407	0.002
Gait speed	-0.643	<0.001	-0.402	0.002	-0.402	0.002	-0.387	0.003
Grip strength	-0.252	0.061	-0.264	0.049	-0.128	0.348	-0.164	0.227
Hamoglobin	-0.200	0.139	-0.237	0.079	-0.306	0.022	-0.218	0.106
Hematocrit	-0.252	0.061	-0.237	0.078	-0.310	0.020	-0.259	0.053
Gamma-glutamyl transferase	0.058	0.673	0.213	0.115	0.094	0.491	0.211	0.118
Alanine aminotransferase	-0.194	0.153	-0.072	0.597	-0.079	0.563	-0.009	0.945
Aspartate aminotransferase	0.010	0.941	-0.056	0.682	0.002	0.988	0.127	0.350
Total Bilirubin	0.026	0.848	0.211	0.119	0.139	0.307	0.175	0.196
Glomerular filtration rate	-0.248	0.066	-0.118	0.385	-0.334	0.012	-0.098	0.471
Creatinine	0.221	0.101	0.191	0.159	0.416	0.001	0.213	0.115
Urea	0.119	0.383	0.206	0.128	0.389	0.003	0.190	0.161
Glucose	0.012	0.930	0.133	0.330	0.345	0.009	0.198	0.143
Insulin	0.104	0.446	-0.062	0.650	0.061	0.657	-0.243	0.072
C-reactive protein	0.144	0.289	0.172	0.206	0.155	0.254	0.071	0.604
Interleukin 6	-0.073	0.593	0.121	0.374	0.232	0.086	0.132	0.331
Tumour necrosis factor a	0.159	0.242	0.070	0.609	0.108	0.428	-0.023	0.868
Insulin-like growth factor 1	-0.389	0.003	0.059	0.665	-0.051	0.709	-0.109	0.423

Figure 12: Correlation analysis for fatigue and psychosocial symptoms

Partial correlation analysis between fatigue, features of fatigue (central/peripheral) and; psychosocial factors (anxiety/depression symptoms), loneliness, anthropometry, body composition, food/alcohol intake, physical function/strength and laboratory markers (n = 56). The colour gradients (blue/red) indicate positive and negative correlations respectively while intensity and circle size represent the value of Pearson correlation coefficients. IPAQ: international physical activity questionnaire, METs: metabolic equivalent of task.

4.1.15 Multiple linear regression analysis

Next, we carried out multiple regression analysis to assess the relationship between fatigue in LC patients and its predictor variables. Based on previous results, we identified loneliness status, waist circumference (as indication of ascites), handgrip strength, gait speed, anemia status, IGF-1, inflammation cluster, intake of proton-pump inhibitors, walking METs and dietary fiber as viable parameters for the multiple regression analysis. Anxiety and depression symptoms were in the analysis beforehand excluded due to multicollinearity.

In a backward stepwise regression, the least important variable were rejected including loneliness status, handgrip strength (HGS), anemia status, inflammation cluster, intake of proton-pump inhibitors (PPI), walking METs and dietary fiber as shown in Table 27. The detailed single model summaries 1-8 and stepwise elimination of the least important variables are shown in Appendix A1. Waist circumference, gait speed and IGF-1 were identified as independent predictors of fatigue in this cohort of LC patients explaining 58% of the variation (95 % CI 1.91, 6.80, F = 23.5, p = 0.00).

Table 27: Backward stepwise regression results for dependent variable fatigue

Variables	Models							
	1	2	3	4	5	6	7	8
Loneliness status	✓	✓	✓	✓	× Lonely	× Lonely	× Lonely	× Lonely
Waist circumference	✓	✓	✓	✓	✓	✓	✓	✓
Handgrip strength	✓	✓	✓	✓	✓	× HGS	× HGS	× HGS
Gait speed	✓	✓	✓	✓	✓	✓	✓	✓
Anemia status	✓	✓	✓	✓	✓	✓	× Anemia	× Anemia
IGF-1	✓	✓	✓	✓	✓	✓	✓	✓
Inflammation cluster	✓	✓	✓	× INF	× INF	× INF	× INF	× INF
PPI	✓	✓	× PPI	× PPI	× PPI	× PPI	× PPI	× PPI
Walking METs	✓	× METs	× METs	× METs	× METs	× METs	× METs	× METs
Dietary fiber	✓	✓	✓	✓	✓	✓	✓	× Fiber
	Dependent variable – Fatigue Model 8							
Predictor		*β* unstandardized		*p*	95% CI		*R* square	Adjusted *R* square
Waist circumference		0.031		**0.004**	0.010	0.052	0.58	0.55
Gait speed		-2.30		**<0.001**	-3.02	-1.59		
IGF-1		-0.010		**0.016**	-0.019	-0.002		

4.2 Intervention Study

The aim of this post-hoc analysis in the interventions study was to evaluate the course of fatigue symptoms, psychosocial factors and fatigue indicators along an intensified intersectoral nutritional therapy over 6 months in a small group of malnourished cirrhotic patients.

4.2.1 Study Workflow

The recruitment workflow for the analysis of malnourished, fatigued LC patients in the intervention study is shown in Figure 13. In total,6 LC patients could be included in the analysis.

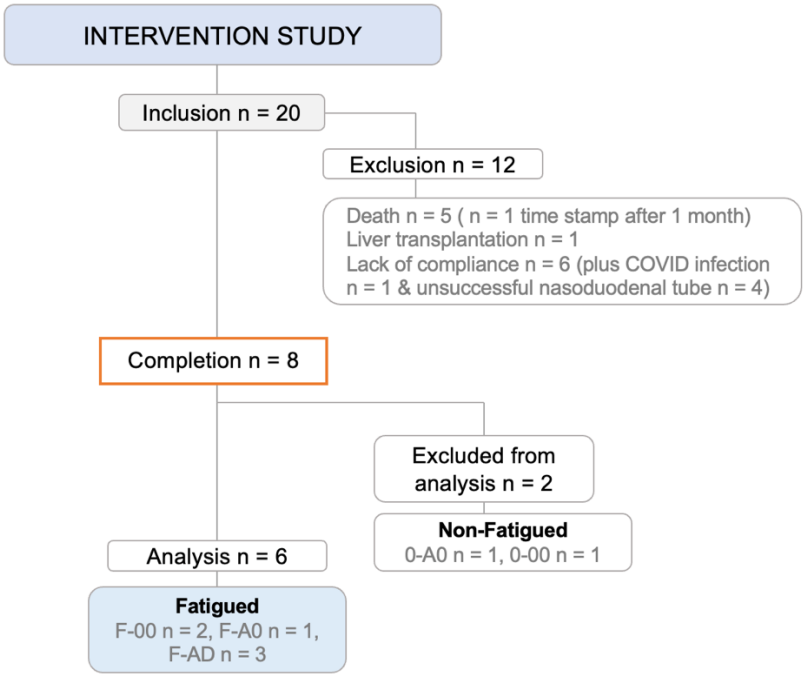


Figure 13: Flow diagram for the recruitment in the intervention study

F: fatigue, Non-F: non-fatigue, F-00: only fatigued/without psychosocial factors (anxiety/depression symptoms), F-AD: fatigued with psychosocial factors (anxiety/depression symptoms).

4.2.2 General characteristics

The majority of patients were female, middle-aged nonsmokers with an average BMI of 24.8 kg/m^2 and advanced liver cirrhosis (Child-Pugh C) (Table 28). The average length of hospital stay was 29 days. Most patients had a shorter duration of education, were

unemployment or retired, and abstinent. The primary etiology was excessive alcohol consumption followed by non-alcoholic steatohepatitis in 1 patient.

Table 28: General characteristics (only LC)

Parameters	% (n), Mean ± Standard deviation n = 6
Sex male	17 % (1)
female	83 % (5)
Age (years)	54 ± 5.6
Body mass index (kg/m^2)	24.8 ± 2.8
Education DURATION	
≤ 10 years	83 % (5)
> 10 years	17 % (1)
Employment	
Employed	33 % (2)
Unemployed OR Retired	67 % (4)
Nicotine CONSUMPTION	
Smoker	33 % (2)
Non-Smoker	67 % (4)
Ex-Smoker	0 % (0)
Alcohol CONSUMPTION	
regular to occasionally	33 % (2)
not anymore	67 % (4)
Disease etiology	
Ethyltoxic	83 % (5)
Non-alcoholic steatohepatitis	17 % (1)
Child Pugh Score	
Child-Pugh A	17 % (1)
Child-Pugh B	17 % (1)
Child-Pugh C	67 % (4)
Length of hospital stay (days)	29.0 ± 13.2

4.2.3 Primary Analysis

4.2.3.1 Trajectory of fatigue, anxiety and depression symptoms

Trajectory of fatigue, anxiety and depression symptoms from institutional care, through the 3-month supportive ambulant nutrion therapy (SaNT) program to follow-up (6 months after discharge) are shown in Table 29 and Figure 14. Significant improvements were seen over time in fatigue, anxiety and depression scores. The proportion of patients with improving fatigue and anxiety i.e., non-fatigued or anxious during the final follow-up was 83 % each. None of the patients was depressed during the final follow-up however, only 1 patient was still fatigued or mildly anxious at the final follow-up.

Table 29: Trajectories of fatigue severity, anxiety and depression scores

Parameters % (n), Mean ± SD	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-up	
FSS Score	5.67 ± 0.89	4.38 ± 1.86	4.00 ± 1.82	3.08 ± 1.68	2.65 ± 1.25	**0,006**[a]
Fatigue STATUS						
fatigue	100 % (6)	50 % (3)	33 % (2)	17 % (1)	17 % (1)	**0,006**[b]
non-fatigue	0 % (0)	50 % (3)	67 % (4)	83 % (5)	83 % (5)	
Anxiety Score	10.5 ± 5.47	5.17 ± 3.06	5.00 ± 3.29	3.67 ± 2.25	3.33 ± 2.34	**0,020**[a]
Anxiety STATUS						
mod.-sev.(≥11)	67 % (4)	0 % (0)	0 % (0)	0 % (0)	0 % (0)	**0,019**[a]
mild (8-10)	0 % (0)	33 % (2)	17 % (1)	0 % (0)	17 % (1)	
no anxiety (0-7)	33 % (2)	67 % (4)	83 % (5)	100 % (6)	83 % (5)	
Depression Score	8.50 ± 3.73	4.67 ± 3.88	3.83 ± 4.17	1.50 ± 1.38	1.17 ± 1.47	**0,001**[a]
Depression STATUS						
mod.-sev.(≥11)	33 % (2)	17 % (1)	0 % (0)	0 % (0)	0 % (0)	0,072[a]
mild (8-10)	17 % (1)	17 % (1)	17 % (1)	0 % (0)	0 % (0)	
no depr. (0-7)	50 % (3)	67 % (4)	83 % (5)	100 % (6)	100 % (6)	

SD: standard deviation, SaNT:supportive ambulant nutrion therapy, Mod. – sev.: moderate to severe anxiety/depression symptom (a) Friedman-test, (b) cochran-q-test. Statistical significance was set at p value < 0.05 (shown in bold face).

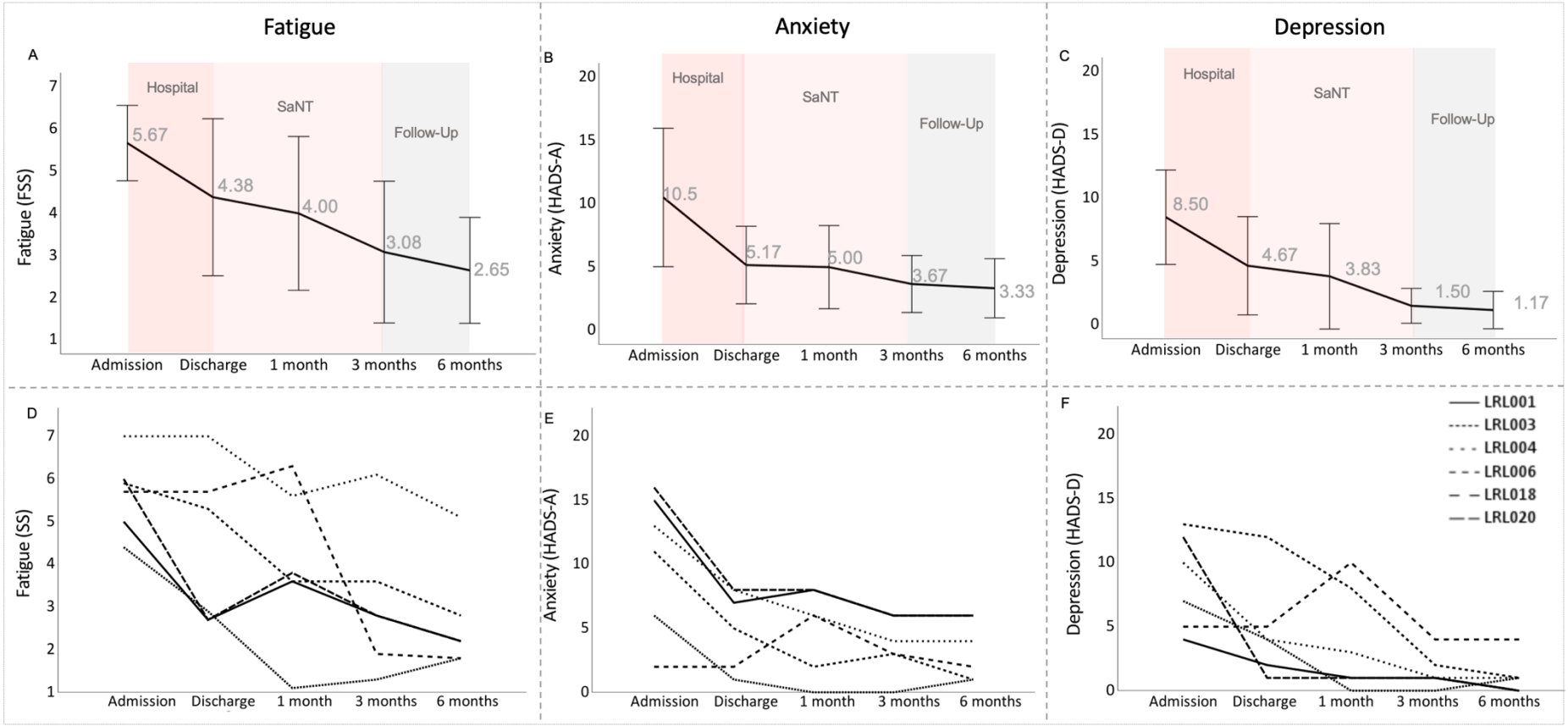


Figure 14: Trajectories of fatigue severity score, anxiety and depression symptoms

A) Average fatigue score of all subjects, (B) Average fatigue score based on patients, (C) Average anxiety score of all subjects, (D) Average depression score of all subjects, (E) Average anxiety score based on patients and (F) Average depression score based on patients from admission to 6 months after discharge, SaNT: supportive ambulant nutrion therapy,

4.2.3.2 Complications of cirrhosis (edema/ ascites) and Anthropometrics

Edema/ascites and corresponding anthropometry are summarized in Table 30. As expected, 5/6 patients had both edema and ascites at baseline while all patients had at least one of the two conditions. Over time, ascites, waist circumference and waist hip ratio (WHR) improved significantly.

Table 30: Trajectory of the complications of cirrhosis (edema and ascites) and Anthropometrics

Parameters % (n), Mean ± SD	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-up	
Edema	83 % (5)	83 % (5)	50 % (3)	33 % (2)	17 % (1)	0,074[b]
Ascites	83 % (5)	100 % (6)	83 % (1)	17 % (1)	0 % (0)	**0,002**[b]
Edema OR Ascites	100 % (6)	100 % (6)	100 % (6)	33 % (2)	17 % (1)	**0,002**[b]
Waist circumference (cm)	96.1 ± 9.07	97.6 ± 6.67	90.0 ± 5.42	86.1 ± 7.03	87.0 ± 6.46	**0,009**[a]
Waist Hip Ratio (WHR)	1.01 ± 0.07	1.01 ± 0.08	0.94 ± 0.08	0.90 ± 0.05	0.90 ± 0.04	**0,007**[a]

SD: standard deviation, SaNT: supportive ambulant nutrion therapy, (a) Friedman-Test, (b) Cochran-Q-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.2.3.3 Insulin-like growth factor (IGF-1)

Similarly, insulin-like growth factor (IGF-1) significantly improved and normalised over time in all but one LC patient as shown in Table 31 and Figure 15.

Table 31: Trajectory of insulin-like growth factor (IGF-1)

Parameters % (n), Mean ± SD	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-up	
IGF 1 (ng/ml)	26.8 ± 11.8	30.3 ± 10.9	54.2 ± 27.9	67.7 ± 34.7	81.6 ± 39.8	**0,002**[a]

SD: standard deviation, SaNT: supportive ambulant nutrion therapy, (a) Friedman-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

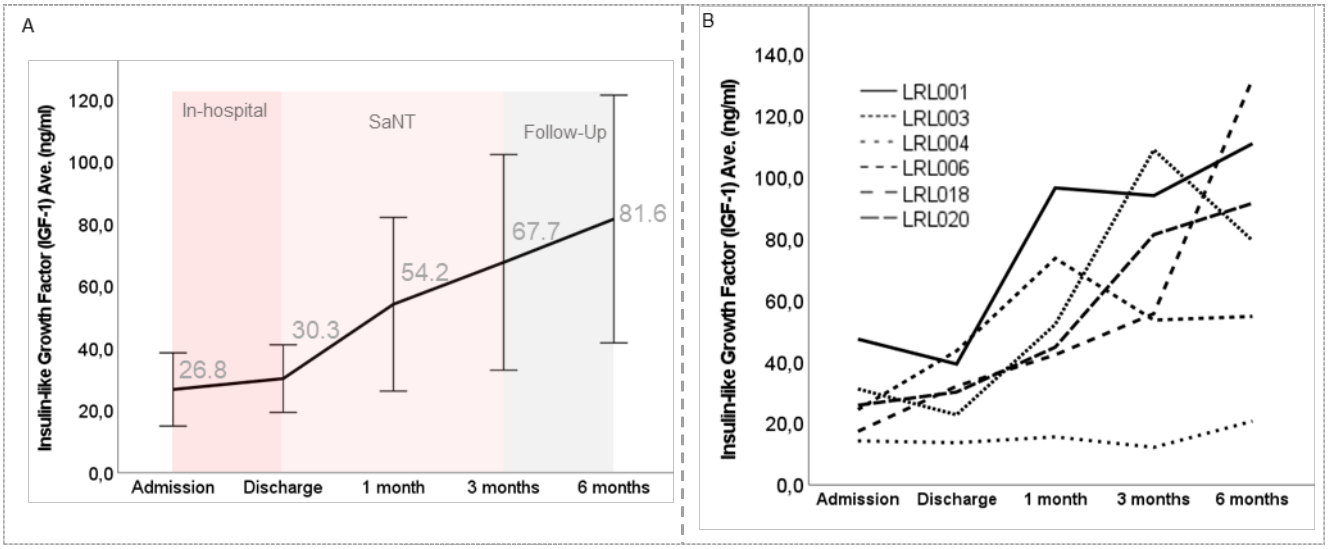


Figure 15: Trajectory of insulin-like growth factor 1 (IGF-1)

(A) Average IGF-1 values of all subjects, (B) Average IGF-1 values based on patients from admission to 6 months after discharge. SaNT: supportive ambulant nutrion therapy.

4.2.3.4 Malnutrition Diagnosis – GLIM

All patients were malnourished at discharge from the hospital (Table 32). One patient had an unclear malnutrition diagnosis at admission due to severe edema and ascites. At the final follow-up, significant improvements were seen in the malnutrition status with only one patient still being malnourished. Improvements were mainly due to significant positive changes in etiological criteria of malnutrition, i.e., reduced food intake and inflammation.

Table 32: Trajectory of malnutrition diagnosis (GLIM)

Parameters % (n), Mean ± SD	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-up	
Malnutrition STATUS						
malnourished	83 % (5)	100 % (6)	100 % (6)	50 % (3)	17 % (1)	**0,009**[a]
not malnourished	0 % (0)	0 % (0)	0 % (0)	50 % (3)	83 % (5)	
unclear	17 % (1)	0 % (0)	0 % (0)	0 % (0)	0 % (0)	
Weight loss > 5% 6 Mo OR > 10% beyond 6 Mo	83 % (5)	100 % (6)	100 % (6)	100 % (6)	83 % (5)	0,558[a]
BMI < 20% (<70) OR < 22 (>70)	0 % (0)	0 % (0)	33 % (2)	17 % (1)	0 % (0)	0,255[a]
FFMI < 15 (♀) OR < 17 (♂)	17 % (1)	33 % (2)	83 % (5)	67 % (4)	33 % (2)	0,103[a]
Food intake < 50% ER > 1Wk. OR Food intake reduction > 2wk.	100 % (6)	67 % (4)	67 % (4)	17 % (1)	0 % (0)	**0,003**[a]
Chronic GI condition	0 % (0)	0 % (0)	0 % (0)	0 % (0)	0 % (0)	-
Inflammation CRP (≥ 5 mg/l)	100 % (6)	100 % (6)	100 % (6)	50 % (3)	17 % (1)	**0,003**[a]

GLIM Phenotypic criteria, GLIM Etiological criteria, GLIM: global leadership initiative on malnutrition, SD: standard deviation, SaNT: supportive ambulant nutrion therapy, (a) Cochran-Q-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.2.3.5 Physical function and strength

At baseline, all patients had a low physical activity level with an average total METs of 99.0 (Table 33). The walking activity increased significantly over time leading to a significant increment in total METs and all but one LC patient had a moderate to high physical activity level at the final follow-up.

Table 33: Physical activity trajectory (IPAQ)

Parameters % (n), Mean ± SD	Admission n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
		SaNT		Follow-up	
IPAQ categories					
low	100 % (6)	50 % (3)	50 % (3)	17 % (1)	
moderate	0 % (0)	50 % (3)	33 % (2)	33 % (2)	**0,006**[a]
high	0 % (0)	0 % (0)	17 % (1)	50 % (3)	
Total METs/week	99.0 ± 166	1085 ± 1092	1324 ± 1430	2672 ± 1044	**0,005**[a]
Vigorous METs/week	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	413 ± 771	0,112[a]
Moderate METs/week	0.00 ± 0.00	560 ± 784	20.0 ± 49.0	380 ± 627	0,070[a]
Walking METs/week	99.0 ± 166	525 ± 430	1304 ± 1439	1878 ± 1114	**0,003**[a]

SD: standard deviation, SaNT: supportive ambulant nutrion therapy, IPAQ: international physical activity questionnaire, METs: metabolic equivalent of task, (a) Friedman-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

Handgrip strength improved significantly over time (Table 34). Gait speed numerically improved over the follow-up period of 6 months (already between admission and discharge), however, this was not statistically significant.

Table 34: Trajectories of gait speed and handgrip strength

Parameters % (n), Mean ± SD	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-ip	
Gait speed (m/s)	1.03 ± 0.63	1.22 ± 0.41	1.25 ± 0.34	1.33 ± 0.26	1.24 ± 0.36	0,281[a]
Handgrip strength (kg)	22.6 ± 9.04	22.1 ± 6.70	23.5 ± 5.87	27.1 ± 9.43	25.2 ± 8.23	**0,012**[a]

SD: standard deviation, (a) Friedman-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.2.4 Secondary Analysis

4.2.4.1 Inter-sectoral Nutrition Intervention

Inpatient Nutrition Therapy: Details of the in-patient nutrition therapy are shown in Table 35. On average, patients had 12.5 days of nasoduodenal tube feeding with a mean volume of 501 ml enteral nutrition per day (average energy intake: 753 kcal/d, protein intake: 34.8 g/d, respectively). In addition to tube feeding, patients were also allowed to have food.

Table 35: Inpatient nutrition therapy

	Inpatient duration (Admission to after tube feeding) (days)	Tube feed Duration (days)	Average Enteral feeding volume (ml/d)	Protein Intake (g/d)	Energy Intake (kcal/d)
LRL001	32	27	907	50.8	1361
LRL003	43	15	500	37.5	750
LRL004	32	10	750	56.3	1125
LRL006	23	9	406	30.5	609
LRL018	5	0	0	0	0
LRL020	17	14	446	33.5	669
Mean ± SD	25.3 ± 13.3	12.5 ± 8.87	501 ± 313	34.8 ± 19.8	753 ± 470

SD: standard deviation

Compliance/adherence to the Supportive Ambulant Nutritional Therapy (SaNT)–Program: An overview of the SaNT program is shown in Table 36. On average, the patients were readily accessible by telephone with each patient attaining a score of more than 80 % and a 90% study retention day score (maximum duration: 180 days = 90 days SaNT and follow-up after 90 days). While, short-term dietary goals implementation was successful in more than 3/4 of the coaching sessions. On average, the patients also achieved 58 % in punctuality for the in-person control visits at UMR with deviations from the schedule resulting in a reduction in the overall score. This was consequent to unavoidable circumstances for example re-hospitalisation. In the final category 5 ‘oral nutritional supplements target intake’, the patients achieved 71 % of the daily intake. This was set at ≥ 400 kcal and ≥ 20 g protein per day based on existing literature [120, 170]. In summary, feasibility with adherence to the SaNT program was defined a priori with a cumulative score of at least ≥ 70%. This was fulfilled in all fatigued LC patients (mean total score of 81 %) and calculated by summing all 5 categories with equal weightage of all components (20 % each).

Table 36: Overview SaNT (Intervention)

Subject code Mean %	Category 1 Telephone availability	Category 2 Remaining in SaNT	Category 3 Nutritional therapy goals (short-term goals)	Category 4 In-person control visit	Category 5 ONS compliance	Total SaNT score
LRL001	100.0	100.0	-	60.0	98.2	89.6
LRL003	80.0	100.0	-	70.0	96.4	86.6
LRL004	100.0	100.0	-	70.0	58.9	82.2
LRL006	100.0	99.0	-	70.0	0.00	67.3
LRL018	87.5	70.0	71.4	40.0	89.3	71.6
LRL020	92.5	100.0	85.7	40.0	85.7	80.8
Mean ± SD	93.3 ± 8.32	94.8 ± 12.2	78.6 ± 10.1	58.3 ± 14.7	71.4 ± 37.7	79.7 ± 8.63

SD: standard deviation

4.2.4.2 Trajectory of loneliness

The majority of the patients (4/6) were not lonely at baseline, and the proportion remained unchanged until the last follow-up (Table 37). Still, the degree of loneliness improved significantly over time.

Table 37: Trajectory of loneliness

Parameters % (n), Mean ± SD	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-up	
Loneliness Score	2.44 ± 0.57	2.72 ± 0.85	2.00 ± 0.67	1.78 ± 0.33	1.92 ± 0.92	**0,012**[a]
Loneliness STATUS						
Lonely	33 % (2)	33 % (2)	17 % (1)	0 % (0)	17 % (1)	0,478[b]
Not Lonely	67 % (4)	67 % (4)	83 % (5)	100 % (6)	83 % (5)	

SD: standard deviation, SaNT: supportive ambulant nutrion therapy, (a) Friedman-Test, (b) Cochran-Q-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.2.4.3 Additional parameters: Anthropometry and body composition

In line with the improvement of edema and ascites, there was significant decrease in body weight and an alleviation in upper arm circumference. All body composition parameters except resistance value improved significantly over time as shown in Table 38.

Table 38: Additional parameters to anthropometry and body composition

Parameters % (n), Mean ± SD	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-up	
Weight (kg)	69,9 ± 3,94	68,0 ± 4,18	62,4 ± 3,38	62,3 ± 4,83	65,5 ± 7,05	**0.003**[a]
Upper arm circumference (cm)	25.5 ± 4.25	23.4 ± 2.63	22.7 ± 2.77	24.0 ± 2.66	25.6 ± 2.57	**0.006**[a]
Fat Free Mass Index (kg/m^2)	17.2 ± 1.51	17.1 ± 2.60	15.1 ± 0.83	15.0 ± 0.76	16.0 ± 1.76	**0.012**[a]
Skeletal Muscle Mass Index (kg/m^2)	6.25 ± 1.35	5.89 ± 1.34	5.35 ± 1.29	5.93 ± 1.14	6.67 ± 1.11	**0.023**[a]
Total Body Water (TBW)	35.1 ± 4.42	34.1 ± 5.56	30.2 ± 4.20	30.8 ± 3.83	33.1 ± 5.82	**0.003**[a]
Extracellular water (ECW) l	18.3 ± 1.67	18.2 ± 3.15	15.7 ± 1.46	15.1 ± 0.72	15.9 ± 2.46	**0.017**[a]
ECW/TBW (%)	52.6 ± 6.11	53.7 ± 6.14	52.7 ± 6.68	49.4 ± 5.12	48.2 ± 3.29	**0.009**[a]
Resistance (R)	605 ± 101	632 ± 152	676 ± 77.6	677 ± 55.3	641 ± 110	0.308[a]
Reactance (Xc)	35.4 ± 12.7	35.4 ± 18.0	41.0 ± 13.7	47.9 ± 10.3	47.2 ± 14.9	**0.023**[a]
Phase angle °	3.30 ± 0.78	3.04 ± 0.84	3.41 ± 0.79	4.03 ± 0.70	4.14 ± 0.70	**0.005**[a]

SD: standard deviation, SaNT: supportive ambulant nutrion therapy, (a) Friedman-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.2.4.4 Sarcopenia diagnosis trajectory (EWGSOP2)

As compared to malnutrition, sarcopenia was less prevalent at baseline with only 1/3 of the patient being sarcopenic (Table 39). Although no significant improvements were evident, all patients were diagnosed as none sarcopenic a month after discharge. Three patients showed reduced gait speed at hospital admission, which already normalized at discharge from the hospital (> 0.8 m/s).

Table 39: Sarcopenia diagnosis trajectory (EWGSOP2)

Parameters % (n), Mean ± SD	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-up	
Sarcopenia STATUS						0.171[a]
sarcopenic	33 % (2)	17 % (1)	0 % (0)	0 % (0)	0 % (0)	
non-sarcopenic	67 % (4)	83 % (5)	100 % (6)	100 % (6)	100 % (6)	
↓Handgrip strength (kg) (<27 ♀<16♂)	33 % (2)	17 % (1)	0 % (0)	0 % (0)	0 % (0)	0.171[a]
↓Skeletal Muscle Index (kg/m^2)	67 % (4)	67 % (4)	83 % (5)	67 % (4)	50 % (3)	0.504[a]
↓Gait speed (m/s) (≤ 0,8)	50 % (3)	0 % (0)	0 % (0)	0 % (0)	0 % (0)	**0.017**[a]

SD: standard deviation, SaNT: supportive ambulant nutrion therapy, (a) Cochran-Q-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.2.4.5 Additional parameters: Clinical chemistry

At baseline all but one patients were anemic (Table 40) and the proportion decrased to 50% at the last follow up. Inflammation markers and liver markers improved significantly over time. Interestingly, there was significant rise in insulin and total cholesterol over time. All other laboratory markers were not distinguishable over time.

Table 40: Clinical chemistry – trajectories

% (n), Mean ± SD *Anemia*	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
			SaNT		Follow-up	
Hemoglobin (mmol/l) ♀: 7.5 - 9.9 ♂: 8.4 - 10.9	5.83 ± 0.90	5.87 ± 0.98	6.57 ± 1.16	7.25 ± 1.30	7.48 ± 1.02	**< 0.001**[a]
Anemic *Non anemic*	83 % (5) 17 % (1)	83 % (5) 17 % (1)	67 % (4) 33 % (2)	67 % (4) 33 % (2)	50 % (3) 50 % (3)	0.115[b]
mild *moderate* *severe* *life-threatening*	17 % (1) 50 % (3) 17 % (1) -	17 % (1) 50 % (3) 17 % (1) -	17 % (1) 50 % (3) - -	33 % (2) 33 % (2) - -	33 % (2) 17 % (1) - -	
MCV (fl)	102 ± 8.78	101 ± 9.64	98.7 ± 8.87	91.6 ± 6.45	92.7 ± 8.53	**0.010**[a]
Microcytic *Normocytic* *Macrocytic*	0 % (0) 33 % (2) 67 % (4)	0 % (0) 50 % (3) 50 % (3)	0 % (0) 67 % (4) 33 % (2)	0 % (0) 100 % (6) 0 % (0)	0 % (0) 83 % (5) 17 % (1)	**0.040**[a]
Haematocrit (--)	0.28 ± 0.03	0.28 ± 0.05	0.32 ± 0.05	0.35 ± 0.05	0.36 ± 0.04	**< 0.001**[a]
Inflammation	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
Albumin (g/l) 37 – 53	27.8 ± 5.32	33.4 ± 6.79	35.0 ± 6.52	38.8 ± 7.05	38.8 ± 6.46	**0.004**[a]
Transthyretin (g/l) ♀: 0.26 – 0.28 ♂: 0.29 - 0.32	0.04 ± 0.01	0.07 ± 0.02	0.10 ± 0.04	0.13 ± 0.06	0.14 ± 0.07	**0.002**[a]
Leucocytes (Gpt/l) 3.9 – 10.2	10.3 ± 7.13	10.5 ± 4.84	7.73 ± 2.31	5.48 ± 1.45	6.33 ± 2.21	**0.015**[a]
C-reactive protein (mg/l) < 5	36.3 ± 20.5	29.8 ± 19.6	11.0 ± 4.93	6.81 ± 6.10	3.98 ± 3.22	**0.007**[a]
Interleukin-1β (pg/ml) < 5	n = 5 12.9 ± 10.1	23.1 ± 27.5	21.8 ± 20.2	17.4 ± 15.0	110 ± 241	0.434[a]
Interleukin 6 (pg/ml) < 7	35.3 ± 11.9	30.7 ± 15.6	19.8 ± 19.3	14.1 ± 21.1	7.03 ± 9.92	**0.001**[a]
Tumor Necrosis Factor α (pg/ml) < 8.1	n = 5 10.3 ± 6.53	19.5 ± 10.4	12.8 ± 4.18	26.8 ± 39.9	27.7 ± 39.7	0.084[a]
Liver and coagulation	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
ASAT (U/l) ♀: < 35 ♂: < 59	95.4 ± 34.9	94.0 ± 50.0	56.3 ± 14.1	47.7 ± 11.5	43.3 ± 12.3	0.050[a]
ALAT (U/l) ♀: < 35 ♂: < 50	37.3 ± 14.2	35.8 ± 20.2	32.0 ± 12.9	26.8 ± 7.12	25.8 ± 9.88	0.388[a]

Gamma-GT (U/l) ♀: < 40 ♂: < 60	338 ± 377	162 ± 102	216 ± 282	227 ± 292	224 ± 289	0.856[a]
AP (U/l) ♀: 35 - 104 ♂: 40 - 129	163 ± 18.4	138 ± 40.7	144 ± 46.4	133 ± 29.7	135 ± 39.5	0.615[a]
Pseudo-cholinesterase (kU/l) 0.001	2.50 ± 1.07	1.59 ± 0.40	2.95 ± 1.24	4.48 ± 2.13	4.81 ± 1.79	**< 0.001**[a]
Bilirubin. total (µmol/l) < 21	429 ± 250	115 ± 61.0	69.6 ± 57.0	50.3 ± 58.1	45.8 ± 60.9	**0.002**[a]
INR 0.80 – 1.20	1.69 ± 0.46	1.56 ± 0.28	1.45 ± 0.23	1.29 ± 0.27	1.27 ± 0.27	**0.005**[a]
Renal function	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
Creatinine (µmol/l)♀: 73-109 ♂:82.127	72.5 ± 38.0	79.2 ± 20.3	76.3 ± 32.4	70.7 ± 15.9	74.2 ± 21.6	0.569[a]
CKD-EPI-GFR	89.2 ± 33.6	82.0 ± 22.7	84.3 ± 33.3	86.8 ± 23.3	83.8 ± 27.2	0.470[a]
Urea (mmol/l) < 8.3	4.79 ± 3.00	5.62 ± 3.27	6.48 ± 2.83	5.53 ± 1.86	6.09 ± 2.24	0.281[a]
Uric acid (µmol/l) < 420	357 ± 315	387 ± 154	457 ± 249	429 ± 193	403 ± 166	0.856[a]
Cardiovascular risk	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
Glucose (mmol/l) 3.3 – 5.5	5.72 ± 0.47	6.59 ± 1.48	6.59 ± 1.48	6.73 ± 1.50	6.41 ± 1.92	0.388[a]
Insulin (µIU/ml) 2.6 – 24.9	7.09 ± 3.45	12.3 ± 6.34	38.7 ± 24.9	37.5 ± 27.6	40.3 ± 34.6	**0.004**[a]
Total cholesterol (mmol/l)< 5.2	2.07 ± 1.05	2.75 ± 0.60	4.38 ± 1.84	6.13 ± 2.98	5.78 ± 2.51	**< 0.001**[a]
Triglycerides (mmol/l) < 2.3	1.94 ± 1.20	1.07 ± 0.36	0.97 ± 0.28	1.33 ± 0.55	1.22 ± 0.38	0.255[a]
Electrolytes and trace elements	Admission n = 6	Discharge n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
Sodium (mmol/l) 133 - 146	136 ± 2.10	137 ± 5.75	135 ± 3.20	136 ± 1.79	137 ± 2.74	0.570[a]
Calcium (mmol/l) 2.15-2.50	2.03 ± 0.10	2.21 ± 0.12	2.38 ± 0.15	2.37 ± 0.15	2.43 ± 0.13	**< 0.001**[a]
Magnesium (mmol/l) 0.7 – 1.1	0.78 ± 0.19	0.67 ± 0.10	0.74 ± 0.06	0.68 ± 0.08	0.68 ± 0.08	0.146[a]
Potassium (mmol/l) 3.5-5.1	3.30 ± 0.57	3.67 ± 0.44	3.78 ± 0.31	3.87 ± 0.29	3.85 ± 0.21	0.383[a]
Zinc (µmol/l) 9.18 – 18.3	6.31 ± 5.69	7.38 ± 1.79	6.29 ± 2.09	7.25 ± 2.97	8.66 ± 2.04	0.113[a]

SD: standard deviation, MCV : mean corpuscular volume ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase, gamma-GT: gamma-glutamyl transferase, AP: alkaline phosphate, INR: international normalized ratio, CKD-EPI-GFR: chronic kidney disease epidemiology collaboration glomerular filtrate rate, (a) Friedman-Test, (b) Cochran-Q-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

4.2.4.6 Nutrient intake and alcohol consumption trajectories

Total calories and carbohydrate intake were comparable overtime, while protein intake improved markedly in absolute numbers but showed only trends for statistical significance. Dietary fibre and folic acid intake increased significantly and alcohol intake decreased (Table 41).

Table 41: Nutrient intake and alcohol consumption trajectories

Parameters % (n). Mean ± SD	Admission n = 6	1 Month n = 6	3 Months n = 6	6 Months n = 6	p Value
		SaNT		Follow-up	
Total calories (kcal)	2126 ± 968	2139 ± 374	2562 ± 1142	2562 ± 716	0.615[a]
Protein (g)	56.0 ± 51.6	66.8 ± 19.0	102 ± 47.3	93.2 ± 17.2	0.061[a]
Total fats (g)	64.5 ± 77.8	62.5± 17.0	87.5 ± 40.2	87.3± 21.2	0.051[a]
Carbohydrates (g)	174 ± 64.8	266 ± 80.7	310 ± 149	310 ± 118	0.155[a]
Dietary fiber (g)	13.3 ± 7.47	23.5± 4.42	28.2± 6.74	29.7± 6.19	**0.031**[a]
Alcohol (g)	75.0 ± 153	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.41	**0.043**[a]
Folic acid (µg)	171 ± 103	312 ± 74.2	373 ± 123	396 ± 136	**0.014**[a]

SD: standard deviation, SaNT: supportive ambulant nutrion therapy, (a) Friedman-Test. Statistical significance was set at p value < 0.05 (shown in bold face).

5 Discussion

Recent years have seen an increasing interest in fatigue symptoms and its influencing factors in chronic liver disease patients [20, 21]. In this COVID/post-COVID era, symptoms of fatigue often neglected have gained further acknowledgement as an entity that needs addressing [171]. The aim of this work was to evaluate the prevalence and severity of fatigue symptoms in LC patients and associations with malnutrition, sarcopenia, anxiety and depression symptoms as well as possible indicators of fatigue responsive to nutritional therapy. The results in this study indicate that fatigue is a major symptom in LC patients and confirmed prevalence of fatigue like published data [67, 164, 172-175]. Overall, 68 % of the LC patients in this study were fatigued and this correlated positively with anxiety and depression symptoms. Contrary to general assumption [176], we found no association between fatigue and disease related malnutrition or sarcopenia. Waist circumference, gait speed and insulin-like growth factor 1 (IGF-1) were identified as independent predictors (indicators) of fatigue in the multiple linear regression analysis, explaining 58 % of the variance. Importantly, these independent indicators were also responsive to the intensified intersectoral nutritional therapy and correlated with improvements in fatigue prevalence and severity. Significant improvements were also seen in anxiety and depression symptoms, with 83 % of LC patients no longer fatigued or anxious and none depressed at the final follow-up 3 months after the end of the nutritional therapy.

The prevalence of fatigue symptoms in this study was 50 % with severe and 18 % with moderate symptoms. These results are in good agreement with previous studies that have reported fatigue prevalence ranging from 33 % to 85 % in patients with liver cirrhosis, non-alcoholic steatohepatitis and chronic hepatitis B/C viral infection [58-63]. Regarding features of fatigues, unexpectedly, both central and peripheral fatigue were equally manifested in our LC patients (63 % and 73 %, respectively). Furthermore, none of the patients presented with only central fatigue while 11 % had only peripheral fatigue. These results contradict the current literature that points towards central rather than peripheral fatigue in patients with chronic liver disease [15, 20, 22, 164]. In these studies, central fatigue is suggested to be consequent to alterations in the serotonergic and corticotropin neurotransmitter systems. Using a serotonin receptor antagonist - ondansetron, a randomized, placebo control trial in chronic hepatitis C patients found significantly reduced fatigue symptoms by 32 % on day 15 and 38 % by day 60 (intervention end), as compared to only 6 % and 20 % in the placebo group [83]. Swain et al. experimentally demonstrated effects of enhancement of central serotonergic neurotransmission in which bile duct resected (BDR) (cholestatic) and sham (controls – non cholestatic) rats treated with 5HT1A receptor agonist resulted in a significantly increased overall activity levels in cholestatic rats but not in the sham [82]. The role of the serotonergic neurotransmitter system seems to be specific for antagonist treated

cholestatic rodents as further supported by Nguyen and colleagues [81], the authors found cholestatic rodents treated with tropisetron (5-HT3 receptor antagonist) showed improvements in fatigue-like behavior, evidenced by an increase in locomotor.

Further supporting the mechanism of central fatigue consequent to alterations in the serotonergic and corticotropin neurotransmitter systems are studies using non-invasive techniques in clinical settings. This is evidenced in fatigued patients with primary biliary cirrhosis following transcranial magnetic stimulation showing no decrease in motor evoked response, unlike an increase in stimulation during fatigue exercise in healthy control subjects [85]. This is consequent to transcranial magnetic stimulation applying magnetic stimulation to the motor cortex and optimized to activate the interest muscle [177]. Unlike in our study, the aforementioned studies used different methods of analyzing fatigue features including; murine models, administration of antagonists and non-invasive techniques such as transcranial magnetic stimulation and not validated questionnaires. This could to some extent be a possible explaination for the discrepancy from our study; that suggests the existence of both central and peripheral fatigue in LC patients. Nonetheless, in concordance with our study, Weinstein el al., [42] in 106 patients with non-alcoholic fatty liver disease and hepatitis C virus using factor analysis between the FSS and medical outcomes study short-form (SF-36v2), found both central and peripheral fatigue to be present these patients. A recent systematic review and meta-analysis [178], in patients with liver cirrhosis, identified fatigue as a modifiable factor that was negatively associated with functional status which was measured in part by the international physical activity questionnaire (IPAQ). A decrease in functional status was defined as the inability “of the individual with liver cirrhosis to perform defined tasks in physical, psychological, social, and role functioning” [178]. This may therefore further suggest the presence of peripheral fatigue in cirrhotic patients. The results of our study indicate that the intervention resulted in clinically relevant improvements, thereby strongly suggesting that these mechanisms and problems can be effectively addressed by modifying dietary habits and increasing physical activity.

A potential mechanism involved in central fatigue is autonomic dysfunction [21]. Autonomic dysfunction impairs muscle responsiveness by increasing protons/lactate efflux from cells and outflux from tissues [69]. Previous studies showed that there is universal autonomic dysfunction in primary biliary cirrhosis [179]. Autonomic dysfunction, specifically vasomotor dysfunction, is associated with impaired cerebral autoregulation [88]. A UK based cross-sectional study in 2353 patients with primary biliary cirrhosis [89], showed significant correlations between vasomotor autonomic symptoms and fatigue ($r = 0.51$), cognitive symptoms ($r = 0.48$) and sleep disturbance ($r= 0.36$, all = $p < 0.0001$). The pathogenesis of peripheral fatigue in chronic liver disease has previously been demonstrated, with peripheral muscle dysfunction as a component of fatigue as a contributing mechanisms

[21]. This is suggested to be due to increased muscle acidosis during exercise, impaired aerobic capacity and mitochondrial impairment. Hollingsworth and colleagues [91, 92] showed that the capacity of repeating just a simple activity over time is reduced in fatigued patients with primary biliary cirrhosis. This is because acidosis affects muscle contraction by slowing the attachment of myosin and detachment rates from actin [96]. A significant association was found between acidosis recovery and fatigue severity [91, 92]. In LC patients, fatigue of the peripheral muscles can occur during daily living tasks and hamper the patients' quality of life [93]. Patients often describe this as "the inability to sustain physical activity, loss of energy or feeling of batteries being run down" [180]. Such findings were replicated in LC patients by Goldblatt et al. [181] who found a significant decrease in muscle function with repetitive physical activity that strongly correlated with fatigue severity. Ekerfors and colleagues [65] also demonstrated that low physical activity levels were associated with poor muscle performance. Although talterations in autonomic dysfunction, serotonergic and corticotropin neurotransmitter were not evaluated in this study, they may at least in part explain our findings.

Secondly, we found anxiety and depression symptoms were more prevalent in LC patients compared to healthy controls. Over 50 % of LC patients had mild, moderate to severe anxiety and depression symptoms. These results extend previous literature on anxiety and depression symptoms, that have reported prevalence between 16 % - 70 % in patients with liver cirrhosis, non-alcoholic liver disease, autoimmune hepatitis [172, 182-184]. High levels of both anxiety and depression symptoms are expected in these populations given frequent complications, hospitalization, high morbidity and mortality [4, 8]. Although we did not conduct in-depth analysis of the cause of anxiety or depression, we found positive correlations to fatigue symptoms consistent with our hypothesis. This is in line with Mells et al. [61], who found in large cohort with primary biliary cirrhosis, correlations between fatigue severity and anxiety/depression symptoms using the same hospital anxiety and depression scale similar to the scale we used it our study. An earlier cross-sectional study in the same patient population, also found depression to be more prevalent in fatigued patients [63]. However, Lima and colleagues [185] in their cross-sectional study in end-stage liver disease patients found no correlation between fatigue and anxiety/depression. This is possibly explained by the low anxiety/depression scores observed in their study measured using the HADS questionnaire, Portuguese version. In further analysis, we chose fatigue as the key psychosocial symptom because anxiety/depression is secondary to altered central neurotransmission resulting from central fatigue [24, 164]. This addition of anxiety/depression symptoms made hardly any apparent difference in the clinical characteristics of our LC patients. Probably because anxiety/depression can exhibit psychological mechanisms like fatigue [21, 186]. Further studies with a larger sample size

are required to examine these relationships on a longitudinal level and establish temporality and causality.

Waist circumference, gait speed and IGF-1 were identified as independent indicators of fatigue, whereby gait speed is associated with peripheral fatigue. In contrast, IGF-1 is linked to both central and peripheral aspects of fatigue, whereas the role of waist circumference in this context remains inconclusive. Waist circumference was chosen in the multiple regression analysis as a measurable proxy for visceral water retention since ascites can hardly be quantified in clinical practice [187]. Furthermore, ascites as a dichotomous variable would not have allowed for any statements regarding the extent of water accumulation. Nevertheless, we found a higher prevalence of ascites in fatigued LC patients with 87 % compared with 56 % in non-fatigued counterparts. These results were confirmed by 3 previous studies [68, 91, 188] who found the presence of ascites to be associated with fatigue. However, Salama et al., [60], found similar fatigue results in LC patients with and without ascites despite the correlation they found to disease severity according to the Child-Pugh classification. There are other studies that confirm these associations. Wittmer and colleagues [189] found that ascites drainage in LC patients reduced fatigue and improved pulmonary volumes and thoracic expansion. Similarly, an observational study in patients with malignant disease showed improvements in fatigue after cell-free and concentrated ascites reinfusion therapy (CART) [190]. CART is implemented in refractory ascites requiring repeated paracentesis [190]. One potential mechanism explaining the relationship between fatigue and ascites is the loss of protein, mainly albumin, from the vascular system into the interstitial space of the peritoneal cavity [190-193]. In ascites and inflammation, serum albumin, the main component of plasma protein in the blood, decreases [194]. However, our fatigued and non-fatigued LC patients showed comparable plasma albumin concentrations, making this an unlikely main mechanism. Recently Liu et al., [195] found active inflammation, defined as inflammation grade ≥2 using histological analysis, to be an independent risk factors for fatigue in their cross-sectional study in patients with chronic liver disease. We found insignificantly higher leucocyte counts and positive acute phase protein concentrations in our fatigued LC patients compared with non-fatigued LC patients. However, after clustering inflammation biomarkers we found no statistically significant association to fatigue frequency and severity in our study.

Another possible cause for the occurrence of fatigue symptoms in the presence of ascites is the severity of liver dysfunction. In LC patients, a decrease in liver function combined with portal hypertension leads to ascites [196]. The development of ascites is a signpost for progression to the decompensated phase of cirrhosis. However, our LC patients showed no association between disease severity, indicated by Child-Pugh model and MELD scores, and fatigue symptoms. This corresponds to the results of three other studies, all concluding

that fatigue in chronic liver disease is not correlated with disease activity or disease severity [195, 197, 198].

Reduced gait speed was the second independent indicator of fatigue we identified. In this study fatigued LC patients had a lower gait speed than non-fatigued LC patients. The results confirm previous research [199, 200], showing that LC patients walk with a slower gait speed compared to healthy controls. An association between gait speed and fatigue was also reported in multiple sclerosis patients [201]. Gait speed was utilized as a proxy for physical function instead of walking METs, which was subjectively estimated by the patients. It was also chosen on account of its integration in sarcopenia assessment as being indicative of a decline in physical performance and functional mobility [155]. Similarly, results from multivariable linear regression analysis reveal muscle performance tests, such as standing heel-rise test or timed up and go test, to be independently associated with the severity of fatigue in patients with chronic liver disease [65].

The inverse correlation of gait speed and fatigue in our LC patients was linear, indicating that the higher the degree of fatigue, the slower the gait. Soto and colleagues [202] found that LC patients with a decreased gait speed had 3-fold higher risk of death as compared to LC patients with a normal gait speed. Gait speed is therefore considered a robust prognostic factor for mortality in LC patients. Additionally, fatigability, i.e. susceptibility to fatigue, is a known risk factor of slower gait speed [203]. Recently, fatigue severity in chronic liver disease has been associated with lower muscle performance and physical activity due to peripheral muscle dysfunction [65]. Strong inverse correlation has also been found between handgrip strength and fatigue severity in patients with primary biliary cirrhosis [204]. We, however, failed to identify a significant difference between handgrip strength in fatigued and non-fatigued LC patients. A possible explanation for this may be that handgrip strength reflects explosive strength, i.e., the rate at which a force is generated [65, 205] rather than lower extremity muscle function.

IGF-1 was identified as the third independent indicator of fatigue in this study. IGF-1 is an anabolic 70-amino acid hormone produced and approximately 75 % of circulating IGF-1 is produced by the liver [206, 207]. Hepatic IGF-1 regulates mitochondrial function, systemic insulin sensitivity and oxidative stress [208]. In our study serum IGF-1 levels were significantly lower in LC patients compared with the healthy controls, confirming previous findings of Assy et al. [209]. Such was expected, since IGF-1 deficiency is a widespread phenomenon in LC patients [206]. This deficiency results from a decrease in growth hormone receptors in the liver coupled with a gradual decline in the liver's ability to synthesize IGF-1 [206]. IGF-1 insufficiency has been linked to insulin resistance, altered lipid metabolism, oxidative damage and neuro-hormonal axis deregulation [206]. In patients with liver cirrhosis this insufficiency plays a key role in intrahepatic metabolic disorders

[210]. Our fatigued LC patients showed trends for lower IGF-1 values than non-fatigued patients. Additionally, IGF-1 correlated negatively with fatigue symptoms, suggesting a relationship between serum IGF-1 levels and the intensity of fatigue in LC patients. These findings are novel, as to our knowledge, no previous studies have investigated the association between IGF-1 and fatigue in LC patients. However, similar negative correlations between IGF-1 and fatigue were previously reported in patients with multiple sclerosis [66] and children with subclinical inflammatory bowel disease [67]. The underlying mechanisms explaining these observations merit further research. IGF-1 represents a potential avenue for further investigation as a therapeutic option for the management of fatigue in cirrhosis. However, additional research is required, including the utilisation of animal models and conclusive human studies, to gain a comprehensive understanding of the role of IGF-1 in the context of fatigue in cirrhosis.

Sustainable improvement in fatigue was observed during the intensified nutritional intervention in 6 malnourished and fatigued LC patients, with no LC patient being fatigued at the end of the intervention and only 1 LC patient three months later at the final follow up. In addition, anxiety and depression symptoms paralleled these improvements in fatigue. Preliminary results of a multimodal intervention including nutrition in patients with progressive multiple sclerosis reported clinically significant improvements in perceived fatigue in 7/9 patients [211]. Another small study by the same authors using another multimodal intervention revealed significant reductions in perceived fatigue in 20 patients with progressive multiple sclerosis [212, 213]. The latter included modified Paleolithic diet with supplements, stretching, electrical stimulation of trunk and lower limb muscles, meditation and massage. in a duration of 12 months [211, 212]. Due to the small sample size and uncontrolled study designs, future randomized controlled trials are necessary to examine the impact of nutritional intervention on fatigue frequency and severity.

The strengths of this study are the strict inclusion criteria that limits confounding, well-characterized sample from two medical centers, complete data set and use of validated tools for measuring fatigue. Additionally, we designed the intervention as proof of principle to test the feasibility of a novel and complex intersectoral nutritional therapy model over 3 months with a follow-up after 6 months. Furthermore, the characteristics of our LC patients were similar tallying with the etiological profile of cirrhotic patients in Europe and Germany specifically [3, 4]. Therefore, our results on fatigue can be transferred to cirrhotic patient population in this part of the world. Limitations include, that cross-sectional studies do not warrant cause-effect relationships and the independent indicators we identified point to the presence of fatigue rather than cause. A further limitation is the single-armed design of intervention study. When designed, we could not exclude the failure of feasibility and randomized controlled trials are required to confirm our findings in a larger sample size. The

small sample size together with the lack of controls affected the significance of the results, as we cannot exclude that improvements might have occurred anyway in the course of time independent of the nutritional therapy. However, the effects observed were of a substantial clinical relevance and consistent with our hypotheses. As we used a highly complex intervention model, our results cannot be generalized for other nutritional interventions. Furthermore, we did not take into account additional factors related to fatigue such as quality of sleep, although a number of studies in cirrhotic patients [67], have identified existent associations.

6 Conclusion

In conclusion, we confirm that fatigue is a prevalent symptom/syndrome in patients with liver cirrhosis and demonstrate its multifactorial nature as seen with the prevalence of also anxiety and depression symptoms. Our research strongly suggested that reduced gait speed, presence of ascites and lower IGF-1 levels are implicated in fatigue in LC patients. However, further studies are still needed to understand the exact mechanisms involved. Our findings revealed easily identifiable fatigue factors in clinical practice, i.e., ascites and gait speed, that may benefit future care of fatigued cirrhotic patients. The identification of these indicators can possibly reduce the underreporting of fatigue in routine clinical practice and aid quick identification of fatigue risk patients and adapt their nutritional therapy and communication accordingly. Furthermore, we provided preliminary evidence that an intensified nutritional intervention program is feasible and alleviates fatigue symptoms in this patient population. This program worked out well including, good compliance of patients, well-functioning intersectoral cooperation between institutions, and well managed interface challenges. Future randomized controlled trials are however required to confirm our findings with a larger sample size. This study is among a few that address fatigue in combination with anxiety/depression symptoms in LC patients. To our knowledge, this study is the first to assess simultaneously, potentially modifiable fatigue indicators within an intensified intersectoral nutritional therapy. Of particular interest is the role of IGF-1 in addressing fatigue in patients with liver cirrhosis, which represents a novel approach in this context. Taken together, these findings emphasize the importance of fatigue in LC patients and provides a starting point for future research in the field.

7 Summary

Rationale: Fatigue is a common and prevalent symptom among patients with liver cirrhosis (LC pts). The etiology and management of fatigue in LC pts remain poorly understood. This study aimed to evaluate the prevalence and severity of fatigue symptoms in LC pts and to identify biomarkers indicative of fatigue without the necessity for routine fatigue screening. Additionally, it aimed to observe the progression of fatigue under intensified nutritional therapy over a six-month period in malnourished LC pts.

Method: A total of 56 LC pts (male: 63 %, age: 57 ± 11 years, body mass index (BMI) 28.0 ± 5.1 kg/m²) were examined in a secondary cross-sectional analysis of the European Social Fund-funded project "Enteral Nutrition in Malnutrition Due to Diseases of the Gastrointestinal Tract: From Basic Understanding to Innovative Treatment Concept" (EnErGie) between 05/2019 and 01/2022. The evaluation included the assessment of total, peripheral and central fatigue (FSS), anxiety and depression symptoms (HADS-D), loneliness (De Jong Gierveld), malnutrition (GLIM), sarcopenia (EWGSOP2), laboratory parameters, waist circumference, body composition (SECA-mBCA 515/25), walking speed and hand grip strength (Jamar, dynamometer). Six malnourished and fatigued LC pts (male 17 %, age 54 ± 6 years, BMI 24.8 ± 2.8 kg/m²) were enrolled in a single-armed proof-of-principle intervention trial consisting of in-hospital nutritional support, oral nutrition supplementation for 1 month, 8-12 nutritional coaching sessions within 3 months after discharge and follow-up after 6 months.

Results: Overall, 68% (n = 38) of LC pts exhibited moderate to severe fatigue symptoms with similar proportions of central (63%) and peripheral fatigue (74%). Fatigued (F-) and non-fatigued (Non-F) LC pts were similar in age, BMI, body composition, disease etiology and severity, prevalence of malnutrition and sarcopenia and handgrip strength. However, F-LC pts compared with non-F-LC pts showed a higher intake of proton pump inhibitors (82% vs. 50%, $p = 0.025$) and antibiotics (42% vs. 11%, $p = 0.031$), lower gait speed (0.90 ± 0.34 vs 1.48 ± 0.54, $p < 0.001$) and a higher prevalence of edema or ascites (92% vs. 56%, $p = 0.003$), the latter resulting in a higher waist circumference (109 ± 17 cm vs 99 ± 14 cm, $p = 0.038$). Fatigue severity correlated positively with anxiety ($r = 0.377$, $p = 0.004$) and depression symptoms ($r = 0.451$, $p < 0.001$), loneliness ($r = 0.331$, $p = 0.013$) and waist circumference ($r = 0.377$, $p = 0.004$). Conversely, negative correlations were identified between gait speed ($r = -0.643$, $p < 0.001$) and physical activity ($r = -0.321$, $p = 0.016$). The results of the multiple regression analysis showed that three independent predictors explained 55% of the variance in fatigue: namely, walking speed ($p < 0.001$), waist circumference ($p = 0.004$), and insulin-like growth factor (IGF-1) ($p = 0.016$). The intervention study resulted in statistically significant and sustainable improvements in fatigue, anxiety- and depression symptoms, as well as gait speed, waist circumference and IGF-1.

Conclusion: In clinical practice, gait speed and ascites are readily identifiable indicators of fatigue. Further investigation is required to determine the precise mechanism between fatigue and IGF-1, as this could represent a potential avenue for therapeutic options for fatigue in LC pts. Intensified nutritional therapy has the potential to alleviate malnutrition and fatigue symptoms in LC pts, thus representing a potential additional avenue of investigation.

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Appendix

A 1: MLR Analysis - Stepwise elimination variables

Dependent variable - Fatigue	Predictor	*β* unstandardized	*p*	95% CI	*R* square	Adjusted *R* square
Model 1	Loneliness status	0.283	0.563	-0.695 | 1.26	0.59	0.50
	Waist circumference	0.030	**0.015**	0.006 | 0.053		
	Handgrip strength	-0.013	0.537	-0.054 | 0.029		
	Gait speed	-2.07	**< 0.001**	-3.099 | -1.048		
	Anemia status	0.267	0.575	-0.685 | 1.219		
	IGF-1	-0.011	**0.036**	-0.022 | -0.001		
	Inflammation cluster	0.095	0.820	-0.738 | 0.928		
	PPI	0.091	0.843	-0.830 | 1.011		
	Walking METs	< 0.001	0.921	0.000 | 0.000		
	Dietary fiber	- 0.011	0.601	-0.052 | 0.030		
Model 2	Loneliness status	0.279	0.563	-0.685 | 1.243	0.59	0.51
	Waist circumference	0.030	**0.013**	0.007 | 0.053		
	Handgrip strength	-0.012	0.535	-0.051 | 0.027		
	Gait speed	-2.055	**<0.001**	-2.998 | -1.113		
	Anemia status	0.265	0.573	-0.675 | 1.206		
	IGF-1	-0.011	**0.032**	-0.022 | -0.001		
	Inflammation cluster	0.094	0.818	-0.729 | 0.918		
	PPI	0.101	0.820	-0.787 | 0.989		
	Dietary fiber	-0.011	0.592	-0.051 | 0.030		
Model 3	Loneliness status	0.264	0.576	-0.680 | 1.209	0.59	0.52
	Waist circumference	0.030	**0.010**	0.008 | 0.053		
	Handgrip strength	-0.012	0.542	-0.050 | 0.027		
	Gait speed	-2.078	**<0.001**	-2.989 | -1.167		
	Anemia status	0.291	0.520	-0.612 | 1.194		
	IGF-1	-0.012	**0.021**	-0.021 | -0.002		
	Inflammation cluster	0.081	0.841	-0.725 | 0.886		
	Dietary fiber	-0.012	0.548	-0.051 | 0.027		
Model 4	Loneliness status	0.267	0.568	-0.667 | 1.201	0.59	0.53
	Waist circumference	0.030	**0.007**	0.009 | 0.052		
	Handgrip strength	-0.011	0.555	-0.049 | 0.026		
	Gait speed	-2.061	**<0.001**	-2.947 | -1.175		
	Anemia status	0.300	0.501	-0.589 | 1.189		
	IGF-1	-0.012	**0.020**	-0.021 | -0.002		
	Dietary fiber	-0.013	0.483	-0.050 | 0.024		
Model 5	Waist circumference	0.032	**0.005**	0.010 | 0.053	0.58	0.53
	Handgrip strength	-0.009	0.611	-0.046 | 0.027		
	Gait speed	-2.157	**<0.001**	-2.971 | -1.344		
	Anemia status	0.233	0.585	-0.618 | 1.085		
	IGF-1	-0.011	**0.022**	-0.021 | -0.002		
	Dietary fiber	-0.012	0.515	-0.048 | 0.025		
Model 6	Waist circumference	0.031	**0.005**	0.010 | 0.052	0.58	0.54
	Gait speed	-2.221	**<0.001**	-2.989 | -1.452		
	Anemia status	0.210	0.617	-0.630 | 1.051		
	IGF-1	-0.011	**0.021**	-0.020 | -0.002		
	Dietary fiber	-0.013	0.465	-0.049 | 0.023		
Model 7	Waist circumference	0.031	**0.005**	0.010 | 0.051	0.58	0.55
	Gait speed	-2.222	**<.001**	-2.984 | -1.459		
	IGF-1	-0.010	**0.020**	-0.019 | -0.002		
	Dietary fiber	-0.012	0.503	-0.047 | 0.023		
Model 8	Waist circumference	0.031	**0.004**	0.010 | 0.052	0.58	0.55
	Gait speed	-2.30	**<0.001**	-3.022 | -1.585		
	IGF-1	-0.010	**0.016**	-0.019 | -0.002		