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Transient elastography for assessing and monitoring of liver steatosis and fibrosis

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Für Dr. Inge und Nafis Arslanow

“For the liver is a source of many diseases. It is a noble organ which serves many other parts of the body, indeed nearly all of them. If it suffers, this is no small matter, but rather a great one which leads to many things.”

Paracelsus (Theophrastus Bombastus von Hohenheim, 1493−1541)
taken from Kuntz and Kuntz, Hepatology Textbook and Atlas,
Springer-Verlag, Berlin, Heidelberg 2008

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Abbreviations

ALT	alanine aminotransferase / Alanin-Aminotransferase/ Alanin-Aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase/ Aspartat-Aminotransferase
AUROC	area under the receiver operating characteristic
BFFM	body fat free mass
BFM	body fat mass
BIA	body impedance analysis/ Körperimpedanzanalyse
BMI	body mass index
CAP	controlled attenuation parameter
CI	confidence interval
CLD	chronic liver disease
DBP	diastolic blood pressure
DGVS	Deutsche Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten
E	glutamic acid
F	Frau
FXR	farnesoid X receptor
F1…4	fibrosis stage 1…4
γ-GT	gamma-glutamyl transferase/ gamma-glutamyl transpeptidase/ γ-Glutamyltransferase
GLC	Glukose
HCC	hepatocellular carcinoma
HDL	high-density lipoprotein
HOMA-IR	insulin resistance index
HS	Harnsäure
HSROC	hierarchical summary receiver operating characteristic
HU	Hüftumfang
HWE	Hardy-Weinberg equilibrium
I	Isoleucine
INR	International Normalized Ratio
IQR	interquartile range/ Interquartilsabstand
K	Lysine
kcal	kilocalorie
KFM	Körperfettmasse
kJ	kilojoule
KW	Körperwasser
LDL	low-density lipoprotein

LS	liver stiffness/ Lebersteifigkeit
LSM	liver stiffness measurement/ Lebersteifigkeitsmessung
M	Methionine/ Mann
mBCA	medical body composition analyzer
N	Number
NAFL	Non-alcoholic fatty liver/ Nichtalkoholische Fettleber
NAFLD	Non-alcoholic fatty liver disease/ Nichtalkoholische Fettlebererkrankung
NASH	Non-alcoholic steatohepatitis
PNPLA3	patatin-like phospholipase domain containing 3
OR	odds ratio
P	p-value/ P-Wert
PASH	PNPLA3-associated steatohepatitis
PChE	pseudocholin-esterase
r_p	Pearson-Korrelationskoeffizient
r_s	Spearman's rank coefficient
SBP	systolic blood pressure
SMM	Skelettmuskelmasse
S0…3	steatosis grade 0…3
TBW	total body water
TC	total cholesterol/ Cholesterin
TE	transient elastography/ Transiente Ealstographie
TG	Triglycerides/ Triglyzeride
THV	Taille-Hüfte-Verhältnis
TM6SF2	transmembrane 6 superfamily member 2
TU	Taillenumfang
UKS	university medical center Saarland/ Universitätsklinikum des Saarlandes
VCTE	vibration-controlled transient elastography
VF	Viszerales Fettvolumen
VFI	visceral fat index
WC	waist circumference
WHO	World Health Organization

Summary

Transient elastography (TE) is a novel method to assess and monitor non-alcoholic fatty liver disease (NAFLD) non-invasively by simultaneously quantifying steatosis using the controlled attenuation parameter (CAP) and liver stiffness using the liver stiffness measurement (LSM). We carried out four studies using TE as the central method to explore its value for research and clinical practice.

We found a positive correlation between CAP and ultrasonography in the detection of steatosis in a cohort of 174 patients with chronic liver diseases. Furthermore, patatin-like phospholipase domain containing 3 (*PNPLA3*) p.148M, a known genetic risk factor for NAFLD, was associated with CAP, but not with LSM. No association was observed for CAP and LSM and transmembrane 6 superfamily member 2 (*TM6SF2*) p.167K, which is another known genetic risk factor for NAFLD. However, for both variants an association with serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities was demonstrated. Overall, carriers of the *PNPLA3* risk allele [M] had an increased risk for the development of hepatic phenotypes in general and more specifically an increased odds of 2.4 for steatosis. This is the first time steatosis assessed by CAP was used in a genetic study.

To investigate the short-term modulation of liver fat, we carried out the first intervention study using TE. In 60 patients with hepatic steatosis, fourteen days of a hypocaloric high-fiber, high-protein diet resulted in significant reductions in liver fat by 14% and in body weight by 4.8%. There is evidence that *PNPLA3* modulated this dietary-related response. Furthermore, liver stiffness, body composition, serum liver enzymes and metabolic markers improved significantly.

The UKS-study availed of CAP and LSM during occupational health check-ups and suggests that the prevalence of NAFLD is underdiagnosed in 104 hospital employees. The study also observed that TE is superior to serum parameters for diagnosing hepatic steatosis and fibrosis. By use of body impedance analysis (BIA) and a tape measure, we were able to demonstrate that body fat mass and waist circumference are the strongest predictors for steatosis. This is the first study that availed of CAP and LSM during occupational health check-ups.

The recommendations for the use of TE to assess fibrosis are weaker in the German guideline as opposed to the European guideline on NAFLD. Neither of the guidelines however, provides a statement on assessing steatosis using CAP. Consequently, we analyzed almost 6,700 TE measurements obtained over a period of 3.5 years in patients and the general population. The results reflect the feasibility of TE and subsequently, we introduced the term “EGK des Hepatologen”, translating to “ECG of the hepatologist”.

Zusammenfassung

Die transiente Elastographie (TE) ist eine neuartige Methode zur nichtinvasiven Beurteilung und Überwachung der nichtalkoholischen Fettlebererkrankung (NAFLD), bei der gleichzeitig die Leberverfettung mittels Controlled Attenuation Parameter (CAP) und die Lebersteifigkeit mittels Lebersteifigkeitsmessung (LSM) quantifiziert wird. Die Bedeutung der Methode für Forschung und klinische Praxis wurde in vier Studien untersucht.

Bei 174 Patienten mit chronischen Lebererkrankungen konnte bei der Detektion der Steatose eine positive Korrelation zwischen CAP und der Messung mittels Ultraschall gezeigt werden. Für Patatin-ähnliche Phospholipase-Domäne 3 (*PNPLA3*) p.148M, ein bekannter genetischer Risikofaktor für NAFLD, konnte ein Zusammenhang mit CAP, nicht aber mit LSM, gezeigt werden. Für Transmembran-6-Superfamilienmitglied 2 (*TM6SF2*) p.167K, einem weiteren NAFLD-Risikofaktor, konnten weder für CAP, noch für LSM ein Zusammenhang nachgewiesen werden. Eine Assoziation mit Serum-Alanin-Aminotransferase (ALT) und Aspartat-Aminotransferase (AST) Aktivitäten wurde hingegen für beide genetische Varianten gezeigt. Träger des Risikoallels [M] hatten ein erhöhtes Risiko für die Entwicklung eines hepatischen Phänotyps und eine erhöhte Wahrscheinlichkeit für die Entwicklung einer Steatose um das 2,4-Fache. In dieser Studie wurde die CAP zum ersten Mal zur Detektion der Steatose in einer genetischen Studie eingesetzt.

Die kurzfristige Modulation von Leberfett wurde in der ersten Interventionsstudie mit TE durchgeführt. Bei 60 Patienten mit Steatose führten vierzehn Tage einer hypokalorischen, ballaststoffreichen und proteinreichen Diät zu einer signifikanten Reduktion des Leberfetts um 14% und des Körpergewichts um 4,8%. Es gibt Hinweise darauf, dass *PNPLA3* die ernährungsbedingte Reduktion beeinflusst. Weiterhin verbesserten sich Lebersteifigkeit, Körperzusammen-setzung, Serumleberenzyme und metabolische Marker signifikant.

Die UKS-Studie konnte mithilfe von CAP und LSM im Rahmen der betrieblichen Gesundheitsförderung zeigen, dass die Prävalenz von NAFLD bei 104 Krankenhaus-angestellten unterdiagnostiziert ist und dass TE den Serumparametern zur Diagnose von Steatose und Fibrose überlegen ist. Dabei wurde weiterhin gezeigt, dass gemessen mittels Körperimpedanzanalyse (BIA) und einem einfachen Maßband, Körperfettmasse und Taillenumfang die stärksten Prädiktoren für die Steatose sind. Dies ist die erste Studie, bei der bei arbeitsmedizinischen Vorsorgeuntersuchungen auf CAP und LSM angewendet wurde.

Die Empfehlungen zur Verwendung von TE zur Beurteilung der Fibrose sind in der deutschen Leitlinie gegenüber der europäischen Leitlinie zu NAFLD schwächer ausgeprägt.

Keine der Leitlinien macht eine Aussage zur Beurteilung der Leberverfettung mittels CAP. Eine Auswertung von fast 6.700 TE-Messungen bei Patienten und der Allgemeinbevölkerung über einen Zeitraum von 3,5 Jahren macht die Durchführbarkeit der transienten Elastographie deutlich und lässt sich durch den dafür eingeführten Begriff "EGK des Hepatologen" trefflich beschreiben.

1. Introduction

1.1. Definition of Non-alcoholic fatty liver disease

The liver, as the body's largest solid organ, plays an essential role in synthesis and retention of glucose, fat and protein and consequently the allocation of energy. Core functions of the liver are the storage of vitamins, the secretion of bile, the formation of clotting factors as well as the detoxification of ammonia, alcohol and drugs (Kuntz and Kuntz, 2008).

Non-alcoholic fatty liver disease (NAFLD) is the most frequent cause of liver disease worldwide (Rinella, 2015). Also, patients with NAFLD have a lower quality of life compared to a general healthy population (David et al., 2009). NAFLD summarizes the conditions of non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL, also referred to as steatosis, occurs if the liver contains more than 5% of triglycerides in the hepatocytes. The combination of steatosis, inflammation and liver cell injury with or without fibrosis characterizes NASH (European Association for the Study of the Liver et al., 2016; Younossi et al., 2012). NASH was first described in detail in a 1980 publication by Ludwig and colleagues (Ludwig et al., 1980). An alcohol consumption of less than 20 grams per day in women and 30 grams daily in men is required for the diagnosis of NAFLD (European Association for the Study of the Liver et al., 2016).

1.2. Prevalence and progression of Non-alcoholic fatty liver disease

The estimated prevalence of NAFLD worldwide, as assessed by ultrasonography, computed tomography and magnetic resonance imaging, is 25.2% based on 45 studies. Here, the frequency ranges from 13.5% in Africa to 31.8% in the Middle East (Younossi et al., 2016). In Europe, rates of up to 50% have been reported when NAFLD was diagnosed by ultrasonography only (Blachier et al., 2013).

After a median follow-up period of 6.6 years, 42% of patients with NAFLD developed fibrosis, irrespective of NAFLD or NASH at baseline. Specifically, 44% with NAFL progressed to NASH and 22% to stage 3 fibrosis (F3 = severe fibrosis) (McPherson et al., 2015). Overall, the fibrosis progression in NASH patients is almost 41% according to a meta-analysis, (Younossi et al., 2016). The rate of progression varies: after 5.9 years, one out of five patients progresses rapidly from no fibrosis to severe fibrosis (F3 fibrosis) or cirrhosis (F4 fibrosis), whereas 80% progress slowly to mild or moderate fibrosis (F1 or F2 fibrosis, respectively). This meta-analysis also calculated a progression by one stage after 14.3 years in NAFL and 7.1 years in NASH (Singh et al., 2015). Furthermore, the presence of diabetes increases fibrosis progression (Adams et al., 2005c). An increase in weight of more than five

kilograms, insulin resistance indicated by a higher insulin resistance index (HOMA-IR) and increased liver fat separated between progressive and non-progressive fibrosis after a mean follow up of 14 years (Ekstedt et al., 2006). Overall, fibrosis is the key prognostic factor for liver-related events, liver transplantation and overall mortality in patients with NAFLD (Angulo et al., 2015). NAFLD can further progress to hepatocellular carcinoma (HCC). In 2003, only a few cases of NAFLD-related HCC were reported but numbers increased over time and reached 35% of 118 cases in 2010 (Dyson et al., 2014). NAFLD-related HCC can also occur in the absence of cirrhosis, as reported in an Italian study where 67 of 145 cases presented with NAFLD-HCC and no prior cirrhosis (Piscaglia et al., 2016). A 2013 review sums up data on liver disease incidence and prevalence from 260 studies published between 2007 and 2013 as follows: 170,000 people die due to cirrhosis and 47,000 die because of liver cancer in Europe every year (Blachier et al., 2013). **Figure 1** presents a summary of the progression of NAFLD and a further specification of the histological differences of the separate stages (Cohen et al., 2011).

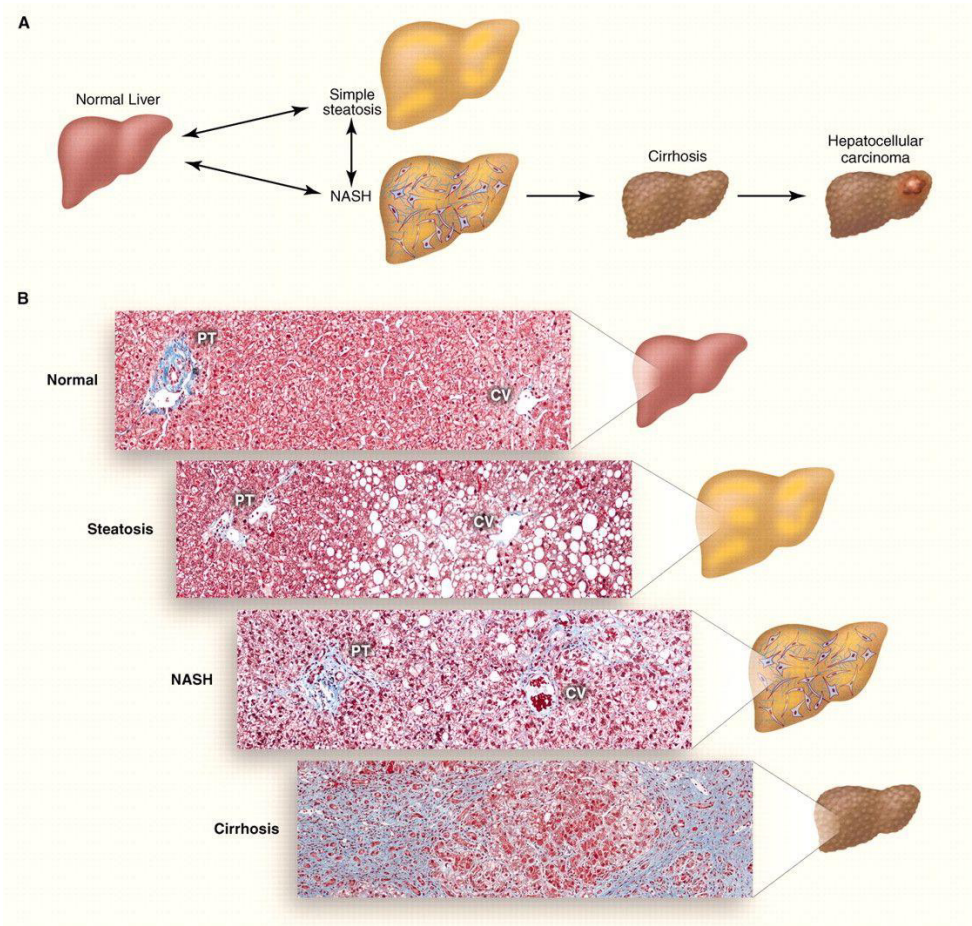

Fig. 1: Progression of NAFLD (**A**) and a further specification of the histological differences of the NAFLD stages (**B**) (Cohen et al., 2011)

The main reason for death in patients with NAFLD is cardiovascular events. A Swedish long-term study with a mean follow-up of 13.7 years of 129 NAFLD patients reported 26 (20.2%) cases of death. Overall, 16 (12.4%) died of cardiovascular diseases and only two patients died of HCC and variceal hemorrhage (Ekstedt et al., 2006). A Danish study in 170 NAFLD patients prolonged the duration for follow-up to 20 years and confirmed the findings from Sweden: 48 cases of death were reported (28.2%), and the main cause of death was arteriosclerosis in 18 cases (10.6%), one person died of cirrhosis, and no case of liver cancer was reported (Dam-Larsen et al., 2009).

1.3. Risk factors for Non-alcoholic fatty liver disease

NAFLD is a multifactorial disease (Younossi et al., 2018). Steatosis and fibrosis were found to be, in part, heritable traits, independent of age, sex and ethnicity (Loomba et al., 2015). Patatin-like phospholipase domain containing 3 (*PNPLA3*) and transmembrane 6 superfamily member 2 (*TM6SF2*) have been identified as potential candidate genes for the development of NAFLD. The greatest attributable risk has been reported for *PNPLA3* p.I148M (rs738409) in a genome-wide association study (Romeo et al., 2008). This variant increases the risk for steatosis, fibrosis, NASH, cirrhosis and HCC (Krawczyk et al., 2011; Liu et al., 2014a; Valenti et al., 2010). The minor allele frequency, the frequency of the rare allele in the population, is 0.24 for the *PNPLA3* risk allele [M] (Romeo et al., 2008). *TM6SF2* p.E167K (rs58542926), identified in an exome-wide association study (Kozlitina et al., 2014), increases the risk for steatosis but not fibrosis (Krawczyk et al., 2017; Sookoian et al., 2015). It is less frequent with a minor allele frequency of 0.12 for the risk allele [K] (Liu et al., 2014b). The frequency of both genetic risk variants differs within ethnic groups. *PNPLA3* p.148M prevalence is highest in Hispanics (26.6%), medium in European Americans (4.1%) and lowest in African-Americans (1.8%) (Romeo et al., 2008). In contrast, *TM6SF2* p.167K is most common in Europeans, Americans (7.2%), medium in Hispanics (4.7%) and lowest in African-Americans (3.4%) (Kozlitina et al., 2014).

In carriers of the *PNPLA3* risk allele [M], the risk for NAFLD was independent of sex, body mass index (BMI) and diabetes (Valenti et al., 2010). The term PASH – *PNPLA3*-associated steatohepatitis – is suitable, for those who have NAFLD, are heterozygous or homozygous carriers of the risk allele [M] and present without environmental risk factors (Krawczyk et al., 2013b).

BMI is the most prominent factor in the development of NAFLD and reaches a 3.5-fold NAFLD risk in obese subjects (Li et al., 2016a). The World Health Organization (WHO) defines obesity as a BMI of 30 kg/m² or above (World Health Organization, 2000), with BMI calculated as the proportion of body weight in kilograms divided by the square of body height

in meters. The increase of BMI is linked to an obesogenic diet, which is hypercaloric, rich in processed foods and low in fruits and vegetables, and also reduced physical activity (Barrera and George, 2014; Thorburn et al., 2014). These lifestyle aspects have a profound influence on the development and progression of NAFLD. Between 1998 and 2010, obesity rates in Germany have increased from 18.8% to 23.3% in men and 22.5% to 23.9% in women (Mensink et al., 2013). In 2014, the mean BMI of adults in 200 countries worldwide was 24.2 kg/m² in men and 24.4 kg/m² in women, and 10.8% and 14.9% of the population were obese, respectively. The frequency for obese men and women is expected to exceed the 18% and 21% thresholds by 2025 (NCD Risk Factor Collaboration, 2016).

Even in children, the prevalence for NAFLD is 7.6% in the general population and increases to 34.2% when assessed in obesity clinics for children with higher rates for boys than girls in both populations, showing that the risk for NAFLD is 13 times higher in overweight and obese children as compared to those who have a normal BMI (Anderson et al., 2015). Overweight and obese children may show signs of depression, which are significantly worse when NAFLD is also present (Kerkar et al., 2013). In pediatric cohorts, *PNPLA3* genotype did not affect biopsy-proven NAFLD, although they were related to higher serum alanine aminotransferase activities (Krawczyk et al., 2015; Rotman et al., 2010). Children carrying the risk allele [M] tend to be younger (11 months) by the time they received a liver biopsy as compared to children without the risk allele. In this pediatric cohort, the diagnosis of steatosis was more likely than NASH as compared to adults (Rotman et al., 2010).

Obesity is a common risk factor for NAFLD and diabetes, which are intertwined through insulin resistance (Loria et al., 2013). Type 2 diabetics have more liver fat and reduced hepatic and adipose tissue insulin resistance (Kotronen et al., 2008). In patients with type 2 diabetes, the prevalence of NAFL as assessed by ultrasound ranged from 61% to 70% (Leite et al., 2009; Lv et al., 2013; Targher et al., 2007). By means of transient elastography, NAFL was detected in 72.8% of 1309 cases and the percentage of affected patients increased with steatosis grade 1, 2 and 3 (S1, S2 and S3). Simultaneously, 18% of diabetics presented with F3 fibrosis or F4 cirrhosis (Kwok et al., 2016). Diabetes and fibrosis were independent risk factors for the progression of fibrosis (Adams et al., 2005c).

NAFLD is not only triggered by genetics and obesity, but also by a large number of secondary causes and after intake of steatogenic or fibrogenic drugs, as highlighted in **table 1** (Adams et al., 2005a; Kneeman et al., 2012; Torres et al., 2012).

Table 1: Overview of secondary causes of Non-alcoholic fatty liver disease (Adams et al., 2005a; Kneeman et al., 2012; Torres et al., 2012).

Hepatology	Severe weight loss after intestinal
Hepatitis C infection	bypass surgery
Human immunodeficiency virus	**Cardiology**
Wilson's disease	Cardiovascular disease
Gastroenterology	**Endocrinology**
Celiac disease	Polycystic ovarian syndrome
Inflammatory bowel disease	**Medication**
Hyperuricemia	Tamoxifen
Total parenteral nutrition	Corticosteroids
Starvation	Amiodarone
Vitamin D deficiency	Estrogen

1.4. Diagnosis of Non-alcoholic fatty liver disease

Liver biopsy is considered to be the "gold standard" for the detection of steatosis, fibrosis, ballooning and inflammation, although it is an invasive method, has a risk of bleeding and causes pain in 20% of patients. Biopsy is also affected by sampling errors and represents only about 1/50,000th of the liver volume (Cadranel et al., 2000; Campbell and Reddy, 2004; Ratziu et al., 2005; Terjung et al., 2003).

In 51 paired liver samples, staging for fibrosis was equal in 59% but left 41% with staging results differing by ≥ 1 stage. For activity grading, there was a match in 57% and a difference by one grade in 43%. The authors concluded that agreement was moderate and low with Cohen's kappa coefficients of 0.47 and 0.18, respectively (Ratziu et al., 2005). In addition, three samples showed cirrhosis but this finding was not confirmed in two of three samples, indicating that two patients may or may not have been diagnosed with cirrhosis depending on the biopsied part of the liver. For steatosis, 22% of paired biopsies were not concordant, and in 18% the difference was higher than 20% (Ratziu et al., 2005). Furthermore, up to 21% of biopsy samples were not suitable for histological assessment (Myers et al., 2012a; Sandrin et al., 2003). All in all, biopsy does not allow regular follow-up measurements due to its invasive nature and its high costs and is therefore not suitable for monitoring of progression or therapeutic outcome or for screening large population studies.

The most widely used method in Europe to assess the prevalence of steatosis is ultrasonography (Blachier et al., 2013) because it is non-invasive, inexpensive and easily

accessible (Schwenzer et al., 2009). On the contrary, ultrasonography does not clearly differentiate between steatosis and fibrosis (Schwenzer et al., 2009), is semiquantitative and strongly depends on operator experience (Polyzos and Mantzoros, 2014).

Computed tomography and magnetic resonance imaging are reliable imaging techniques (Schwenzer et al., 2009) for the quantification of liver fat although they are not suitable for the detection of early stages of fibrosis (Martinez et al., 2011). In addition, computed tomography causes radiation exposure and imaging quality depends on dosage. Magnetic resonance imaging represents a radiation-free method but scanners are not widely available. The bore diameter is 60 cm and therefore limited when used for obese patients, and scanners with an increased diameter are even fewer. In addition, the procedure is expensive, time consuming and noisy and therefore not patient friendly (Schwenzer et al., 2009).

Often, steatosis and fibrosis are diagnosed using serum surrogate markers. Alanine aminotransferase (ALT) is a common marker to evaluate liver health because of its high specificity for hepatocellular injury. In contrast, patients with liver diseases can also present with ALT levels that are normal. In 386 and 458 cases of biopsy-proven NAFLD, normal levels of ALT were found in 13% and 14%, respectively. Of those, 6 and 8 cases were diagnosed with cirrhosis (Fracanzani et al., 2008; Mofrad et al., 2003). Findings differ in the general population, where data was assessed outside the hospital routine. Here, 40.0% to 74.6% presented with fibrosis and normal ALT values (Harris et al., 2017). Transient elastography (TE) provides an alternative to liver biopsy, ultrasonography, computed tomography, magnetic resonance imaging and serum surrogate markers.

1.5. Transient elastography as a novel approach for liver diagnostics

Transient elastography (TE) was first mentioned in a 2003 publication by Sandrin and colleagues from France (Sandrin et al., 2003) and described as “a noninvasive, painless, rapid and objective method to quantify liver fibrosis” (Sandrin et al., 2003). The new technology for the assessment of liver stiffness was explained in detail and verified against liver biopsy in 67 chronic hepatitis C patients (Sandrin et al., 2003). TE is performed by Fibroscan®, a device by the French company Echosens.

During the assessment, the patient lies on his or her back, while the right arm is stretched and placed behind the head. The operator places the tip of the probe in the space between the ribs perpendicular to the xyphoid and horizontally on the midaxillary line and creates an ultrasonic shear-wave by pushing a button on the probe. The propagation of the shear wave in the liver rises with the intensity of liver stiffness, resulting in values between 1.5 kPa and

75.0 kPa. The expression of liver stiffness in kPa is based on the Young's modulus $E = 3 \rho V_s^2$, where the speed of the shave is multiplied by the density of the liver of 1.000 kg/m³, which is assumed to be the density of water (Sandrin et al., 2003).

When the technology was introduced, probes in two different sizes were available for the measurement of liver stiffness (LS): the S probe for use in children and the M probe for use in adults. In 2010, the XL-probe was announced for measurements in obese patients, because the M-probe was prone to high failure rates in patients with obesity (De Ledinghen et al., 2010). The same year, the controlled attenuation parameter (CAP) for the quantification of liver steatosis was introduced, allowing the simultaneous detection of LS and CAP. Herein, CAP expresses the attenuation of of ultrasonic waves by fat based on a patented algorithm (Sasso et al., 2010). CAP values are expressed in dB/m and range from 100 dB/m to 400 dB/m. At the time, the use of CAP was limited to the M-Probe. The ability to quantify steatosis using CAP with the XL probe was presented in early 2016 (Sasso et al., 2016).

Transient elastography measurements have been carried out in different patients with liver diseases, in particular NAFLD (Chan et al., 2014; Friedrich-Rust et al., 2012), chronic hepatitis C (Sandrin et al., 2003; Sasso et al., 2012; Ziol et al., 2005) or hepatitis B virus infections (Marcellin et al., 2009; Mi et al., 2015), alcoholic liver disease (Nahon et al., 2008), primary biliary cholangitis and primary sclerosing cholangitis (Corpechot et al., 2006). Other studies performed measurements in cohorts of patients with various liver diseases, foremost NAFLD and hepatitis B and C (De Ledinghen et al., 2012; Myers et al., 2012a; Yilmaz et al., 2014). Smaller studies in children with NAFLD or Wilson's disease have also been executed (Cho et al., 2015; Desai et al., 2016; Stefanescu et al., 2016).

The European guideline on non-invasive testing in liver disease, published in 2015, lists several advantages for the use of transient elastography: commonly used, validated, user-friendly, good reproducibility, excellent performance for cirrhosis with an AUROC > 0.9 compared to biopsy. In contrast, the guideline also states the lack of differentiation of intermediate fibrosis stages and points to the influence of factors such as prior food intake, acute hepatitis and extra-hepatic cholestasis (European Association for the Study of the Liver and Asociación Latinoamericana para el Estudio del Hígado, 2015). The German guideline on diagnostics in NAFLD, also published in 2015, suggests the use of TE, Acoustic Radiation Force Impulse Imaging, Elast-PQ and Supersonic Shear-Wave Elastography for the non-invasive diagnosis of fibrosis. There is a strong consensus for TE to exclude advanced fibrosis and cirrhosis, and biopsy is recommended for verification (Roeb et al., 2015).

1.6. Management of Non-alcoholic fatty liver disease

As previously described, steatosis is not related to liver-associated morbidity and mortality but may progress to NASH, fibrosis and further to cirrhosis. Patients with NAFLD often present with co-morbidities, including obesity, dyslipidemia, insulin resistance and type 2 diabetes, referred to as components of the metabolic syndrome (Krawczyk et al., 2010; Loria et al., 2013). Therefore, the therapeutic goal for these patients consists in the improvement of steatosis, fibrosis and co-morbidities, the prevention of disease progression and consequently better quality of life. Currently, the management of NAFLD involves permanent lifestyle changes, including diet, physical activity and behavior. As recommended by the German, European and American guidelines, the foundation of a successful therapy for NAFLD is weight loss in overweight/obese patients by 3% to 5% of body weight to improve steatosis (Chalasani et al., 2012) and a further reduction of up to 10% to improve NASH (Chalasani et al., 2012; European Association for the Study of the Liver et al., 2016; Roeb et al., 2015). Although weight loss as the main driver for improvements in NAFLD is recommended, no widely accepted dietary therapy exists to date.

Bariatric surgery showed improvements in morbily obese adults and biopsy-proven NASH: After one year, BMI decreased from 49.3 kg/m² to 37.4 kg/m², steatosis declined from 60% to 10%, fibrosis improved in 33.5% of cases, and NASH resolved in 85% of patients (Lassailly et al., 2015). The level of liver fat reduction may depend on genetic risk factors. In 84 obese patients undergoing bariatric sugery, median weight loss after 1 year was 40 kg, and the reduction in liver fat was associated with the *PNPLA3* variant. Carriers of both risk alleles showed a reductionof 85.5%, whereas carriers without the risk allele reduced by 64.1% (Krawczyk et al., 2016). This finding, together with bariatric surgery as treatment option, needs to be further elucidated in future research.

To date, no pharmacological therapy on NAFLD is available but several potential drugs are under review. Pioglitazone has a positive effect on steatosis but not fibrosis in patients with diabetes, whereas vitamin E improved NASH but not fibrosis. Albeit the positive effects, weight gain was reported in both groups (Sanyal et al., 2010), which is contrary to the recommended therapy for NAFLD to reduce body weight (Chalasani et al., 2012; European Association for the Study of the Liver et al., 2016; Roeb et al., 2015). Metformin decreases body weight but no improvement on liver function tests, fibrosis and steatosis was found (Haukeland et al., 2009). Since NAFLD is mostly associated with dyslipidemia, the use of statins may be indicated to treat commonly associated comorbidities but the effect on NAFLD has not been confirmed yet due to a limited number of randomized controlled trials (Eslami et al., 2013). Obeticholic acid, a farnesoid X receptor (FXR) agonist, improves

insulin sensitivity, hepatic inflammation and fibrosis by after 72 weeks via reduction of bile synthesis (Neuschwander-Tetri et al., 2015). However, pruritus was reported in 23% of patients and total cholesterol and LDL cholesterol concentrations in blood increased (Neuschwander-Tetri et al., 2015). Elafibranor, a PPARα/δ agonist, resolved NASH in 19% of 91 patients, showed a tendency of improvement of steatosis over fibrosis and had positive effects on cardiometabolic risk factors, i.e. triglycerides, LDL- and HDL-cholesterol (Ratziu et al., 2016). Resolution of NASH was also achieved in patients on liraglutide, a glucagon-like peptide-1-receptor agonist, while a small proportion showed progression of fibrosis (Armstrong et al., 2016).

2. Objective of the doctoral thesis

The aim of this doctoral thesis is to explore whether transient elastography as a non-invasive method can be applied for the assessment and monitoring of patients with NAFLD. Four consecutive studies were planned, executed, analyzed and published in peer-reviewed journals and consequently, they are the framework for this cumulative doctoral thesis.

At first, we studied the association of the controlled attenuation parameter to assess hepatic steatosis by transient elastography with the common steatogenic risk variants *PNPLA3 p.I148M* and *TM6SF2 p.E167K*. We carried out an observational study of 174 NAFL patients who were admitted for diagnostic work-up and treatment of chronic liver diseases (CLD) to the tertiary referral center of the Department of Internal Medicine II at Saarland University Medical Center in Homburg, Germany. The manuscript of the study is presented in chapter 3 as ***The common PNPLA3 variant p.I148M is associated with liver fat contents as quantified by controlled attenuation parameter (CAP)***.

Second, we monitored the effect of a short-term hypocaloric high-fiber, high-protein diet in 60 patients with hepatic steatosis by transient elastography and body composition using body impedance analysis while surveying the response of carriers of the *PNPLA3* risk variant p.148M to the diet. The study was carried out in cooperation with four nutrition centers in the Saarland and Palatinate region in Southwest Germany. The manuscript of the study is presented in chapter 4 as ***Short-Term Hypocaloric High-Fiber and High-Protein Diet Improves Hepatic Steatosis Assessed by Controlled Attenuation Parameter***.

Next, we carried out a study in cooperation with the occupational doctors of Saarland University Medical Center in 133 employees undergoing occupational health check-ups. Here, the focus was to determine whether transient elastography is superior for the detection of hitherto unknown steatosis and fibrosis compared to commonly used serum surrogate markers. The manuscript of the study is presented in chapter 5 as ***Nichtinvasive Früherkennung von Lebererkrankungen im Rahmen der betrieblichen Gesundheitsförderung (Noninvasive early detection of liver diseases as part of occupational health check-ups)***.

Finally, we published a response to the recent Germany S2k guideline on non-alcoholic fatty liver disease to underpin the feasibility and practicability in assessing liver health using transient elastography in clinically obtained data in almost 6,700 measurements in patients with CLD and healthy subjects at the Department of Internal Medicine II at Saarland University Medical Center in Homburg, Germany. The manuscript of the study is presented in chapter 6 as ***Zur Diagnose der Fettleberkrankheit (Diagnosis of fatty liver disease)***.

3. The common PNPLA3 variant p.I148M is associated with liver fat contents as quantified by controlled attenuation parameter (CAP)

Published in Arslanow A, Stokes CS, Weber SN, Grünhage F, Lammert F, Krawczyk M. *Liver International* 2016 Mar;36(3):418-26

The published manuscript can be found in Appendix 1.

3.1. Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is becoming the most prevalent liver disorder. The *PNPLA3* (adiponutrin) variant p.I148M has been identified as common genetic modifier of NAFLD. Our aim was to assess the relationships between genetic risk and non-invasively measured liver fat content.

Methods: Hepatic steatosis was quantified by transient elastography, using the controlled attenuation parameter (CAP) in 174 patients with chronic liver diseases (50% women, age 18–77 years). In addition, a cohort of 174 gender-matched healthy controls (50% women, age 32–77 years) was recruited. The *PNPLA3* mutation as well as the novel NAFLD-predisposing genetic variant (*TM6SF2* p.E167K) were genotyped with allele-specific probes.

Results: The *PNPLA3* genotype correlated significantly (P = 0.001) with hepatic CAP measurements. The p.148M risk allele increased the odds of developing liver steatosis (OR = 2.39, P = 0.023). In multivariate models, BMI and *PNPLA3* mutation were both independently associated with CAP values (P < 0.001 and P = 0.007, respectively). Carriers of the *TM6SF2* risk allele presented with increased aminotransferase activities (ALT: P = 0.007, AST: P = 0.004), but the presence of this variant did not affect CAP values.

Conclusions: The *PNPLA3* p.I148M variant represents the most important prosteatotic genetic risk factor. NAFLD carriers of this variant should be followed up carefully, with elastography and CAP being ideally suited for this purpose.

3.2. Key points

1. The common *PNPLA3* p.I148M variant is associated with increased CAP levels in patients with chronic liver diseases.
2. CAP represents a novel non-invasive method to characterize NAFLD patients and measure liver fat in genetic studies.
3. The *PNPLA3* and *TM6SF2* variants are associated with serum surrogate markers of liver injury.
4. *PNPLA3* remains the most clinically significant genetic marker of hepatic steatosis.

3.3. Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease (CLD) in Western countries and its prevalence continues to increase. NAFLD prevalence varies among ethnic groups in Europe and may reach 50% of the general population (Blachier et al., 2013). Fatty liver is defined by the abnormal accumulation of fat in

hepatocytes, which can progress to steatohepatitis, fibrosis and cirrhosis (Castera et al., 2013; Michelotti et al., 2013). The diagnosis and assessment of hepatic steatosis has been until recently based primarily on abdominal ultrasonography and liver biopsy. Ultrasonography is widely available (Cheung et al., 2014), but is only semiquantitative, strongly depends on operator experience and is associated with intra- and interoperator variability (Polyzos and Mantzoros, 2014). Liver biopsy is generally considered to represent the 'gold standard' for the detection and assessment of steatosis, although it represents only about 1/50,000th of the liver volume and is affected by sampling errors (Campbell and Reddy, 2004; Ratziu et al., 2005). In addition, biopsy is costly, carries specific risks such as bleeding and does not allow regular follow-up measurements. Magnetic resonance imaging (MRI) and computed tomography (CT) as non-invasive steatosis imaging techniques are limited by availability and costs.

The controlled attenuation parameter (CAP) represents a novel quantitative parameter based on the attenuation of the ultrasound signal by liver fat (Castera et al., 2013) determined during non-invasive transient elastography. It is a non-invasive, reliable and patient-friendly tool for the quantification of liver fat contents (Sasso et al., 2010) and can be readily integrated into routine work-up and follow-up of patients with CLD. Recently, CAP measurements have been evaluated in patients with NAFLD, non-alcoholic steatohepatitis as well as chronic hepatitis B and C virus infections (Carvalhana et al., 2014; De Ledinghen et al., 2012; Ferraioli et al., 2014a; Masaki et al., 2013; Sasso et al., 2010; Sasso et al., 2012).

The occurrence of NAFLD is generally attributed to different interacting factors, in particular insulin resistance, diabetes and obesity but may also occur without these obvious environmental challenges (Hwang et al., 2010; Krawczyk et al., 2010). In a genome-wide association study (Romeo et al., 2008), the variant p.I148M rs738409 of the triglyceride hydrolase patatin-like phospholipase domain containing 3 (*PNPLA3*) was demonstrated to increase the susceptibility for hepatic steatosis, steatohepatitis, fibrosis and cirrhosis. For example, Valenti et al. (Valenti et al., 2010) assessed the influence of this risk variant on liver histopathology and reported that it was associated with the grade of steatosis in 574 adults, conferring an odds ratio (OR) of 1.35. Our group availed of non-invasive assessment of liver fibrosis by transient elastography, demonstrating that the *PNPLA3* variant increases liver stiffness and represents a risk factor for developing cirrhosis (OR = 1.56) in 899 patients with various CLDs (Krawczyk et al., 2011). This finding is supported by a recent meta-analysis reporting that the [M] allele results in an elevated cirrhosis risk as compared to the [I] allele (OR = 1.86) (Shen et al., 2015b). As demonstrated in *Pnpla3* p.148M knock-in mice, the mutation leads to the accumulation of PNPLA3 on lipid droplets and an increase in

triglyceride concentrations in liver in the presence of a dietary overload (Smagris et al., 2015). In fact, the gene variant may confer a substantial risk for hepatic steatosis and consecutive inflammation in humans consuming Westernized diets, as highlighted by the term *PNPLA3*-associated steatohepatitis (PASH) (Krawczyk et al., 2013b). Very recently, the novel but less common gene variant *TM6SF2* p.E167K has been reported to increase NAFLD risk (OR = 1.37) (Sookoian et al., 2015).

To date, no study has been carried out to show the association between non-invasively quantified hepatic fat content assessed by CAP and the steatogenic risk variants of the *PNPLA3* and *TM6SF2* genes. Hence, the specific aim of this study was to determine if these variants influence elastography-based phenotypes such as hepatic steatosis and fibrosis and to analyse the correlation between these novel phenotypes, risk genotypes and conventional non-invasive surrogate markers of liver injury.

3.4. Patients and methods

Patient cohorts

This observational study recruited 174 patients admitted to the Department of Internal Medicine II at Saarland University Medical Center (Homburg, Germany), a tertiary referral centre, between September 2010 and September 2014 for diagnostic work-up and treatment of CLD. We excluded patients who presented with viral and cholestatic liver diseases (primary biliary cirrhosis, primary sclerosing cholangitis) or liver tumors. **Table 1** summarizes the clinical characteristics of the cohort. The cohort comprised 50% women, median age was 50 years (range 18–77 years) and median body mass index (BMI) was 28.4 kg/m² (range 17.2–47.3 kg/m²). Overall, 70.7% of the patients were diagnosed with NAFLD, 21.8% presented with cryptogenic CLD and 7.5% suffered from alcohol-induced liver injury. In addition, we studied 174 individuals matched by gender (50% women; median age 59 years, range 32–77 years), who underwent colonoscopy without abnormal findings at our unit and served as the control group for the comparison of *PNPLA3* genotype frequencies in the population.

All patients underwent a clinical examination, and blood samples were drawn in the fasted state. Liver function tests and other analyses were performed with standard clinical chemical assays in the certified central laboratory of our university hospital. Informed consent was obtained from each patient included in this study. The protocol of this study conforms to the ethical guidelines of the Declaration of Helsinki as reflected in *a priori* approval by the institution's human research ethics committee (Ärztekammer des Saarlandes; ref 62/09 and 271/11)

Table 1: Clinical characteristics of individuals with chronic liver diseases

Variables	Patient characteristics (*n* = 174)
***n* men / women**	87 (50%) / 87 (50%)
Age (years)	50 (18–77)
BMI (kg/m²)	28.4 (17.2–47.3)
Underweight BMI < 18.5	2 (1.2%)
Normal weight 18.5 ≤ BMI < 25	15 (8.6%)
Overweight 25 ≤ BMI < 30	50 (28.7%)
Obese BMI ≥ 30	50 (28.7%)
BMI data unavailable	57 (32.8%)
Primary liver disease	
NAFLD	123 (70.7%)
Cryptogenic CLD	38 (21.8%)
Alcoholic liver disease	13 (7.5%)
Transient elastography	
CAP (dB/m)	285 (100–398)
LSM (kPa)	6.1 (1.6–69.1)
Ultrasonography	
Grade 0	62 (35.6%)
Grade 1	55 (31.6%)
Grade 2	42 (24.2%)
Grade 3	15 (8.6%)
Liver function tests	
ALT (U/l)	47 (9–272)
AST (U/l)	35 (17–179)
AST/ALT ratio	0.73 (0.21–3.56)
γ-GT (U/l)	79 (11–1287)
AP (U/l)	78 (33–345)
Bilirubin (mg/dl)	0.6 (0.2–9.4)
Albumin (g/l)	46 (22–53)
Glucose (mg/dl)	98 (75–375)
Creatinine (mg/dl)	0.85 (0.47–1.95)
Platelets ($x10^9$/l)	232 (39–443)
INR	0.99 (0.84–2.61)
Lipid metabolism	
Total cholesterol (mg/dl)	207 (95–403)
Triglycerides (mg/dl)	125 (39–1618)
LDL cholesterol (mg/dl)	124 (17–313)
HDL cholesterol (mg/dl)	55 (16–153)

Values are displayed as medians (ranges). ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CLD, chronic liver disease; γ-GT, gamma-glutamyl transferase; INR, International Normalized Ratio; LSM, liver stiffness measurement; NAFLD, non-alcoholic fatty liver disease.

Non-invasive phenotyping of liver steatosis and fibrosis

Hepatic steatosis and liver stiffness were assessed non-invasively by vibration-controlled transient elastography (VCTE) (FibroScan®, Echosens, Paris) and expressed as CAP and liver stiffness measurement (LSM) respectively. During elastography, a shear-wave is being generated by a mechanical impulse of the transducer, which is in contact with the patient's skin. The dispersion of the shear-wave through the liver is monitored by ultrasound signals sent by the probe. As the ultrasound waves propagate through the liver, the alteration of the amplitude is determined at 3.5 MHz (M-probe) or 2.5 MHz (XL-probe for obese patients). A decrease in amplitude secondary to an increase in liver fat content is measured and expressed as CAP value in dB/m, currently only available with the M-probe. In addition, the propagation speed of the shear-wave, which increases with liver fibrosis, is determined and used for LSM calculation (expressed in kPa) using both, the M- and XL-probes.

Patients were placed in the dorsal decubitus position with the right arm stretched behind their head. All measurements were carried out in fasted patients. The probe was placed on the skin between the ribs at the midaxillary line vertical to the xyphoid process. At least 10 measurements were performed per patient. In order to include a patient's result in the study analysis, a success rate of ≥ 60% based on at least 10 valid measurements and an interquartile range (IQR)/median LSM ≤ 30% were required (Castera et al., 2008). According to a recent study, the IQR/LSM ratio was not taken into account in patients with median LSM < 7.1 kPa (Boursier et al., 2013).

For this study, we used the CAP cut-off values reported by Sasso et al. (Sasso et al., 2010), as follows: Hepatic steatosis grade S1 (corresponding to 11–32% liver fat) is defined by CAP 238–258 dB/m, grade S2 (33–65% liver fat) corresponds to CAP 259–291 dB/m, and grade S3 (≥ 66% liver fat) is indicated by CAP ≥ 292 dB/m.

For comparison, we assessed hepatic steatosis by conventional ultrasonography. Abdominal ultrasound examinations were carried out by experienced physi-cians on the Hitachi EUB-8500 system (Hitachi Medical Systems, Wiesbaden, Germany). The semiquantitative assessment of hepatic steatosis was based on the visual impression of the liver echogenicity and expressed as grades S0 to S3: grade S0 indicates the absence of steatosis, grade S1 corresponds to minimal, grade S2 to moderate and grade S3 to severe steatosis (Gerstenmaier and Gibson, 2014).

Genotyping of risk variants

Genomic DNA was isolated from the EDTA-anticoagulated blood samples using the membrane-based QIAamp DNA extraction protocol (Qiagen, Hilden, Germany). Genotyping of *PNPLA3* rs738409 and *TM6SF2* rs58542926 polymorphisms was performed using PCR-based assays with 5'-nuclease and fluores-cence detection (TaqMan®, Life Technologies, Darmstadt, Germany; rs738409: C__7241_10; rs58542926: C__89463510_10).

Statistical analyses

All statistical analyses were performed with SPSS 20.0 (SPSS, Munich, Germany) or GRAPHPAD PRISM 5.0 (GraphPad Software Inc., San Diego, CA, USA), unless stated otherwise. Two-sided P-values < 0.05 were regarded as significant. Kolmogorov–Smirnov tests were used to determine whether data were normally distributed. Quantitative data were expressed as median and ranges. Correlations were tested using Spearman's rank correlation tests. Quantitative traits were assessed using Mann–Whitney *U* or Kruskal–Wallis tests, as apropriate. The associations of categorical variables were tested in contingency tables. The genotype frequencies of all polymorphisms were tested for consistency with Hardy–Weinberg equilibrium using exact tests (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). De Finetti diagrams with Hardy–Weinberg parabola were generated using the online software tool (https://finetti.meb.unibonn.de). Allele and genotype frequency differences were assessed by chi-squared and Armitage's trend tests, respectively. Genetic associations were tested by univariate logistic and linear regression analyses; herein, significant parameters underwent fur-ther evaluation in multivariate models.

3.5. Results

Liver steatosis assessed by CAP correlates with semiquantitative ultrasonography scores

In total, we performed elastography measurements in 174 CLD patients. Eighteen cases (10.3%) were excluded as a result of an invalid elastography measurement. An LSM value was determined for all of the other patients and a CAP value for 148 patients, because in eight patients (5.1%), the XL-probe was used, for which no CAP measurement has been implemented. Median liver stiffness was 6.1 kPa (range 1.6–69.1 kPa) and median CAP was 285 dB/m, ranging from 100 to 398 dB/m. **Figure 1a** illustrates the distribution of CAP values in our cohort.

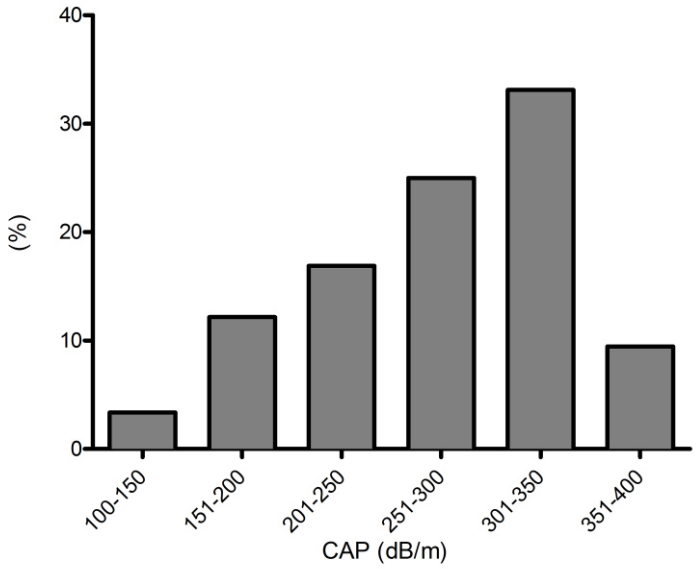

Fig. 1a: Hepatic fat distribution in 148 CLD patients. Liver steatosis was quantified using controlled attenuation parameter (CAP) during transient elastography. Values range from 100 to 398 dB/m with a median CAP of 285 dB/m.

Based on conventional ultrasonography, 62 cases with CLD (35.6%) showed no pronounced liver steatosis. Steatosis grade S1 was observed in 55 patients (31.6%), steatosis was classified as grade S2 in 42 cases (24.2%) and 15 patients (8.7%) presented with steatosis grade S3.

Overall, the prevalence of hepatic steatosis in our cohort as assessed by CAP (≥ 238 dB/m) or ultrasonography (≥ S1) was 74.3% and 64.4%, respectively. Cases were identified by both methods as positive in 67.8% and as negative in 18.6%; conflicting results were obtained in 13.6% of the patients. **Figure 1b** illustrates the significant positive correlation of CAP values and steatosis grades ($r_s = 0.442$, $P < 0.001$): Median CAP values were 279 dB/m for patients with steatosis grade S1, 317 dB/m for grade S2 and 335 dB/m for grade S3. Moreover, CAP was positively correlated with BMI and serum ALT activities (both $P \leq 0.001$) as well as serum glucose and triglyceride concentrations and LSM (all $P < 0.05$).

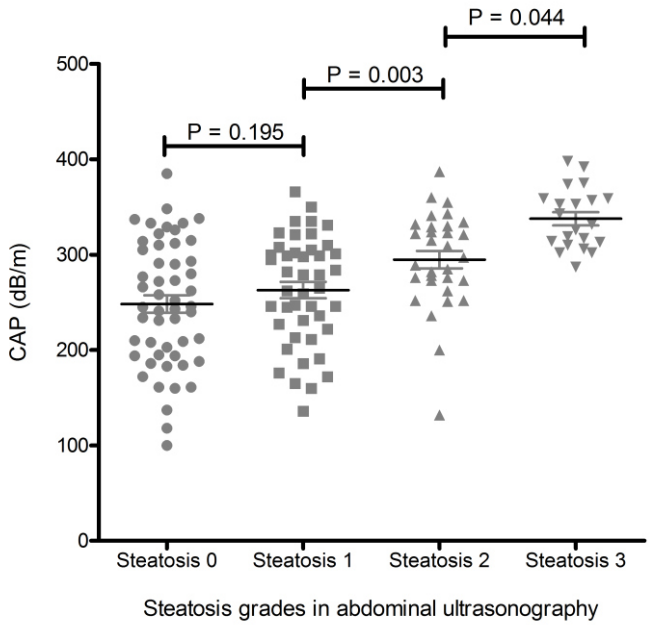

Fig. 2b: Non-invasive assessment of hepatic steatosis. Controlled attenuation parameter (CAP) correlates positively with the steatosis grade in liver ultrasonography (grade 0–3). Symbols represent the value for each patient.

Liver fat contents measured by CAP are associated with the *PNPLA3* risk variant

In this cohort, the frequencies of wild-type, heterozygous and homozygous genotypes were [II]: 87, 50.0%; [IM]: 67, 38.5%; [MM]: 20, 11.5% for the *PNPLA3* variant and [EE]: 139, 79.9%; [EK]: 33, 19.0%; [KK]: 2, 1.1% for the *TM6SF2* variant. None of the genotype distributions deviated from Hardy–Weinberg equilibrium ($P > 0.05$, exact tests; **Fig. S1a, b**), and all were consistent with the frequencies published in the *Entrez* database.

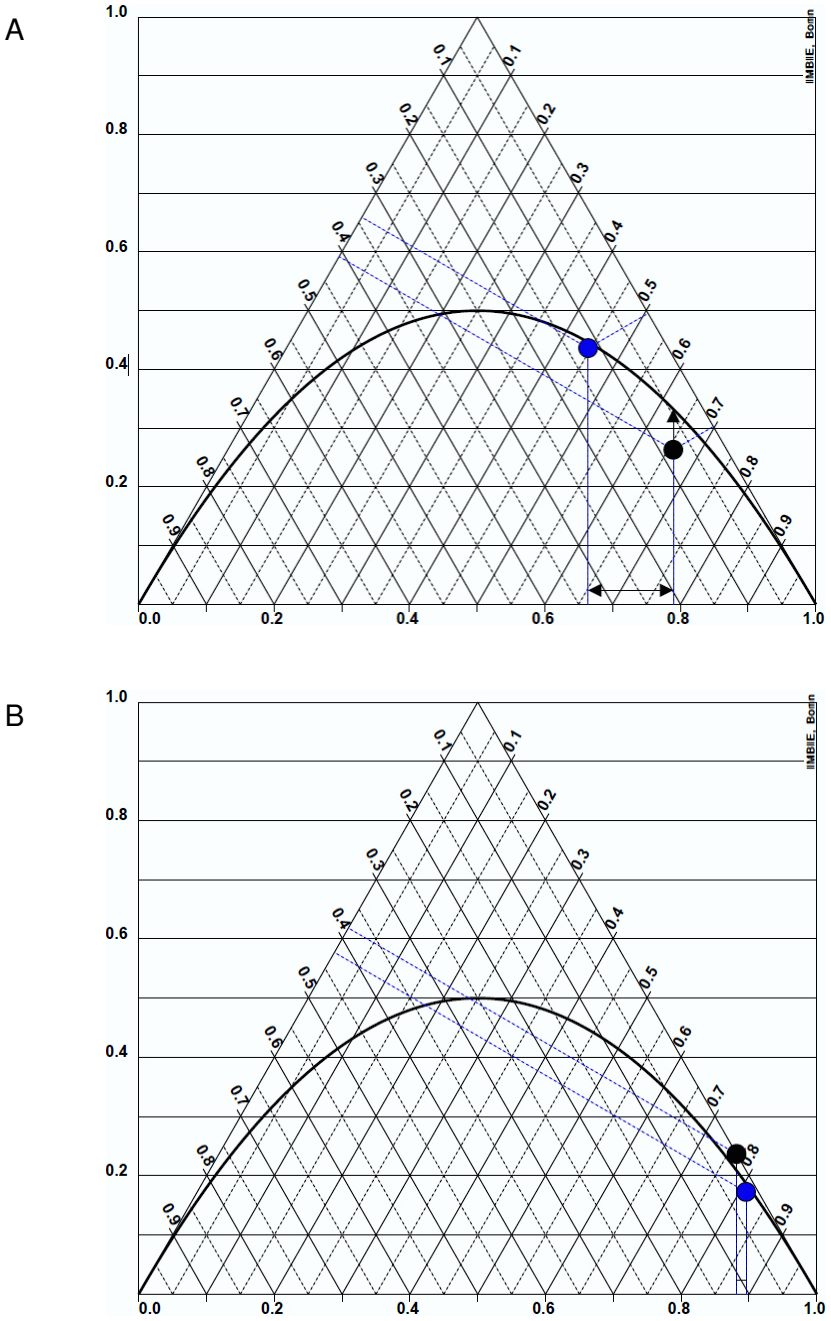

Fig. S1 – Supplementary Fig. 1a, b: De Finetti diagram with Hardy-Weinberg parabola illustrating the distributions of *PNPLA3* p.I148M (**A**) and *TM6SF2* p.E167K (**B**) genotypes in patients with hepatic steatosis, as defined by CAP ≥ 238 dB/m (blue dot), and without hepatic steatosis (CAP < 238 dB/m; black dot). The vertical axis represents the frequency of carriers of the heterozygous genotype p.I148M (**A**) / p.E167K (**B**), and the side lines of the triangle indicate the frequencies of homozygous carriers (left: wild-type allele p.I148 (A) / p.E167 (**B**); right: mutated allele p.148M (**A**) / p.167K (**B**)). The *PNPLA3* p.I148M and *TM6SF2* p.E167K genotype frequencies in cases and controls plot near the parabola (curved line), indicating that they are both in line with Hardy-Weinberg equilibrium (P > 0.05, exact tests). The frequency of the allele p.I148 (horizontal abscissa) is significantly higher in patients without than in those with hepatic steatosis (66.4% vs. 79.0%, P = 0.040; **Table 2A**). No association of the p.E167K variant with steatosis assessed using CAP was detected (P > 0.05).

Overall, CAP values showed a positive correlation with *PNPLA3* p.I148M genotypes (r = 0.302, P = 0.001). **Figure 2a** illustrates the significant differences in median CAP measurements across the carriers of distinct *PNPLA3* genotypes, which were 261 dB/m (100–392 dB/m), 308 dB/m (137–398 dB/m) and 318 dB/m (208–359 dB/m) in patients with genotpyes [II] (n = 74), [IM] (n = 58) and [MM] (n = 16), respectively. The data are consistent with a dominant model of inheritance with respect to the liver steatosis phenotype, as the differences in CAP between carriers of *PNPLA3* genotypes [II] and [MM] as well as genotypes [II] and [IM] were significant (P = 0.004 and P = 0.003, respectively), whereas CAP did not differ between heterozgyous and homozygous carriers of the risk allele p.148M (P > 0.05). The same associations were observed for men in a subgroup analysis, however, for women, significant CAP values were only detected between carriers of genotypes [II] and [MM] (data not shown).

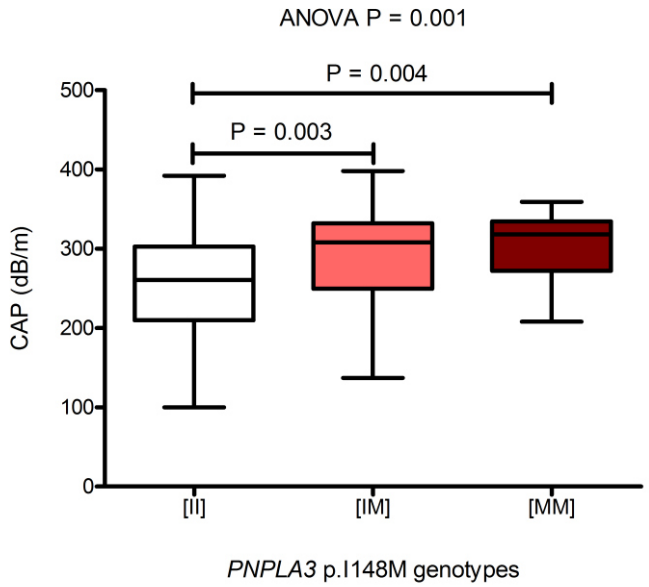

Fig. 2a: Relationship between controlled attenuation parameter (CAP) values among carriers of different *PNPLA3* p.I148M genotypes: [II] (*n* = 74), [IM] (*n* = 58) and [MM] (*n* = 16).

Figure 2b depicts the distributions of *PNPLA3* genotypes in relation to CAP levels. Of note, the frequency of individuals carrying the *PNPLA3* risk allele [M] increased at higher CAP values. **Table S1A** summarizes the results of the univariate linear regression analysis with CAP being significantly related to BMI and *PNPLA3* genotypes (P < 0.001 and P = 0.023) but not with age, gender or LSM. In the multivariate model (**Table S1B**), CAP remained associated with BMI and *PNPLA3* genotypes (P < 0.001 and P = 0.007).

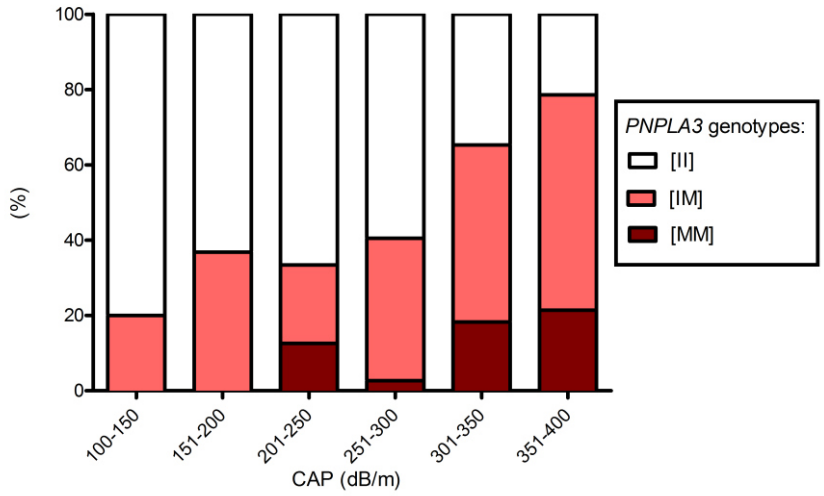

Fig. 2b: Distribution of *PNPLA3* genotypes [II], [IM] and [MM] in relation to CAP classes.

Table S1 – Supplementary table 1: Univariate and multivariate analysis of determinants of CAP and LSM.

	Beta coefficient	***P***
A - Univariate analysis of CAP		
PNPLA3 p.I148M	0.186	**0.023**
Age	0.004	> 0.05
Gender	-0.117	> 0.05
BMI	0.460	**< 0.001**
LSM	-0.098	> 0.05
B - Multivariate analysis of CAP		
PNPLA3 p.I148M	0.249	**0.007**
BMI	0.446	**< 0.001**
C - Univariate analysis of LSM		
PNPLA3 p.I148M	-0.111	> 0.05
Age	0.289	**< 0.001**
Gender	0.118	> 0.05
BMI	0.005	> 0.05
CAP	-0.098	> 0.05

CAP, controlled attenuation parameter; LSM, liver stiffness measurement; *P*, p-value; *PNPLA3*, patatin-like phospholipase domain containing 3. Significant results are highlighted (bold).

The *PNPLA3* risk allele increases the risk of liver steatosis determined by CAP

Table 2A summarizes that in patients with hepatic steatosis, as defined by CAP ≥ 238 dB/m, and in patients with CAP < 238 dB/m, the frequencies of *PNPLA3* p.I148M [II], [IM] and [MM] genotypes were 44.6% vs. 65.8%, 43.6% vs. 26.3%, and 11.8% vs. 7.9%, respectively. Overall, the presence of the *PNPLA3* risk allele, p.148M, significantly increased the odds of

developing hepatic steatosis (odds ratio = 1.90, 95% confidence interval = 1.02–3.53, P = 0.040; **Table 2A**). Of the 174 patients, 25 (14.4%) had a diagnosis of diabetes mellitus type 2 and these patients had a significantly higher median CAP value as compared to the non-diabetics, (314 dB/m vs. 279 dB/m, P = 0.034). The groups of patients with and without steatosis did not differ in age and gender, but the difference in BMI (28.8 kg/m² vs. 25.0 kg/m²) was significant (P < 0.001). In the logistic univariate analysis (**Table S2A**), both *PNPLA3* mutation and BMI (P = 0.042 and P < 0.001, respectively), but not age, gender or LSM (all P > 0.05) were associated with CAP ≥ 238 dB/m. In the multivariate model (**Table S2B**), both the *PNPLA3* mutation and BMI proved to be independently associated with increased CAP values (P = 0.041 and P < 0.001, respectively).

Table 2: Distribution of *PNPLA3* p.I148M alleles and genotypes in **(A)** patients with and without hepatic steatosis; **(B)** CLD patients and healthy controls

(A)	Patients with CAP ≥ 238 dB/m (2N = 220)	Patients with CAP < 238 dB/m (2N = 76)
Counts of alleles/genotypes		
[I]	146 (66.4%)	60 (79.0%)
[M]	74 (33.6%)	16 (21.0%)
[II]	49 (44.6%)	25 (65.8%)
[IM]	48 (43.6%)	10 (26.3%)
[MM]	13 (11.8%)	3 (7.9%)
Association tests	χ^2	***P***
Allele frequency difference test	4.23	0.040
Armitage's trend test	3.94	0.050
OR statistics	**OR**	**95% CI**
[M] ↔ [I]	1.90	1.02–3.53
[MM] ↔ [IM]	2.45	1.06–5.64
[MM] ↔ [II]	2.21	0.58–8.48
[MM+IM] ↔ [II]	2.39	1.11–5.16
[MM] ↔ [IM+II]	1.56	0.42–5.82
(B)	CLD patients (2N = 348)	Healthy controls (2N = 348)
Counts of alleles/genotypes		
[I]	241 (69.3%)	270 (77.6%)
[M]	107 (30.7%)	78 (22.4%)
[II]	87 (50.0%)	104 (59.8%)
[IM]	67 (38.5%)	62 (35.6%)
[MM]	20 (11.5%)	8 (4.6%)
Association tests	χ^2	***P***
Allele frequency difference test	6.19	0.013
Armitage's trend test	5.90	0.015
OR statistic	**OR**	**95% CI**
[M] ↔ [I]	1.54	1.09–2.16
[MM] ↔ [IM]	1.29	0.83–2.02
[MM] ↔ [II]	2.99	1.26–7.12
[MM+IM] ↔ [II]	1.49	0.97–2.27
[MM] ↔ [IM+II]	2.70	1.15–6.30

CAP, controlled attenuation parameter; CI, confidence interval; CLD, chronic liver disease; I, isoleucine; M, methionine; OR, odds ratio; *P*, p-value; *PNPLA3*, patatin-like phospholipase domain containing 3. The [M] allele of the *PNPLA3* variant represents the risk allele for hepatic steatosis (Romeo et al., 2008)

Table S1 – Supplementary table 2: Univariate and multivariate analysis of hepatic steatosis defined as CAP ≥ 238 dB/m.

	OR	95% CI	*P*
A - Univariate analysis			
PNPLA3 p.I148M	2.19	1.03–4.65	**0.042**
Age	1.00	0.97–1.03	> 0.05
Gender	1.38	0.66–2.87	> 0.05
BMI	1.40	1.19–1.66	**< 0.001**
LSM	0.98	0.95–1.02	> 0.05
B - Multivariate analysis			
PNPLA3 p.I148M	3.25	1.05–10.07	**0.041**
BMI	1.40	1.18–1.67	**< 0.001**

CAP, controlled attenuation parameter; CI, confidence interval; I, isoleucine; LSM, liver stiffness measurement; M, methionine; OR, odds ratio; *P*, p-value; *PNPLA3*, patatin-like phospholipase domain containing 3. Significant results are highlighted (bold).

Based on the CAP values ranging from 100 to 400 dB/m, we performed a sensitivity analysis using different CAP thresholds at increments of 10 dB/m (**Fig. 2c**, **Table S3**). The *PNPLA3* p.148M allele was a significant (P < 0.05) risk factor for hepatic steatosis for all CAP thresholds between 190 and 340 dB/m, apart from 240 dB/m where we calculated marginal significance (P = 0.054). In this window, odds ratios (OR) for steatosis range from 3.0 to 2.1 (**Table S3**).

Fig. 2c: Sensitivity analysis of odds ratios (OR) for developing hepatic steatosis in carriers of the *PNPLA3* p.148M risk allele using CAP thresholds between 100 and 400 dB/m in increments of 10 dB/m. OR is given on a log scale. The [M] allele is a significant (P < 0.05) risk factor for hepatic steatosis for CAP thresholds between 190 and 340 dB/m (except for CAP = 240 dB/m, P = 0.054 denoted by ○) with OR for this window ranging from 3.0 to 2.1. Of note, the association with the CAP threshold of 238 dB/m used in this study based on previous publications (Sasso et al., 2010) is also significant (P = 0.040).

Table S3 – Supplementary table 3: Sensitivity analysis of CAP thresholds between 100 and 400 dB/m in carriers of the *PNPLA3* p.148M risk allele (n = 148).

CAP threshold (dB/m)	OR	*P*
100	0.44	1.000
110	2.21	1.000
120	4.02	0.318
130	4.02	0.318
140	4.07	0.292
150	4.07	0.292
160	4.07	0.292
170	2.61	0.121
180	2.57	0.081
190	3.01	**0.022**
200	2.60	**0.023**
210	2.17	**0.036**
220	2.08	**0.033**
230	2.05	**0.032**
240	1.81	0.054
250	2.13	**0.009**
260	2.50	**0.001**
270	2.07	**0.007**
280	2.49	**0.001**
290	2.30	**0.001**
300	3.00	**< 0.001**
310	2.72	**< 0.001**
320	2.54	**< 0.001**
330	2.25	**0.006**
340	2.05	**0.040**
350	1.88	0.104
360	1.04	1.000
370	0.76	1.002
380	0.32	0.459
390	0.76	1.157
400	2.28	1.000

CAP, controlled attenuation parameter; LSM, liver stiffness measurement; M, methionine; OR, odds ratio; *P*, p-value; *PNPLA3*, patatin-like phospholipase domain containing 3. Significant results are highlighted (bold).

The frequencies of the genotypes [EE], [EK] and [KK] in the *TM6SF2* gene were 80.9%, 17.3% and 1.8% vs. 76.3%, 23.7%, 0% and 80.0% in patients with CAP values ≥ 238 dB/m vs. < 238 dB/m, respectively. Overall, this variant did not significantly influence the risk of developing hepatic steatosis (P > 0.05).

Carriers of the *PNPLA3* and *TM6SF2* risk alleles present with increased serum aminotransferase activities

Overall, we detected significantly increased serum activities of ALT (53 U/l vs. 42 U/l, P = 0.014; **Fig. 3a**) and AST (37 U/l vs. 33 U/l, P = 0.008) as well as HDL concentrations (57 mg/dl vs. 50 mg/dl, P = 0.014) in carriers of the *PNPLA3* p.148M risk allele. In carriers of the *TM6SF2* genotypes, serum ALT (57 U/l vs. 44 U/l, P = 0.007; **Fig. 3b**) and AST activities (48 U/l vs. 33 U/l, P = 0.004) were increased.

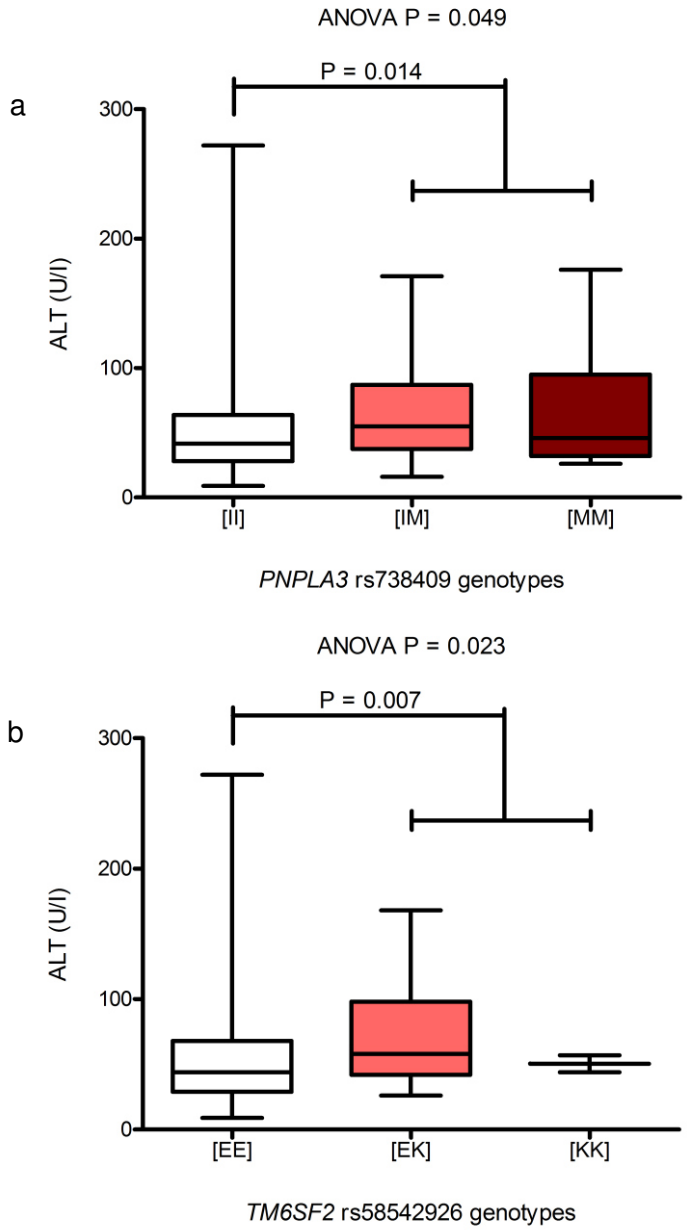

Fig. 3. Serum ALT activities in relation to (**a**) *PNPLA3* and (**b**) *TM6SF2* genotypes in 174 CLD patients.

As expected, a positive association between hepatic steatosis and fibrosis, assessed using CAP and LSM, was observed (r = 0.174, P = 0.035). **Table S1C** demonstrates that LSM values were affected by patients' age (P < 0.001) but not by gender or BMI (both P > 0.05). For carriers of distinct *PNPLA3* genotypes [II] (n = 78), [IM] (n = 62) and [MM] (n = 16), median values were 6.2 kPa (1.6–46.4 kPa), 6.4 kPa (2.5–69.1 kPa) and 5.0 kPa (3.0–12.8 kPa) but did not differ significantly between genotypes (P > 0.05). No association between the *TM6SF2* variant and CAP or LSM results were detected (**Table S4**).

Table S4 – Supplementary table 4: Distribution of *PNPLA3*, and *TM6SF2* genotypes in relation to CAP and LSM.

	***PNPLA3* p.I148M rs738409**		
	[II]	[IM]	[MM]
CAP (dB/m)	261 (100–392)	308 (137–398)	318 (208–359)
LSM (kPa)	6.2 (1.6–46.4)	6.4 (2.5–69.1)	5.0 (3.0–12.8)
	***TM6SF2* p.E167K rs58542926**		
	[EE]	[EK]	[KK]
CAP (dB/m)	283 (100–398)	283 (118–359)	320 (302–338)
LSM (kPa)	6.1 (1.6–52.3)	6.3 (3.0–69.1)	9.3 (4.4–14.1)

LSM and CAP were available in 156 and 148 patients, respectively (*see* Results). Values are displayed as medians (ranges). CAP, controlled attenuation parameter; E, glutamate; K lysine; LSM, liver stiffness measurement; *PNPLA3*, patatin-like phospholipase domain containing 3; *TM6SF2*, transmembrane 6 superfamily member 2.

Carriers of the *PNPLA3* risk allele are at risk of presenting with CLD

Patients referred to our liver clinic displayed significantly higher frequencies of the *PNPLA3* risk allele p.148M as compared to healthy controls (P < 0.05). **Table 2B** summarizes the distribution of alleles and genotypes in these two groups. The *PNPLA3* risk allele conferred a 1.54-fold increased risk of presenting with CLD as compared to the controls (95% confidence interval = 1.26–7.12, P = 0.013). Overall, individuals carrying the prosteatotic *PNPLA3* genotypes were more likely to develop hepatic phenotypes that led to referral to a tertiary reference centre for further work-up.

3.6. Discussion

In previous genetic studies, liver steatosis and fibrosis were mostly quantified using liver biopsy, CT and MRI, or circulating serum surrogate markers (Krawczyk et al., 2013b). In the current report we availed of the non-invasive VCTE-based approach including the recently developed CAP methodology. This allowed us, firstly, to quantify the effects of the *PNPLA3* p.I148M mutation on hepatic fat contents. Secondly, we incorporated the newly detected

genetic risk factor *TM6SF2* for NAFLD in our analysis to further assess the landscape of inherited predisposition to liver steatosis.

Although CAP is a relatively new tool, a recent meta-analysis of nine CAP-based studies has underscored its specificity and sensitivity for the assessment of hepatic steatosis (Shi et al., 2014). VCTE with CAP is a rapid, patient-friendly method providing immediate results concerning not only the degree of steatosis but also liver stiffness. In addition to these advantages, our current study demonstrates that CAP can be used for a timely recruitment of CLD patients for genetic analyses of liver steatosis. In our previous elastography-based studies in different cohorts, we demonstrated that the *PNPLA3* (Krawczyk et al., 2011) and *SREBP1c* (Krawczyk et al., 2013a) variants as well as polymorphisms in the vitamin D pathway (Grünhage et al., 2012) are associated with hepatic injury. Of note, all these studies were per-formed without CAP, which was not available at the time. Herein, we extend these observations by demonstrating a strong association between the *PNPLA3* risk allele and increased CAP values independent from other non-genetic triggers.

CAP is a novel tool for quantifying hepatic steatosis and so far no clear cut-off values for NAFLD have been developed. Indeed, previous studies claimed that CAP results ranging from 215 dB/m (De Ledinghen et al., 2012) to 300 dB/m (Karlas et al., 2014) might be regarded as thresholds for diagnosing steatosis. Based on these uncertainties, we performed a sensitivity analysis demonstrating that the *PNPLA3* mutation is associated with an increased risk of higher CAP values using thresholds between 190 and 340 dB/m. Of note, the ORs for steatosis influenced by *PNPLA3* tend to decrease with higher CAP thresholds, taking into account that values at the extremes are unreliable owing to the small number of cases. This observation indicates stronger genetic effects in patients with moderately elevated CAP but multifactorial effects in severe NAFLD. Hence, this unique analysis, which is possible only in the setting of VCTE-based data acquisition, further supports the role of variant *PNPLA3* as a common risk factor for increased hepatic fat accumulation.

The inclusion of both CAP and LSM in this study gave us the opportunity to investigate the role of *PNPLA3* p.I148M variant in modulating steatosis and fibrosis in CLD. Interestingly, carriers of the *PNPLA3* risk allele, p.148M, showed increased CAP levels but this variant did not significantly affect LSM. This observation contrasts with our previous study in 899 CLD patients (Krawczyk et al., 2011) but is consistent with a report by Shen et al. (Shen et al., 2014), who did not detect a significant association between the *PNPLA3* variant and LSM in 251 NAFLD patients either. The negative LSM results are most likely related to the smaller sample and indicate that the genetic effects on CAP might be stronger than the more indirect effects on liver fibrogenesis. We speculate that the VCTE-based results indirectly

demonstrate that the *PNPLA3* risk allele might have more pronounced effects on hepatic steatosis as compared to liver fibrosis. This underpins a functional analysis performed in *Pnpla3* 148M knock-in mice (Smagris et al., 2015). This study has demonstrated that a high-sucrose diet leads to the accumulation of PNPLA3 on the surface of intrahepatic lipid droplets, which are, in turn, larger in size and contribute to hepatic steatosis (Smagris et al., 2015). The molecular mechanisms that lead to fatty liver and fibrosis, and whether they are secondary to (extra)hepatic fat accumulation or if other *PNPLA3*-related pathways could be involved, have not been elucidated thus far. Recently *PNPLA3* has been identified to play a role as lipase in retinol metabolism in hepatic stellate cells, which are critical for fibrogenesis (Pirazzi et al., 2014). On the other hand, the exact function of *PNPLA3* is to be defined (Dongiovanni et al., 2013) and it remains unclear if the enzyme represents a triglyceride hydrolase (Pingitore et al., 2014) and/or lysophosphatidic acid acyltransferase (Kumari et al., 2012) *in vivo*. Studies in mouse models do not clearly support either gain- or loss-of-function effects of the p.I148M variant (Dongiovanni et al., 2013). Hence, further studies are needed to delineate the role of *PNPLA3* and to elucidate the mechanisms that lead to increased hepatic fat accumulation in carriers of the risk allele p.148M (Anstee and Day, 2013).

In contrast to previous genetic analyses concerning hepatic steatosis and fibrosis, in the current report not only do we use VCTE to measure liver injury but we also extend the number of genotyped variants by including the *TM6SF2* polymorphisms. The increased serum aminotransferase activities in carriers of the risk variant are consistent with previous studies (Dongiovanni et al., 2015; Kozlitina et al., 2014; Liu et al., 2014b; Sookoian et al., 2015). As a result of its low frequency (minor allele frequency around 0.1% in European populations), the *TM6SF2* variant rs58542926 was first identified in a cohort comprising more than 80,000 individuals (Kozlitina et al., 2014). This low frequency of the risk allele, resulting in decreased power of the analysis, is also a potential explanation for the lack of association between this variant and CAP. Similar to *PNPLA3*, *TM6SF2* could be involved in the remodelling of lipid droplets (Mahdessian et al., 2014) but also play a role in VLDL secretion (Kozlitina et al., 2014). Although it remains unclear whether the *TM6SF2* variant is associated with both liver steatosis and fibrosis (Liu et al., 2014b; Sookoian et al., 2015), our results underscore the genetic burden in carriers of the minor allele who are at risk of progressive liver injury in the setting of chronic liver diseases.

In our study we did not perform invasive assessment of liver phenotypes by liver biopsy. On the other hand, we used three non-invasive methods, namely CAP, VCTE and abdominal ultrasonography, to assess liver status in the recruited patients. Although we had a 10% failure rate in obtaining reliable LSM results (mostly because of obesity), the comparison of CAP values and steatosis as quantified by abdominal ultrasonography confirmed the good

correlation between these two methods. Moreover, as we detected increased CAP values in carriers of the prosteatotic *PNPLA3* variant, our results further validate CAP as a reliable method of quantifying liver fat. Comparable results were recently obtained by Karlas et al. (Karlas et al., 2015) in a study performed in transplanted patients, which demonstrated that graft recipients bearing the *PNPLA3* p.148M allele are at risk of increased VTCE-quantified liver steatosis and stiffness.

Mounting evidence suggests that the *PNPLA3* p.I148M variant represents the major genetic determinant of hepatic steatosis (Dongiovanni et al., 2013). Our study expands on these observations by identifying CAP as a new tool to measure liver fat in genetic studies and by showing its value in the carriers of the *PNPLA3* mutation. Given the low frequencies of the *TM6SF2* risk genotypes in the general population, variant *PNPLA3* p.I148M remains, in our view, the most clinically important prosteatotic genetic risk factor. Individuals carrying this mutation should be included in prospective studies with repeat measurements of steatosis and fibrosis progression, which could be accomplished in a timely and non-invasive manner using elastography with CAP.

3.7. References

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4. Short-Term Hypocaloric High-Fiber and High-Protein Diet Improves Hepatic Steatosis Assessed by Controlled Attenuation Parameter

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The published manuscript can be found in Appendix 2.

4.1. Abstract

OBJECTIVES: Non-alcoholic fatty liver disease is one of the most prevalent liver diseases and increases the risk of fibrosis and cirrhosis. Current standard treatment focuses on lifestyle interventions. The primary aim of this study was to assess the effects of a short-term low-calorie diet on hepatic steatosis, using the controlled attenuation parameter (CAP) as quantitative tool.

METHODS: In this prospective observational study, 60 patients with hepatic steatosis were monitored during a hypocaloric high-fiber, high-protein diet containing 1,000 kcal/day. At baseline and after 14 days, we measured hepatic fat contents using CAP during transient elastography, body composition with bioelectrical impedance analysis, and serum liver function tests and lipid profiles using standard clinical–chemical assays.

RESULTS: The median age was 56 years (25–78 years); 51.7% were women and median body mass index was 31.9 kg/m^2 (22.4–44.8 kg/m^2). After 14 days, a significant CAP reduction (14.0%; $P < 0.001$) was observed from 295 dB/m (216–400 dB/m) to 266 dB/m (100–353 dB/m). In parallel, body weight decreased by 4.6% ($P < 0.001$), of which 61.9% was body fat. In addition, liver stiffness ($P = 0.002$), γ-GT activities, and serum lipid concentrations decreased (all $P < 0.001$).

CONCLUSIONS: This study shows for the first time that non-invasive elastography can be used to monitor rapid effects of dietary treatment for hepatic steatosis. CAP improvements occur after only 14 days on short-term low-calorie diet, together with reductions of body composition parameters, serum lipids, and liver enzymes, pointing to the dynamics of hepatic lipid turnover

4.2. Study Highlights

WHAT IS CURRENT KNOWLEDGE

- NAFLD is a global rapidly growing health problem
- Non-inasive methods are increasingly being used to evaluate hepatic steatosis

WHAT IS NEW HERE

- Profound reduction of hepatic steatosis can be detected after only 14 days of dietary intervention using the controlled attenuation parameter
- Calorie reduced high-fiber and high-protein diet causes dynamic short-term changes of hepatic and systemic lipids

- These can be simultaneously and non-invasively assessed by the combination of transient elastography and bioelectrical impedance analysis

4.3. Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of progressive liver conditions in the absence of significant alcohol consumption. Bland steatosis occurs when intra-hepatic triglycerides accumulate in hepatocytes, which may progress to non-alcoholic steatohepatitis (NASH) if accompanied by inflammation (Chalasani et al., 2012). In 10–25% of patients, steatosis advances to hepatic fibrosis, cirrhosis, and end-stage liver disease. In addition, the likelihood of cardiovascular disease is increased when NAFLD occurs, and consequently, these patients have an increased risk of overall and liver-specific mortality (Adams et al., 2005b; Than and Newsome, 2015). NAFLD has emerged as one of the most wide-spread liver diseases in western societies, with prevalence estimates ranging up to 50% and even higher in diabetics (Blachier et al., 2013). This variation is based on differences in screening and detection strategies as well as genetic and environmental risk factors (Loomba et al., 2015; Ratziu et al., 2010). For instance, overweight, type 2 diabetes (Loria et al., 2013) as well as genetic predisposition, such as the patatin-like phospholipase domain containing 3 (*PNPLA3*) variant p.I148M, are all implicated in fatty liver manifestation (Romeo et al., 2008; Stickel et al., 2015). Specifically, carriers of the *PNPLA3* risk allele carry a more than twofold increased steatosis risk (Arslanow et al., 2016) as well as an increased likelihood of developing fibrosis, cirrhosis, and hepatocellular carcinoma (Krawczyk et al., 2011; Liu et al., 2014a).

Currently, treatment options for NAFLD are limited and no accepted standard pharmacotherapy exists. According to AASLD guidelines, reduction of body weight of at least 3–5% through a hypocaloric diet alone or together with increased physical activity has been recommended to reduce steatosis (Chalasani et al., 2012). Lifestyle intervention studies, specifically diet alone (such as, low-fat or low-carbohydrate diets) or combined with physical activity, have shown potential in ameliorating hepatic steatosis (Haufe et al., 2011; Thoma et al., 2012). Specifically, preliminary data suggests that a protein-enriched dietary intervention reduces hepatic steatosis in obese patients (Petersen et al., 2005). However, the majority of studies have employed serum surrogate markers (Angulo et al., 2013), semiquantitative ultrasonography (Mishra and Younossi, 2007), elaborate computer tomography, or magnetic resonance imaging (Patel et al., 2015). Only few studies have used liver biopsy, the “gold standard” for assessing histological changes in hepatic steatosis (Promrat et al., 2010; Tendler et al., 2007), which carries a risk of bleeding and is affected by sampling errors (Ratziu et al., 2005; Terjung et al., 2003). The use of non-invasive and risk-

free techniques to diagnose and monitor hepatic steatosis is highly sought after (Berzigotti, 2014). As such, non-invasive techniques are increasingly being evaluated. During ultrasound-based vibration-controlled transient elastography (VCTE), the attenuation of low-frequency ultrasound waves by liver tissue can be measured. The controlled attenuation parameter (CAP) quantifies liver fat (while simultaneously detecting liver stiffness) (Sasso et al., 2010). VCTE is the most widely validated technique for the detection of liver fibrosis, as documented by the new European Association for the Study of the Liver (EASL) recommendations on non-invasive tests for the evaluation of liver diseases (European Association for the Study of the Liver and Asociación Latinoamericana para el Estudio del Hígado, 2015) .

Until now, no study has compared CAP at baseline and at follow-up in combination with a dietary intervention. The aim of this study was to monitor patients with hepatic steatosis receiving a short-term hypocaloric high-fiber, high-protein diet with the primary outcome of improving liver fat, as quantified with CAP. We hypothesized that this 14-day low-calorie diet would significantly reduce CAP and therefore hepatic steatosis.

4.4. Patients and methods

Study design

This prospective observational pilot study followed patients with fatty liver taking part in a dietary program at four nutrition centers between September 2013 and April 2014 in the Saarland and Palatinate region in Southwest Germany. Specifically, patients received a 14-day hypocaloric high-fiber, high-protein liquid formula diet (HEPAFAST) containing three shakes per day with a total of 786 kcal (41% protein, 29% carbohydrate, 24% fat, and 6% fiber). The formula alone consists of 14.2 g soluble fiber (soluble to insoluble fiber ratio of 3:2) and provides 21 g of total fiber daily. **Table 1** summarizes the full nutrient composition. In addition, one to two portions of non-starchy vegetables were recommended daily, bringing the total energy intake to 1,000 kcal/day. To support digestion of the fiber-enriched product, patients were advised to drink at least 2 l of calorie-free beverages per day. Group meetings were offered at baseline and after seven and 14 days to provide background information and to support compliance with the diet. No other specific dietary or physical activity targets were given. The patients were asked to maintain their habitual level of physical activity.

Table 1: HEPAFAST nutrient composition

	Per 100 g of powdered product	**Per 30 g of powdered product in 350 ml milk (fat content 1.5%)**
Energy and nutrient content		
Energy	324 kcal (1,359 kJ)	262 kcal (1,100 kJ)
Fat	4.8 g	7.0 g
Saturated fat	1.5 g	3.6 g
Total carbohydrate	5.8 g	19.0 g
Sugar	3.5 g	18.0 g
Fiber	24.0 g	7.0 g
Protein	51.0 g	27.0 g
Sodium	101 mg	195 mg
Key ingredients		
L-carnitine	2,000 mg	600 mg
Taurine	2,000 mg	600 mg
Omega-3-fatty acids	1,140 mg	342 mg
Choline	550 mg	165 mg
Oatmeal	20.0 g	6.0 g
β-Glucan	5.6 g	1.7 g
Inulin	7.3 g	2.2 g
Oat fiber	5.0 g	1.5 g

The primary outcome of this study was the effect of the dietary intervention on liver fat contents as measured by CAP. Secondary outcomes included changes in body composition, liver stiffness measurements (LSM), serum lipid concentrations, and cardiovascular risk profile. At the Department of Medicine II of Saarland University Medical Center (Homburg, Germany), we quantified CAP and LSM, and determined body composition. Patients were included in the study if they had a CAP ≥ 215 dB/m at baseline and were excluded from observation if they had any of the following: harmful alcohol intake based on the AUDIT questionnaire (Babor et al., 2001), histologically defined liver cirrhosis or LSM ≥ 13 kPa (Friedrich-Rust et al., 2008), pregnancy, cardiac pace-maker, or stage IV or V chronic kidney disease (National Kidney Foundation, 2002).

Written informed consent was obtained from each partici-pant. The study protocol complies with the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the Saarland Ethics Committee (Ärztekammer des Saarlandes, ref. 271/11).

Anthropometric, clinical and biochemical assessments

After an 8-h overnight fast, the following parameters were measured: height was recorded using a stadiometer (seca 217; Seca, Hamburg, Germany), weight and body composition (body mass index, BMI; body fat mass, BFM; body fat free mass, BFFM; total body water, TBW; visceral fat index, VFI) were assessed using a segmental bioelectrical impedance analyzer (Tanita BC-418MA; Tanita Europe, Sindelfingen, Germany), and waist circumference (WC) was measured using a tape measure aligned at the lowest border of the rib cage in an exhaled and relaxed position. Office systolic and diastolic blood pressure was taken using a digital blood pressure monitor (Visomat; UEBE Medical, Wertheim, Germany). Medical history and current medication were documented. At baseline, 16 patients stated that they took no medication, and 44 listed their current medication, including antihypertensives (N = 28), antidiabetics (N = 14) and lipid-lowering agents (N = 11). At the end of the intervention, antihypertensives were discontinued in five and metformin in three cases; of note, no new medication was started during the intervention.

Fasted blood samples were collected for routine analysis of liver function tests and serum lipids: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl transferase (γ-GT), pseudocholi-nesterase (PChE), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol as well as uric acid, glucose, HbA1c, and kidney function tests. Fatty liver index (FLI), which is based on BMI, WC, γ-GT, and TG, was calculated according to Bedogni et al (Bedogni et al., 2006).

$$FLI = \frac{e^{0.953 \times \log e\,(TG) + 0.139 \times BMI + 0.718 \times \log e\,(\gamma\text{-}GT) + 0.053 \times WC - 15.745}}{1 + e^{0.953 \times \log e\,(TG) + 0.139 \times BMI + 0.718 \times \log e\,(\gamma\text{-}GT) + 0.053 \times WC - 15.745}} \times 100$$

Non-invasive assessment of hepatic steatosis and fibrosis

Hepatic steatosis and liver stiffness were assessed using VCTE (FibroScan, Echosens, Paris). At 3.5 MHz, the M-probe provides CAP values (100–400 dB/m) and LSM values (1.5–75.0 kPa), as described in our previous study (Arslanow et al., 2016). All elastrography measurements were carried out in fasted patients by the same experienced operator (AA, who has carried out > 600 measurements). According to the 2015 EASL-ALEH Clinical Practice Guidelines for non-invasive tests for evaluation of liver disease severity and prognosis (2015), results were only included if they fulfilled the criteria for a valid transient elastography measurement: at least 10 valid shots, a success rate of ≥ 60%, and an interquartile range (IQR)/liver stiffness (LSM) of ≤ 30%.

Genotyping of the *PNPLA3* variant p.I148M

DNA was isolated from EDTA blood according to membrane-based QIAamp DNA extraction protocol (Qiagen, Hilden, Germany). Genotyping of the *PNPLA3* single nucleotide polymorphism rs738409 (c.617G4C, resulting in the amino acid substitution p.I148M) was conducted using a PCR-based assay with 5′-nuclease and fluorescence detection (TaqMan®, Life Technologies, Darmstadt, Germany; rs738409: C__7241_10) as decribed (Krawczyk et al., 2011).

Statistical analysis

All statistical analyses were performed with SPSS 20.0 (SPSS, Munich, Germany) and GraphPad Prism 5.0 (GraphPad Software Inc., CA, USA). A two-sided P-value < 0.05 was regarded as significant. Most of the variables were non-parametric, as assessed using the Kolmogorov–Smirnov tests. Results are presented as medians and ranges, unless stated otherwise, or as frequencies and percentages.

Variables were tested for correlation using the Spearman's rank coefficient r_s. Contingency tables were used to assess for associations between categorical variables. Parameter changes after the dietary intervention are reported as absolute and relative frequencies. Comparisons between two and three unpaired groups were conducted using the Mann–Whitney U and Kruskal–Wallis tests, respectively. The Wilcoxon-signed ranks tests were used for comparisons between two paired groups. Both linear univariate and multivariate regression analysis were employed to detect the influence of baseline variables on absolute CAP changes. We also carried out subgroup analyses assessing for sex-specific differences, patients with and without type 2 diabetes, and comparing CAP responders (defined by CAP reduction) with CAP non-responders (defined by CAP increase).

4.5. Results

Patient characteristics

A total of 84 patients were screened for this open study. As depicted in the flow chart **(Figure 1)**, 24 patients were excluded, mainly due to invalid transient elastography (N = 8) or harmful alcohol use (N = 6), and none of the 60 patients was lost to follow-up. **Table 2** summarizes their clinical characteristics. The cohort comprised 31 (51.7%) women and had a median age of 56 years (25–78 years). Median CAP was 295 dB/m (216–400 dB/m). As stated in *Methods*, the presence of steatosis (steatosis grade ≥ S1) was confirmed based on a CAP ≥ 215 dB/m (De Ledinghen et al., 2012). This cut-off has been validated by de Lédinghen et al. (De Ledinghen et al., 2012) with an area under receiver operating characteristic curve of 0.84 (95% confidence interval; 0.76–0.92) in reference to liver biopsy.

Alternatively, fatty liver can be defined using the FLI, with values ≥ 60 indicating steatosis (Bedogni et al., 2006). At baseline, CAP ≥ 215 dB/m and FLI ≥ 60 were simultaneously detected in 50 (83.3%) patients, and only three cases presented with FLI < 30 (r_s = 0.465, P < 0.001). Three patients had normal weight (BMI 18.5–24.9 kg/m^2), 19 (31.7%) were overweight (BMI 25.0–29.9 kg/m^2), and 38 patients (63.3%) were obese (BMI ≥ 30.0 kg/m^2). In addition, 55 (91.6%) patients had elevated BFM. In total, 57 patients (95.0%) were above the European WC thresholds of 94 cm for men and 80 cm for women (Alberti et al.). Median HbA1c was 5.7% (4.9–10.4%), and type 2 diabetes was present in 14 (23.3%) patients. Overall, 26 patients (43.3%) presented with the metabolic syndrome, defined as elevated WC, TG, blood pressure, fasting plasma glucose, diagnosis of type 2 diabetes and reduced HDL cholesterol, as outlined by the International Diabetes Federation (Alberti et al., 2005). Baseline activity level was monitored through a self-report questionnaire, specifically type, frequency and duration of physical activity. At follow-up, an increase in activity level was reported in a single patient only.

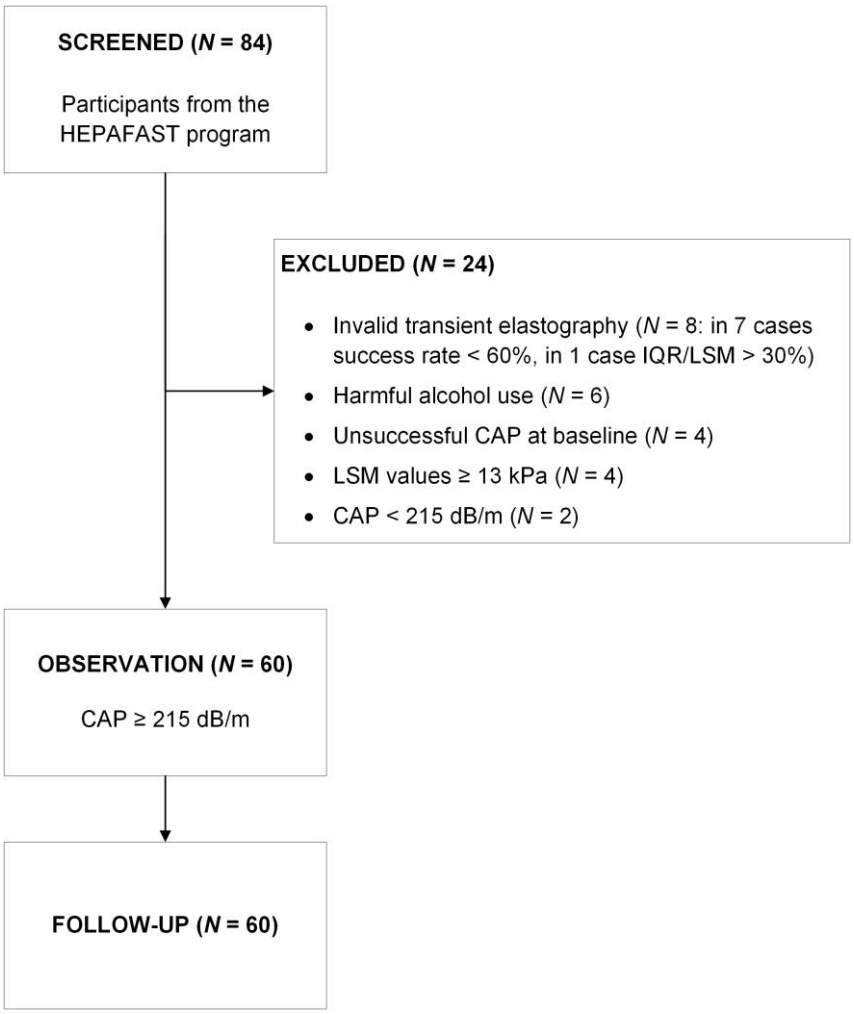

Fig. 1: Flow chart of study recruitment and participation.

Table 2: Clinical characteristics of the study cohort

	At baseline	At follow-up	Relative reduction (%)	P
Sociodemographic characteristics				
N (men/women)	60 (29/31)			
Age (years)	56 (25–78)			
Body composition				
Body weight (kg)	95.1 (60.7–125.6)	90.5 (58.2–120.1)	-4.6 (-8.0– -0.7)	**< 0.001**
BMI (kg/m^2)	31.9 (22.4–44.8)	30.6 (21.3–43.5)	-4.7 (-8.1– -0.6)	**< 0.001**
BFM (kg)	34.5 (16.8–63.4)	31.8 (13.4–59.5)	-6.9 (-27.0–4.6)	**< 0.001**
BFFM (kg)	58.2 (39.5–84.9)	55.3 (39.3–81.9)	-3.3 (-9.1–4.2)	**< 0.001**
TBW (kg)	42.6 (28.9–62.2)	40.5 (28.8–60.0)	-3.3 (-9.1–4.1)	**< 0.001**
WC (cm)	107 (78–127)	103 (76–128)	-4.1 (-9.2–2.2)	**< 0.001**
VFI	13 (5–24)	12 (4–21)	-7.1 (-20.0–11.1)	**< 0.001**
Liver markers				
CAP (dB/m)	295 (216–400)	266 (100–353)	-14.0 (-68.6–38.2)	**< 0.001**
FLI	83 (7–99)	63 (4–98)	-21.3 (-74.0–0.0)	**< 0.001**
LSM (kPa)	6.2 (1.5–11.9)	5.3 (1.5–12.0)	-11.7 (-70.5–43.6)	**0.002**
ALT (U/l)	38 (12–118)	36 (14–150)	0 (-73.1–122.2)	> 0.05
AST (U/l)	25 (10–121)	24 (8–141)	0 (-80.2–464.0)	> 0.05
AP (U/l)	74 (37–159)	64 (32–144)	-11.5 (-43.0–24.1)	**< 0.001**
γ-GT (U/l)	37 (7–335)	26 (7–113)	-26.7 (-77.3–50.0)	**< 0.001**
PChE (kU/l)	10.7 (6.6–17.0)	10.4 (6.7–15.3)	-3.8 (-22.6–19.2)	**0.006**
Metabolic markers				
Glucose (mg/dl)	89 (63–232)	84 (60–126)	-7.1 (-50.4–52.4)	**< 0.001**
TG (mg/dl)	128 (60–419)	83 (48–183)	-34.1 (-84.0–35.9)	**< 0.001**
TC (mg/dl)	214 (147–303)	163 (95–249)	-23.5 (-45.6–10.9)	**< 0.001**
LDL cholesterol (mg/dl)	142 (78–226)	96 (45–193)	-25.3 (-53.1–41.0)	**< 0.001**
HDL cholesterol (mg/dl)	50 (29–110)	45 (28–77)	-13.0 (-66.4–28.9)	**< 0.001**
Uric acid (mg/dl)	6.1 (2.9–8.6)	5.6 (3.1–10.0)	-7.6 (- 40.9–43.5)	**0.024**
SBP (mmHg)	138 (110–175)	130 (104–184)	-5.6 (-28.6–40.5)	**< 0.001**
DBP (mmHg)	92 (74–125)	87 (72–120)	-4.5 (-34.2–18.8)	**0.001**

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BFFM, body fat free mass; BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; FLI, fatty liver index; γ-GT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LSM, liver stiffness measurement; N, number; PChE, pseudocholinesterase; SBP, systolic blood pressure; TBW, total body water; TC, total cholesterol; TG, triglycerides; VFI, visceral fat index; WC, waist circumference. Significant P-values are highlighted in bold.

The dietary intervention has a positive impact on CAP

Overall, the median CAP decreased significantly by 14.0% (47 dB/m, P < 0.001, **Figure 2a**) from 295 dB/m (216–400 dB/m) to 266 dB/m (100–353 dB/m) (**Table 2**). **Figure 2b** displays all CAP values of individual patients.

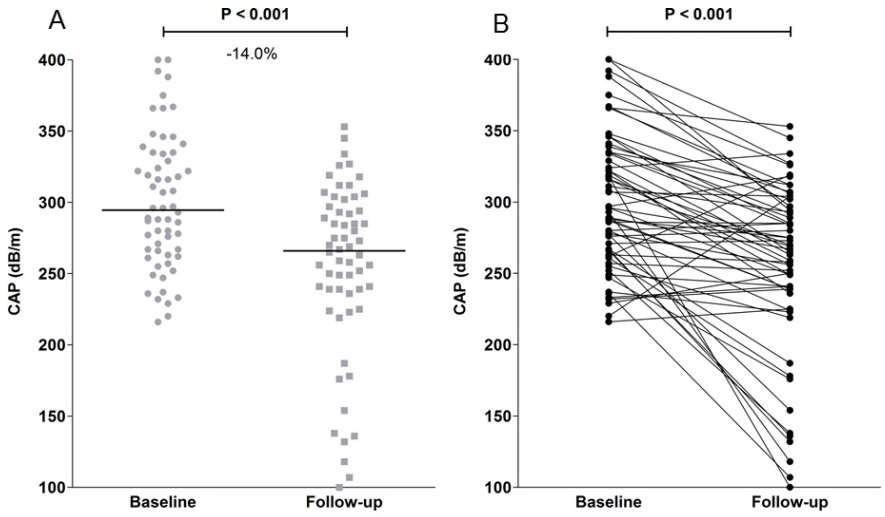

Fig. 2: Changes of CAP in all 60 patients. (**a**) Median and individual CAP at baseline and follow-up at the end of the dietary intervention. The median CAP reduction was 47 dB/m in the entire cohort, corresponding to a relative median reduction of 14.0% ($P < 0.001$). (**b**) Absolute CAP in the individual patients during the dietary intervention.

Of 60 patients, 49 (81.7%) demonstrated a decrease in median CAP of 15.9% (50 dB/m, $P < 0.001$) and were defined as CAP responders (see *Statistical analysis*). Of these, 10 patients (20.4%) showed resolution of steatosis, i.e., they presented with CAP < 215 dB/m after the dietary intervention. Interestingly, 11 demonstrated a median CAP increase of 3.4% (9 dB/m, $P = 0.003$) after the 14-day intervention despite improvements in body composition, thus we classified them as CAP non-responders. Compared to responders, non-responders were mostly women (72.7% vs. 46.9%, $P > 0.05$), younger (51 years vs. 56 years, $P > 0.05$), had a higher baseline BMI (32.2 kg/m^2 vs. 31.2 kg/m^2, $P > 0.05$) and a lower baseline CAP (262 dB/m vs. 308 dB/m, $P = 0.001$). When comparing genetic variation, all five homozygous *PNPLA3* mutation carriers with two prosteatogenic p.148M alleles were responders.

The dietary response might be influenced by PNPLA3

In this cohort, the *PNPLA3* p.148M frequencies for wild-type, heterozygous, and homozygous genotypes were [II]: N = 36, 60.0%; [IM]: N = 19, 31.7%; and [MM]: N = 5, 8.3%. With a minor allele frequency of 0.24 for the risk allele [M], this result is comparable to 0.23 reported in the first genome-wide association study (Romeo et al., 2008). Homozygous carriers of the prosteatogenic [M] allele had a markedly higher baseline CAP (339 dB/m) as

compared to wild-type (291 dB/m) and heterozygous (296 dB/m) patients and as shown in **Table 3**, they had a slightly larger decrease in CAP at 14 days (P > 0.05).

Table 3: Distribution of CAP values across *PNPLA3* genotypes

***PNPLA3* variant p.I148M**	**[II]**	**[IM]**	**[MM]**
Frequency (%)	60.0	31.7	8.3
N	36	19	5
CAP at baseline (dB/m)	291 (216–400)	296 (232–392)	339 (267–367)
CAP at follow-up (dB/m)	269 (100–353)	256 (154–334)	292 (132–306)
Absolute median CAP reduction (dB/m)	-48 (-218–84)	-39 (-123–57)	-49 (-135– -22)
Relative median CAP reduction (%)	-13.8 (-68.6–38.2)	-14.1 (-44.4–21.8)	-14.4 (-50.6– -7.6)

CAP, controlled attenuation parameter; I, isoleucine; M, methionine; N, number; *PNPLA3*, patatin-like phospholipase domain containing 3 (adiponutrin).
The distribution of CAP values and CAP reduction across *PNPLA3* p.I148M genotypes does not differ (all P-values > 0.05).

Factors associated with CAP

CAP at baseline was associated with BMI ($r_s = 0.330$, $P = 0.010$), WC ($r_s = 0.414$, $P = 0.001$), LSM ($r_s = 0.460$, $P < 0.001$), ALT ($r_s = 0.455$, $P < 0.001$), AST ($r_s = 0.412$, $P = 0.001$), and glucose concentrations ($r_s = 0.366$, $P = 0.004$), but not with TG or γ-GT. **Table 4** summarizes the results of univariate regression analysis with absolute reduction of CAP as dependent variable. Age, baseline BFM, BMI, sex, TC, and TG were not correlated, whereas both baseline CAP and HDL cholesterol showed a significant inverse association ($P = 0.035$ and $P = 0.009$, respectively). In multivariate regression analysis, these two variables were independent predictors of absolute CAP reduction ($P = 0.020$ and $P = 0.005$, respectively). As expected, CAP at follow-up correlated with FLI ($r_s = 0.502$, $P < 0.001$).

Table 4: Univariate and multivariate analysis of determinants of CAP reduction

	β coefficient	P
A - Univariate analysis		
Age	0.042	> 0.05
BFM at baseline	0.126	> 0.05
BMI at baseline	0.142	> 0.05
CAP at baseline	-0.273	**0.035**
HDL cholesterol at baseline	-0.335	**0.009**
Sex	0.178	> 0.05
TC at baseline	-0.062	> 0.05
TG at baseline	0.206	> 0.05
B - Multivariate analysis		
CAP at baseline	-0.285	**0.020**
HDL cholesterol at baseline	-0.345	**0.005**

BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; HDL, high-density lipoprotein; TC, total cholesterol; TG, triglycerides Significant P-values are highlighted in **bold**.

Changes of liver stiffness and liver enzymes

Patients presented with a median LSM of 6.2 kPa (1.5–11.9 kPa) at baseline and 5.3 kPa (1.5–12.0 kPa) after the intervention (**Table 2**). Overall, median LSM decreased by 11.7% (0.8 kPa, P = 0.002). With respect to liver function tests, baseline γ-GT activity was elevated in 35% of cases compared to 20% after the intervention. Significant reductions of 26.7 and 11.5% were observed for γ-GT and AP activities, respectively (both P < 0.001; **Table 2**). Although no overall reduction in ALT and AST activities occurred, non-significant (P > 0.05) improvements in patients with elevated baseline activities were detected.

Effects on body composition

All 60 patients were compliant with the program when using weight loss as a marker. Overall, the parameters related to body composition decreased significantly, as summarized in **Table 2** (all P < 0.001). A median weight reduction of 4.6% (4.2 kg, P < 0.001) occurred. A total of 17 (28.3%) patients were reclassified into a lower BMI category after 14 days, whereas 43 (71.7%) remained within their initial category. **Table 2** shows that BFM decreased by 6.9% (2.6 kg, P < 0.001). Overall, 61.9% of the weight reduction was based on loss of BFM. The absolute BFM reduction did not correlate with the reduction of CAP ($r_s = -0.04$, P > 0.05).

Reductions in CAP between patients with a weight reduction ≥ 5 and < 5% were similar (14.9% and 11.4%, both P < 0.001). **Table 5** summarizes within and between group changes.

Overall, 14 (23.3%) patients had a previous diagnosis of type 2 diabetes. When compared to non-diabetics, these patients were older, had higher serum glucose concentrations and higher baseline CAP, but CAP reductions did not differ between the two groups (**Table 6**).

Table 5: Comparison of patients with weight loss ≥ 5% and < 5%

	Weight loss ≥ 5%		Weight loss < 5%		
	At baseline	**At follow-up**	**At baseline**	**At follow-up**	**P**
Sociodemographic characteristics					
N (men/women)	26 (20/6)		34 (9/25)		***
Age (years)	50 (25–66)		58 (33–78)		**
Body composition					
Body weight (kg)	92.8 (61.9–125.6)	86.7 (58.6–115.7)$^{###}$	95.6 (60.7–125.5)	92.1 (58.2–120.1)$^{###}$	***
BMI (kg/m^2)	29.6 (22.4–41.5)	27.8 (21.3–38.5)$^{###}$	32.4 (25.3–44.8)	30.9 (24.2–43.5)$^{###}$	***
BFM (kg)	29.2 (17.0–49.5)	26.0 (13.4–44.1)$^{###}$	36.7 (16.8–63.4)	34.8 (15.4–59.5)$^{###}$	***
BFFM (kg)	68.4 (45.8–84.9)	66.6 (44.4–81.9)$^{###}$	53.2 (39.5–77.1)	51.8 (39.3–78.9)$^{###}$	***
TBW (kg)	50.1 (33.5–62.2)	48.8 (32.5–60.0)$^{###}$	38.9 (28.9–56.4)	37.9 (28.8–57.8)$^{###}$	***
WC (cm)	103 (82–124)	98 (77–120)$^{###}$	107 (78–127)	104 (76–128)$^{###}$	***
VFI	12 (5–24)	12 (5–21)$^{###}$	14 (5–21)	13 (4–20)$^{###}$	**
Liver markers					
CAP (dB/m)	295 (216–400)	251 (100–345)$^{###}$	298 (220–392)	283 (136–353)$^{###}$	n.s.
FLI	82 (30–99)	42 (14–93)$^{###}$	83 (7–99)	71 (4–98)$^{###}$	***
LSM (kPa)	5.9 (1.5–11.9)	5.0 (1.5–12.0)$^{##}$	6.2 (3.8–9.7)	5.4 (3.1–10.0)$^{#}$	n.s.
ALT (U/l)	38 (12–118)	31 (14–150)$^{n.s.}$	37 (18–108)	40 (14–105)$^{n.s.}$	n.s.
AST (U/l)	26 (10–121)	22 (11–141)$^{n.s.}$	24 (10–46)	24 (8–46)$^{n.s.}$	n.s.
γ-GT (U/l)	40 (12–335)	26 (7–99)$^{###}$	36 (7–93)	25 (7–113)$^{###}$	**
Metabolic markers					
Glucose (mg/dl)	87 (71–232)	74 (60–115)$^{###}$	91 (63–164)	85 (72–126)$^{##}$	**
TG (mg/dl)	138 (60–419)	72 (48–148)$^{###}$	119 (63–340)	102 (49–183)$^{###}$	***
TC (mg/dl)	219 (147–303)	161 (95–229)$^{###}$	209 (149–302)	166 (103–249)$^{###}$	*
LDL cholesterol (mg/dl)	143 (84–201)	98 (45–172)$^{###}$	140 (78–226)	95 (47–193)$^{###}$	n.s.
HDL cholesterol (mg/dl)	46 (33–110)	42 (28–75)$^{##}$	54 (29–82)	46 (30–77)$^{###}$	n.s.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BFFM, body fat free mass; BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; FLI, fatty liver index; γ-GT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LSM, liver stiffness measurement; N, number; TBW, total body water; TC, total cholesterol; TG, triglycerides; VFI, visceral fat index; WC, waist circumference.

P-value between baseline and follow-up determined with Wilcoxon-signed-rank test: $^{###}$ P ≤ 0.001, $^{##}$ P ≤ 0.01, $^{#}$ P ≤ 0.05, $^{n.s.}$ P > 0.05

P-value between relative difference of baseline and follow-up value between both groups (weight loss ≥ 5% and weight loss < 5%) determined with Mann-Whitney U test: *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, $^{n.s.}$ P > 0.05

Table 6: Clinical baseline characteristics stratified according to the presence of diabetes

	Patients with diabetes	Patients without diabetes	P
Sociodemographic characteristics			
N (men/women)	14 (6/8)	46 (23/23)	> 0.05
Age (years)	61 (36–74)	54 (25–78)	**0.038**
Body composition			
Body weight (kg)	95.3 (74.5–119.8)	94.5 (60.7–125.5)	> 0.05
BMI (kg/m^2)	31.9 (27.0–44.8)	31.9 (22.4–43.5)	> 0.05
BFM (kg)	34.2 (18.9–53.4)	34.5 (16.8–63.4)	> 0.05
BFFM (kg)	55.4 (46.3–80.9)	61.6 (39.5–84.9)	> 0.05
TBW (kg)	40.6 (33.9–59.2)	45.1 (28.9–62.2)	> 0.05
WC (cm)	109 (96–127)	103 (78–124)	> 0.05
VFI	15 (11–21)	13 (5–24)	> 0.05
Liver markers			
CAP (dB/m)	337 (255–375)	288 (216–400)	**0.025**
FLI	86 (30–99)	76 (7–99)	> 0.05
LSM (kPa)	6.7 (1.5–9.9)	5.9 (3.3–11.9)	> 0.05
ALT (U/l)	42 (12–118)	38 (16–84)	> 0.05
AST (U/l)	26 (10–68)	24 (10–121)	> 0.05
AP (U/l)	75 (48–106)	74 (37–159)	> 0.05
γ-GT (U/l)	47 (17–93)	32 (7–335)	> 0.05
PChE (kU/l)	11.3 (7.9–17.0)	10.4 (6.6–15.0)	> 0.05
Metabolic markers			
Glucose (mg/dl)	115 (63–232)	86 (68–156)	**< 0.001**
TG (mg/dl)	152 (60–273)	122 (65–419)	> 0.05
TC (mg/dl)	210 (149–258)	220 (147–303)	> 0.05
LDL cholesterol (mg/dl)	139 (78–184)	142 (84–226)	> 0.05
HDL cholesterol (mg/dl)	50 (29–82)	51 (33–110)	> 0.05
Uric acid (mg/dl)	6.4 (4.6–8.5)	6.0 (2.9–8.6)	> 0.05
SBP (mmHg)	131 (110–175)	138 (110–175)	> 0.05
DBP (mmHg)	87 (79–175)	93 (74–125)	> 0.05

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BFFM, body fat free mass; BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; FLI, fatty liver index; γ-GT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LSM, liver stiffness measurement; N, number; PChE, pseudocholinesterase; SBP, systolic blood pressure; TBW, total body water; TC, total cholesterol; TG, triglycerides; VFI, visceral fat index; WC, waist circumference. Significant P-values are highlighted in **bold**.

Metabolic effects

At baseline, over half of the cohort (60.0%) presented with TC concentrations ≥ 200 mg/dl. LDL cholesterol concentrations were above 130 mg/dl in 35 (58.6%) patients, and 22 (36.7%) patients had increased TG levels ≥ 150 mg/dl. After the dietary intervention, all lipid parameters decreased by one-third to a quarter apart from HDL cholesterol, which reduced by 13.0% (all P < 0.001; **Table 2**). Specifically, a reduction of 17.1% for HDL cholesterol was noted in 46 patients, whereas 13 patients improved by 9.1% and one case remained stable. Most importantly, LDL cholesterol levels decreased by 25.3%, which corresponds to an absolute change of 32.5 mg/dl. The reduction occurred in 54 patients (90.0%).

Overall, glucose levels decreased significantly by 7.1% ($P < 0.001$). A positive change in systolic and diastolic blood pressure of −5.6% and −4.5% (both $P \leq 0.001$) was observed. The median reductions of key parameters assessed during the study are summarized in **Figure 3**.

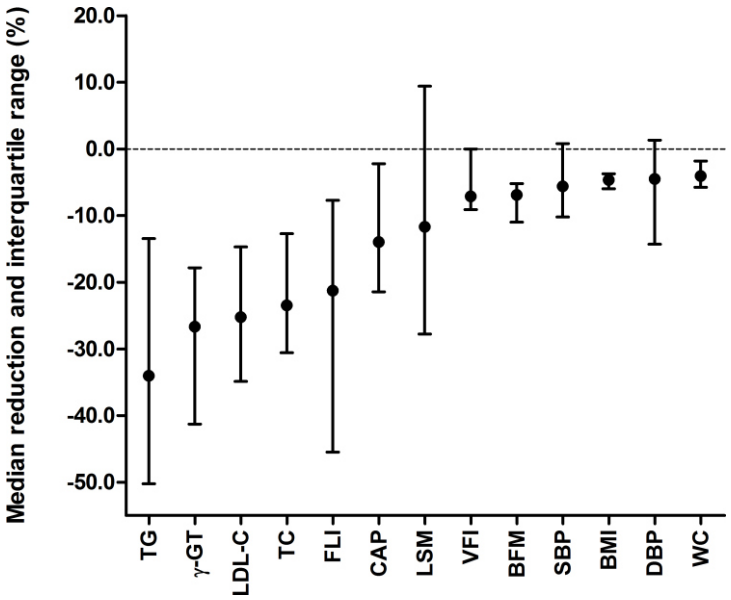

Fig. 3: Summary of significant reductions of key parameters assessed during the dietary intervention. The values are displayed as medians, interquartile range, and ordered based on the extent of reduction. BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; FLI, fatty liver index; γ-GT, gamma-glutamyl transpeptidase; LDL-C, low-density lipoprotein cholesterol; LSM, liver stiffness measurement; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; VFI, visceral fat index; WC, waist circumference.

4.6. Discussion

The aims herein were to simultaneously and non-invasively monitor liver and body fat composition in patients with NAFLD participating in a short-term dietary program, and to avail of CAP to assess dynamic changes in liver fat. After a 14-day hypocaloric high-fiber, high-protein diet, an absolute CAP reduction of 47 dB/m and a relative decrease of 14.0% were achieved. This was accompanied by a significant weight loss of 4.6%, of which almost two-thirds (61.9%) was loss of body fat mass. To our knowledge, this is the first study to monitor rapid changes in hepatic steatosis using CAP, which represents a patient-friendly non-invasive tool.

The results are in line with previous smaller studies using other methodologies to assess changes of liver phenotypes after dietary interventions. According to the AASLD guidelines, a reduction of body weight of at least 3–5% through a hypocaloric diet alone or together with increased physical activity is recommended for ameliorating steatosis (Chalasani et al.,

2012). Colles et al. (Colles et al., 2006) studied a very low-calorie diet (680 kcal/day) for 12 weeks in 32 morbidly obese patients. Body weight was reduced by 10.6% (14.8 kg), and liver volume assessed by computer tomography decreased by 18.7% (0.56 l). Interestingly, after 2 weeks, 80% of the overall decrease in liver volume was detected, whereas body weight improved steadily throughout (Colles et al., 2006). We did not observe a significant association between the extent of weight loss and improvement of liver phenotypes, as reported by others (Patel et al., 2015; Vilar-Gomez et al., 2015), which might be related to less drastic changes in body fat mass (**Figure 3**). Some of the previously reported studies compared overall weight loss and BMI reductions only rather than compartmental changes of body composition. As the interventions were significantly longer, they may have resulted in greater changes of fat mass and corresponding liver-specific effects (Patel et al., 2015; Vilar-Gomez et al., 2015).

In addition to CAP reductions and weight loss, the dietary modification assessed herein also resulted in metabolic improvements, particularly those related to the metabolic syndrome. Besides the expected reduction in triglycerides, the reduction of LDL cholesterol levels by 33 mg/dl after 14 days is remarkable, especially in comparison to a meta-analysis of 174,000 patients, which reported a decrease of 42.5 mg/dl after 1 year of statin therapy (Cholesterol Treatment Trialists' (CTT) Collaboration et al., 2015). Another meta-analysis on dietary fiber reported a reduction of LDL cholesterol of 2.21 mg/dl for each 1 g of soluble fiber per day (Brown et al., 1999), which is reflected in our study with a daily intake of 14 g soluble fiber. HDL cholesterol levels decreased, which has previously been reported during acute weight loss, however HDL subsequently increased in the weight maintenance phase (Jazet et al., 2007; Lammert et al., 2008; Vasankari et al., 2001). Brinton et al. (Brinton et al., 1990) suggested that the reduction in HDL cholesterol might be associated with lower HDL apolipoprotein transport rates, and Aminian et al. (Aminian et al., 2015) observed a decrease in lipoprotein lipase activity by up to 50% during caloric restriction. Once weight stabilized, HDL cholesterol metabolism reversed and led to increased HDL cholesterol concentrations above the pre-intervention levels (Aminian et al., 2015).

The diet given to patients as part of a specifically designed weight loss program contains ingredients known to be potentially beneficial for the liver, including omega-3 polyunsaturated fatty acids (Shapiro et al., 2011), L-carnitine (Malaguarnera et al., 2010), choline (Corbin and Zeisel, 2012), β-glucan (Chang et al., 2013), inulin (Brighenti, 2007), and taurine (Gentile et al., 2011).

A limitation of this study is that we were unable to determine if, and to what extent, these ingredients contributed to the overall effects of liver fat reduction, as compared to the overall effects of the caloric restriction and concomitant weight reduction. Moreover, the significant

improvements observed after 14 days do not afford the opportunity to evaluate the liver-specific long-term effects of the diet. Although several key parameters of the metabolic syndrome improved, we did not specifically assess the effect of the diet on insulin resistance.

Shen et al. (Shen et al., 2015a) studied the effect of a lifestyle modification program in NAFLD patients and observed that patients who carry the *PNPLA3* mutation p.I148M showed a better response as compared to patients with wild-type alleles (Shen et al., 2015a). Although, the current data on genetic associations in our study are hampered by sample size, we also note that hepatic response was observed in all homozygous carriers of the *PNPLA3* risk allele, which should be further evaluated as personalized biomarker for a response to the dietary regimen.

Recent recommendations from a joint AASLD–FDA work-shop pointed out that the use of elastography in subjects with NASH has not been explored in great detail, and that non-invasive measures should be included as secondary or exploratory endpoints in current trials (Sanyal et al., 2015). Our study results illustrate that CAP might represent a reliable alternative for monitoring hepatic steatosis in research and clinical settings (Berzigotti, 2014; Shi et al., 2014).

In conclusion, the 14-day hypocaloric high-fiber, high-protein diet reduced CAP, and hence hepatic steatosis simultaneously to improvements in parameters of the metabolic syndrome. We demonstrated that improvements in hepatic fat contents can be observed after a couple of weeks only, which highlights the possibility for dynamic short-term modulation of liver fat. Whether such a program provides long-term benefits for these patients should be substantiated, but extent and rate of liver fat reduction set the benchmark for pharmacological treatment. Regardless, CAP provides a convenient and patient-friendly method to assess lipid turnover during lifestyle and dietary interventions to combat NAFLD.

4.7. References

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5. Nichtinvasive Früherkennung von Lebererkrankungen im Rahmen der betrieblichen Gesundheitsförderung (Noninvasive early detection of liver diseases as part of occupational health check-ups)

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The published manuscript can be found in Appendix 3.

5.1. Zusammenfassung

Hintergrund

Bis zu 50% der europäischen Bevölkerung haben eine Fettlebererkrankung, die häufig zunächst symptomlos ist, aber ein erhöhtes metabolisches und kardiovaskuläres Risiko anzeigt. Im Rahmen betriebsärztlicher Untersuchungen werden gesundheitliche Risiken bei Mitarbeitern in einem regelmäßigen Turnus erfasst.

Ziel der Arbeit

Es sollte jetzt untersucht werden, ob die nicht-invasive transiente Elastographie im Rahmen der betrieblichen Gesundheitsförderung im Vergleich zu Laborparametern einen Mehrwert hinsichtlich der Diagnose einer Fettlebererkrankung bietet.

Material und Methoden

In dieser prospektiv geplanten Querschnittsstudie wurden 133 Mitarbeitern eines Universitätsklinikums beim Betriebsarzt zusätzliche nicht-invasive Untersuchungen angeboten: Transiente Elastographie zur Quantifizierung der Leberverfettung (CAP-Wert) und Lebersteifigkeit (LS-Wert), Ermittlung der Körperzusammensetzung mittels Bioelektrischer Impedanzanalyse sowie die Erhebung von anthropometrischen Parametern. Laborparameter wurden mit klinisch-chemischen Standardtests bestimmt.

Ergebnisse und Diskussion

Insgesamt 104 Mitarbeiter (74% Frauen, mittleres Alter 35 Jahre) wurden in die Studie aufgenommen. Bei 36% der Mitarbeiter wurde eine Fettleber (CAP-Wert ≥ 243 dB/m) neu diagnostiziert. In einem Drittel dieser Fälle waren ALT- und γ-GT-Aktivitäten normwertig, sodass eine Lebererkrankung durch die Serumparameter alleine nicht erfasst worden wäre. Der CAP-Wert ist mit steigendem BMI assoziiert $r_p = 0{,}615$, $P < 0{,}001$). Die stärksten Prädiktoren für eine Verfettung der Leber waren Körperfettmasse und Taillenumfang (Odds Ratios 1,7 bzw. 3,8; $P = 0{,}033$ bzw. $P < 0{,}001$).

Diese Studie belegt, dass die transiente Elastographie im Vergleich zu Leberenzymen mehr Patienten mit einer Fettlebererkrankung identifiziert und dass die Fettlebererkrankung bei Mitarbeitern im Gesundheitswesen unterdiagnostiziert ist.

5.2. Abstract

Background

Up to 50% of the European population develops fatty liver disease, which although often asymptomatic, is associated with increased metabolic and cardiovascular risk. Thus regular occupational health check-ups for employees might help timely identification of this liver disease.

Objectives

The specific aim of the study was to determine whether measuring liver fat non-invasively using transient elastography is more effective for diagnosing fatty liver disease as compared to serum parameters obtained during occupational health check-ups.

Materials and methods

In this prospective cross-sectional study, 133 employees of a university hospital were offered additional non-invasive tests during occupational health check-ups: Transient elastography to quantify hepatic steatosis (CAP value) and liver stiffness (LS value), bioelectric impedance analysis to determine body composition, assessment of additional anthropometric parameters, and measurement of serum markers by standard clinical chemical tests.

Results and conclusions

In total, 104 employees (74% women, mean age 35 years) were included. Fatty liver (as defined by CAP value ≥ 243 dB/m) was newly diagnosed in 36% of employees. In one third of these cases, ALT and γ-GT activities were within the normal range, therefore liver disease was undetected when using serum parameters alone. The CAP value was associated with increased body mass index ($r_p = 0.615$, $P < 0.001$). The strongest predictors for accumulation of hepatic fat were body fat mass and waist circumference (odds ratios 1.7 and 3.8, $P = 0.033$ and $P < 0.001$, respectively).

This study shows that transient elastography provides additional value in the detection of fatty liver as compared to serum parameters in occupational health check-ups. Furthermore, the study provides evidence that fatty liver disease is underdiagnosed among health care workers.

5.3. Einführung

Patienten mit einer Fettleber sind meist symptomlos, und die Diagnose wird oftmals nur als Zufallsbefund gestellt, wobei es unerkannt zur Progression der Erkrankung bis hin zur Zirrhose und zur Entwicklung eines Leberzellkarzinoms kommen kann. Bisher hat sich keine Methode durchgesetzt, die in der Fläche zur frühzeitigen Erkennung eingesetzt werden kann. Die betriebsärztliche Vorsorgeuntersuchung bietet die Möglichkeit, die Gesundheit der Mitarbeiter regelmäßig zu überprüfen, hierbei auch Lebererkrankungen früh zu erkennen und so das Risiko einer Krankheitsprogression zu senken.

5.4. Hintergrund und Fragestellung

Bis zu 50% der europäischen Bevölkerung haben eine Fettleber, die durch eine Akkumulation von Triglyzeriden über 5% in den Hepatozyten definiert ist (Blachier et al., 2013). Die Fettleber kann zu Steatohepatitis und Zirrhose voranschreiten (Michelotti et al., 2013). Diese drei Entitäten werden als Fettlebererkrankung zusammengefasst (Roeb and Canbay, 2016). Ein Viertel der Erwachsenen in Europa sind vom metabolischen Syndrom betroffen, und die Fettleber wird als hepatische Manifestationen des metabolischen Syndroms verstanden (Salamone and Bugianesi, 2010). Das Sterblichkeitsrisiko für die meisten dieser Patienten ist insbesondere durch kardiovaskuläre Erkrankungen erhöht (Than and Newsome, 2015).

Der Goldstandard zur Beurteilung einer Lebererkrankung ist die (perkutane) Leberbiopsie. Diese Methode trägt ein Blutungsrisiko (Terjung et al., 2003), kann eine beträchtliche Variabilität aufweisen (Roeb et al., 2015) und sollte nur bei einem begründeten Verdacht eingesetzt werden. Im klinischen Alltag hat sich die Bestimmung der Serumaktivitäten von „Leberenzymen“ wie der Alanin-Aminotransferase (ALT) und der γ-Glutamyltransferase (γ-GT) als einfach durchzuführende Methode etabliert, obgleich diese aber auch bei einer bereits bestehenden Lebererkrankung normwertig sein können. Unter 386 und 458 Fällen mit histologisch nachgewiesener NAFLD lagen die Raten der Patienten mit normwertiger ALT bei 13% und 14%, von denen in der Biopsie 6 bzw. 8 Fälle mit Zirrhose diagnostiziert wurden (Fracanzani et al., 2008; Mofrad et al., 2003). Patienten mit Leberzellkarzinom auf dem Boden einer NAFLD können normwertige ALT-Aktivitäten aufweisen (Tateishi et al., 2015).

Arbeitsmediziner beschäftigen sich mit den Wechselwirkungen von Arbeit und Gesundheit, geregelt durch die Verordnung zur arbeitsmedizinischen Vorsorge (ArbMedVV). Die Untersuchungsabstände finden in einem regelmäßigen Turnus statt. 2016 ist in Deutschland das Gesetz zur Stärkung der Gesundheitsförderung und der Prävention, kurz

Präventionsgesetz (PrävG), in Kraft getreten, das die Rolle des Betriebsarztes durch Leistungen zur Gesundheitsförderung in Betrieben (betriebliche Gesundheitsförderung) fördert. Das PrävG bezieht die Primär-, Sekundär- und Quartärprävention in die (arbeits)medizinische Prävention ein (Letzel, 2016). Charakteristisch für die Sekundärprävention sind das Durchführen von Screenings zur Erkennung von Risikofaktoren und Früherkennung von Krankheiten. Die neue Rolles des Betriebsarztes kommt vor allem denjenigen zugute, die nicht an den Präventionsprogrammen der Krankenkassen teilnehmen und aufgrund von ausbleibenden Krankheitssymptomen keinen Arzt aufsuchen (Letzel, 2016).

Zur Untersuchung der Leber im Rahmen der betrieblichen Gesundheitsförderung ist ein invasiver Eingriff wie die Biopsie weder durchführbar noch gerechtfertigt. Die ebenfalls mögliche Untersuchung mittels Magnetresonanztomographie ist nicht-invasiv, jedoch zeit- und ressourcenintensiv und stellt damit auch keine geeignete Methode dar. Bisher gibt es nur wenige Berichte darüber, dass Mitarbeiter im Rahmen der betriebsärztlichen Untersuchungen mittels bildgebender Methoden auf eine Fettlebererkrankung hin untersucht werden. Einzelne Studien zur Untersuchung mittels Ultraschall wurden aus China und Taiwan berichtet (Dai et al., 2009; Lin et al., 2010). Nachteilig bei der Sonografie sind jedoch die nur semiquantitative Beurteilung der Leberverfettung und die unzureichende Beurteilung der Leberfibrose.

Die Fettleber ist häufig mit einer Adipositas und einer erhöhten Körperfett- und Viszeralfettmasse assoziiert (Silaghi et al., 2015). Mit zunehmendem Body Mass Index (BMI) der gesamten Bevölkerung wird mit einer steigenden Prävalenz der Fettleber gerechnet (Müller et al., 2006). Die Progression der Fettleber, bei normwertigen wie auch pathologischen Leberenzymen, ist von Umweltfaktoren, aber auch von genetischen Faktoren abhängig. Es konnte gezeigt werden, dass insbesondere Träger einer häufigen Genvariante des Gens der Triglyceridhydrolase PNPLA3 häufiger eine Fettleber (Arslanow et al., 2016), eine Zirrhose (Krawczyk et al., 2011) und ein Leberzellkarzinom (Liu et al., 2014a) entwickeln.

Mittels transienter Elastographie können nicht-invasiv und innerhalb kurzer Zeit simultan die Leberverfettung und die Lebersteifigkeit zur Abschätzung einer Leberfibrose quantifiziert werden (Arslanow et al., 2016; Sasso et al., 2010). Mit dieser Studie soll primär untersucht werden, ob dieses Verfahren hinsichtlich der frühzeitigen Diagnose einer Fettleber einen Mehrwert im Vergleich zu Laborparametern bietet. Die UKS-Studie ist die erste Studie, bei der die Lebergesundheit in der betrieblichen Gesundheitsförderung bei Krankenhausmitarbeitern in Deutschland unter Verwendung der transienten Elastographie untersucht wird. Zusätzlich sollte untersucht werden, wie hoch die Prävalenz der Fibrose bei

nach eigenen Angaben gesunden Klinikumsmitarbeitern ist und welche Risikofaktoren für die Entstehung einer Fettleber in dieser Kohorte vorliegen.

5.5. Studiendesign und Untersuchungsmethoden

Studiendesign

Im Rahmen der betrieblichen Gesundheitsförderung durch den Betriebsärztlichen Dienst des Universitätsklinikums des Saarlandes (UKS) wurde Mitarbeitern im Zeitraum von Mai 2014 bis Dezember 2015 die freiwillige Teilnahme an der Studie angeboten. Hierzu wurde ein Studieninformationszettel mit wesentlichen Informationen zur Studie ausgehändigt. Schwangere und Träger eines Herzschrittmachers waren von der Studienteilnahme ausgeschlossen. Insgesamt erhielten 133 interessierte Mitarbeiter vom Arbeitsmediziner einen Studiencode zur pseudonymisierten Weiterverwendung der Daten. Davon meldeten sich 105 Mitarbeiter (79%) persönlich zur Teilnahme an der Studie in der Studienzentrale.

Nach der Deklaration von Helsinki in ihrer aktuellen Fassung wurde von jedem Teilnehmer im Anschluss an die schriftliche und mündliche Erläuterung der Studie persönlich eine Einwilligungserklärung unterzeichnet. Unter Verwendung eines Studiencodes wurden anschließend alle Untersuchungen pseudonymisiert durchgeführt. Die Durchführung der transienten Elastographie bei Gesunden wurde durch die Ethik-Kommission des Saarlandes genehmigt (Referenznummer 07/11). Zur Studienteilnahme wurde eine Flüssigkeitsbeschränkung von maximal 200 ml Wasser oder Kaffee und eine Nahrungskarenz von 120 Minuten vorausgesetzt (Arena et al., 2013), wobei 51,4% der Mitarbeiter zum Zeitpunkt der betrieblichen Gesundheitsförderung länger als 8 Stunden nüchtern waren. Mittels eines standardisierten Fragebogens wurden Alter, Geschlecht und Vorhandensein bekannter Erkrankungen (Lebererkrankungen, Diabetes mellitus Typ 2, arterielle Hypertonie) erfragt. Die Einnahme von Medikamenten, Alkoholkonsum und Rauchverhalten wurde nicht dokumentiert, um die Akzeptanz der Studie durch die Mitarbeiter zu erhöhen. Der Zeitaufwand für Aufklärung, Befragung, Durchführung der transienten Elastographie und der Bioelektrischen Impedanzanalyse betrug pro Mitarbeiter ca. 10 Minuten. Das Flowchart in **Abb. 1** fasst den Ablauf der Studie zusammen.

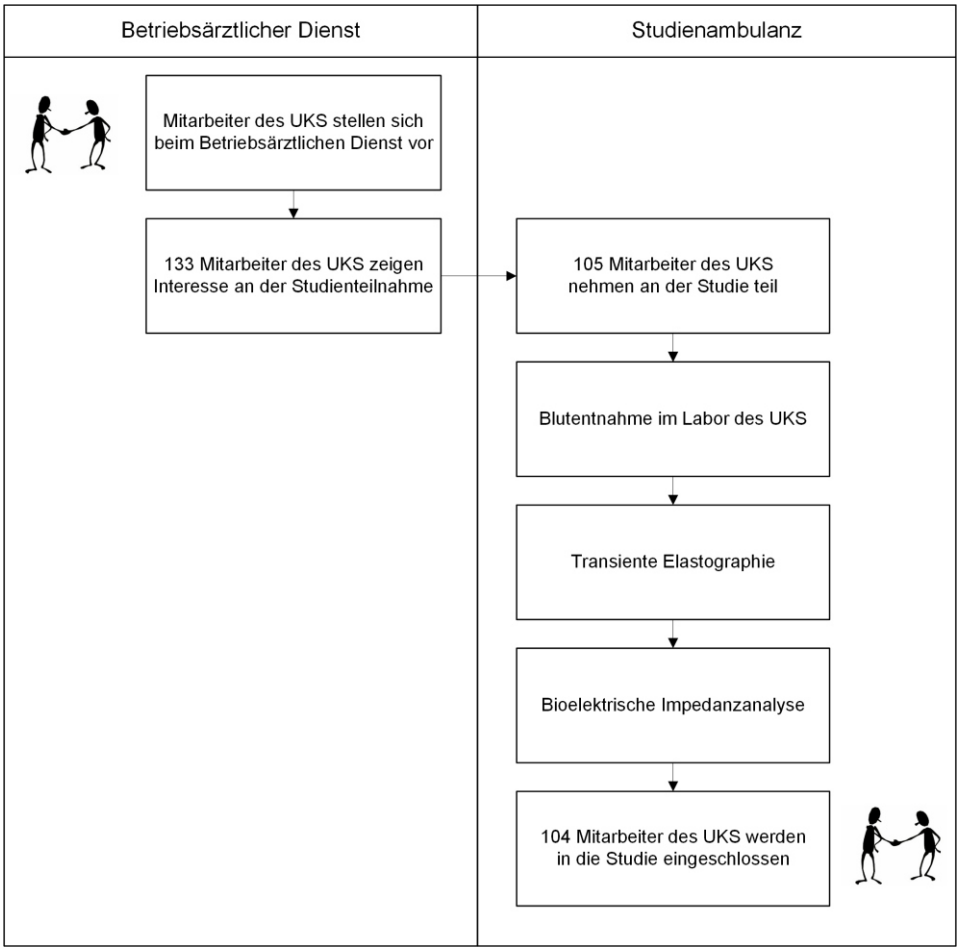

Abb.1: Das Flowchart zeigt graphisch den Ablauf der Studie. Abkürzungen: UKS, Universitätsklinikum des Saarlandes

Laborchemische Untersuchungen

Vom Arbeitsmediziner angeordnet, erfolgte eine Blutentnahme im Labor, die anschließend im Zentrallabor des UKS mittels klinisch-chemischer Standardtests analysiert wurde. Die Anforderung der Parameter unterlag allein dem Arbeitsmediziner anhand der medizinischen Notwendigkeit. Von den bestimmten Parametern wurden folgende in dieser Studie ausgewertet: Cholesterin (TC), Triglyzeride (TG), Glukose (GLC), Harnsäure (HS), Alanin-Aminotransferase (ALT), Aspartat-Aminotransferase (AST) und γ-Glutamyltransferase (γ-GT).

Untersuchungen in der Studienambulanz

Bioelektrische Impedanzanalyse und Anthropometrie

Die Bioelektrische Impedanzanalyse mit 8 Elektroden (seca mBCA515, Seca, Hamburg) ermittelt, an unbedeckten Händen und Füßen auf einer Wiegeplattform stehend, folgende Parameter: Gewicht, BMI, prozentuale Körperfettmasse (KFM), prozentuales Körperwasser (KW), prozentuale Skelettmuskelmasse (SMM) und viszerales Fettvolumen (VF). Die

Körperlänge wurde mittels Stadiometer (seca217, Seca, Hamburg) ermittelt. Taillenumfang (TU) und Hüftumfang (HU) wurden mittels Maßband im stehenden und ausgeatmeten Zustand auf Höhe des letzten Rippenbogens und der Beckenknochen gemessen. Das Taille-Hüfte-Verhältnis (THV) berechnet sich als Quotient aus TU und HU. Als Referenzwerte für Europäer gelten gemäß WHO ein TU ≤ 80 cm bei Frauen und ≤ 94 cm bei Männern sowie ein THV von < 0,85 bei Frauen und < 0,90 bei Männern (World Health Organization, 2011).

Transiente Elastographie

Mittels Ultraschall-basierter transienter Elastographie (FibroScan®, Echosens, Paris), einem jetzt seit über 10 Jahren bewährten Verfahren zur Untersuchung der Lebergesundheit, werden simultan die Leberverfettung und die Lebersteifigkeit quantifiziert. Hierfür wird in Rückenlage im Zwischenrippenraum des rechten Leberlappens an der Sonde ein niedrig-frequenter Ultraschall mittels Scherwelle ausgelöst. Die Verfettung der Leber wird durch die zunehmende Schallabschwächung als Controlled Attenuation Parameter (CAP) quantifiziert und kann Werte von 100 bis 400 dB/m annehmen (Sasso et al., 2010). In einer Studie der Allgemeinbevölkerung wurden Messungen ≥ 243 dB/m als Fettleber gewertet (entspricht Steatosegrad ≥ S1), und CAP-Werte ≥ 303,5 dB/m wurden als moderate Fettleber definiert (Steatosegrad ≥ S2). Die Performance des CAP-Wertes für S1 und S2 wurde mit einem AUROC von 0,94 und 0,95 bestimmt (Carvalhana et al., 2014).

Die Ausbreitungsgeschwindigkeit des Schalls in der Leber wird als Lebersteifigkeit (LS) in kPa ausgegeben; sie nimmt Werte von 1,5 bis 75,0 kPa an und steigt mit zunehmender Fibrosierung (Sandrin et al., 2003). Werte ≥ 7,65 kPa sprechen für ein Fibrosestadium ≥ F2, und Werte ≥ 13,0 kPa zeigen eine Leberzirrhose (F4) an (Friedrich-Rust et al., 2008). Als Gültigkeitskriterium für die Messungen gelten ein Interquartilsabstand zum Median des LS-Wertes ≤ 30% oder > 30% bei einem LS-Wert < 7,1 kPa (Boursier et al., 2013). Zum Zeitpunkt der Messung standen zwei Sonden mit unterschiedlichen Eigenschaften zur Verfügung. Die M-Sonde misst simultan den CAP und LS-Wert. Die XL-Sonde, die bei adipösen Menschen eingesetzt wird, ermittelt nur den LS-Wert. Zur Auswertung der Fettleber mittels CAP-Wert konnten demnach nur Messungen mit der M-Sonde berücksichtigt werden.

Metabolisches Syndrom

Für diese Studie wurden zur Definition des metabolischen Syndroms die Kriterien der International Diabetes Federation verwendet. Diese sind ein erhöhter TU und mindestens zwei der folgenden Kriterien: erhöhte Serum-TG-Konzentrationen, Nüchtern-GLC, Diabetes mellitus Typ 2, arterielle Hypertonie oder vermindertes Serum-HDL-TC (Alberti et al., 2005).

Statistik

Die Auswertung der Daten erfolgte mittels SPSS 20.0 (SPSS, München). Aufgrund der Normalverteilung nach Kolmogorov-Smirnoff-Test werden metrische Daten als Mittelwert±Standardabweichung angegeben und durch die Angabe der Spannweite ergänzt. Für unabhängige Stichproben aus zwei Gruppen wurden die Mittelwerte mittels t-Tests, bei mehr als zwei Gruppen mittels einfaktorieller ANOVA berechnet. Der Zusammenhang zwischen zwei Parametern wird mittels Pearson-Korrelationskoeffizient (r_p) dargestellt. Die Verteilung der Häufigkeiten zweier nominal verteilter Parameter wurde mittels χ^2-Test oder Fishers Exact Test (wie in **Tab.3 und 4** angegeben) berechnet. Parameter, die den CAP-Wert beeinflussen, wurden mittels univariater linearer Regressionsanalyse ermittelt, und signifikante Faktoren wurden in multivariaten Analysen erneut überprüft. Als signifikant wurden P-Werte < 0,05 definiert.

5.6. Ergebnisse

Studienkollektiv

An der UKS-Studie nahmen 104 Mitarbeiter, darunter 77 Frauen (74%), im Alter von 21–63 Jahren (mittleres Alter 35,1±12,5 Jahre), teil. Bei allen konnten die Bioelektrische Impedanzanalyse und die transiente Elastographie durchgeführt werden. In einem Fall konnte nur die XL-Sonde eingesetzt werden, sodass der fehlende CAP-Wert zum Ausschluss eines Mitarbeiters aus der Studie führte. Der durchschnittliche BMI lag bei 24,0±4,0 kg/m^2 (17,5–37,0 kg/m^2), wobei 67% der Teilnehmer einen BMI < 25 kg/m^2, 24% einen BMI zwischen 25 und 29,9 kg/m^2 und 9% einen BMI ≥ 30 kg/m^2 hatten. Initial gaben drei Teilnehmer (3%) vor den Untersuchungen an, dass eine Lebererkrankung bekannt sei (Fettleber bei N = 1, chronische Hepatitis B Virus-Infektion bei N = 2). Weiterhin hatten drei Teilnehmer (3%) einen Diabetes mellitus Typ 2 und sechs (6%) eine arterielle Hypertonie.

Laborchemische und Parameter der Körperzusammensetzung

Im Rahmen der betrieblichen Gesundheitsförderung waren bei 32,6% und 7,7% der Mitarbeiter die TC- bzw. die TG-Konzentrationen im Serum erhöht. Nüchtern-GLC-Werte (N = 47) lagen bei 14,9% der Studienteilnehmer oberhalb von 100 mg/dl. Der HS-Wert war bei 10,2% der Mitarbeiter erhöht. Die Parameter zur Bestimmung der Lebergesundheit (ALT, AST und γ-GT) waren bei 7,5%, 7,6% und 4,3% der Teilnehmer auffällig (**Tab. 1A**).

Bei 26,5% der Mitarbeiter lag der TU über dem WHO-Grenzwert, wobei er bei 14,7% erhöht (> 80 cm in Frauen und > 94 cm in Männern) und bei 11,8% stark erhöht (> 88 cm in Frauen und > 102 cm in Männern) war; das THV lag bei 49,0% der Mitarbeiter oberhalb der Norm. Mittels Bioelektrischer Impedanzanalyse wurde bei den Mitarbeitern folgende Parameter ermittelt: 28,8% KFM, 52,4% KW, 33,4% SMM und 1,4 l VF (**Tab. 1B**).

Tabelle 1: Laborchemische und Parameter der Körperzusammensetzung der Studienkohorte

	Mittelwert ± Standardabweichung (Spannweite)	**Referenzbereich**	**Außerhalb des Referenz-bereiches (%)**
A – Laborchemische Parameter			
TC (mg/dl)	189±38 (115–313)	< 200	32,6
TG (mg/dl)	100±56 (28–363)	< 150	7,7
Nüchtern-GLC (mg/dl)	91±9 (68–109)	60–100	14,9
HS (mg/dl)	4,6±1,3 (1,9–9,6)	2,4–5,7 (F), 3,4–7,0 (M)	10,2
ALT (U/l)	23±12 (11–81)	10–35 (F), 10–50 (M)	7,5
AST (U/l)	25±9 (12–69)	10–35 (F), 10–50 (M)	7,6
γ-GT (U/l)	23±17 (8–108)	< 40 (F), < 60 (M)	4,3
B – Parameter der Körperzusammensetzung			
BMI (kg/m²)	24,0±4,0 (17,5–37,0)		
TU (cm)	80±11 (61–119)	≤ 80 (F), ≤ 94 (M)	
THV	0,87±0,07 (0,74–1,09)	< 0,85 (F), < 0,90 (M)	
KFM (%)	28,8±9,2 (7,9–51,1)	---	
KW (%)	52,4±6,3 (37,0–67,7)	---	
SMM (%)	33,4±5,0 (23,1–45,6)	---	
VF (l)	1,4±1,3 (0,2–4,8)	---	

Abkürzungen: ALT, Alanin-Aminotransferase; AST, Aspartat-Aminotransferase; BMI, Body Mass Index; GLC, Glukose; γ-GT, γ -Glutamyltrans-ferase; F, Frau; HS, Harnsäure; KFM, Körperfettmasse; KW, Körperwasser; M, Mann; SMM, Skelettmuskelmasse; TC; Cholesterin; TG, Triglyzeride; THV, Taille-Hüfte-Verhältnis; TU, Taillenumfang; VF, viszerales Fettvolumen

Fettleberdiagnostik mittels CAP-Messung

Mittels transienter Elastographie konnte bei 104 Mitarbeitern mit einer durchschnittlichen Messdauer von 2,7±3,0 Minuten eine Messung mit der M-Sonde durchgeführt werden (siehe Methoden). Der mittlere CAP betrug 224±61 dB/m (100–400). In 38 Fällen (36,5%) lag eine Fettleber vor (CAP ≥ 243 dB/m; **Abb. 2A**), wovon 26,9% und 9,6% auf die Steatosegrade S1 und S2 entfielen (**Abb. 3A**). Unter Ausschluss der drei Teilnehmer mit bekannten Leberkrankheiten betrug die Häufigkeit einer neu diagnostizierten Fettleber 35,6%.

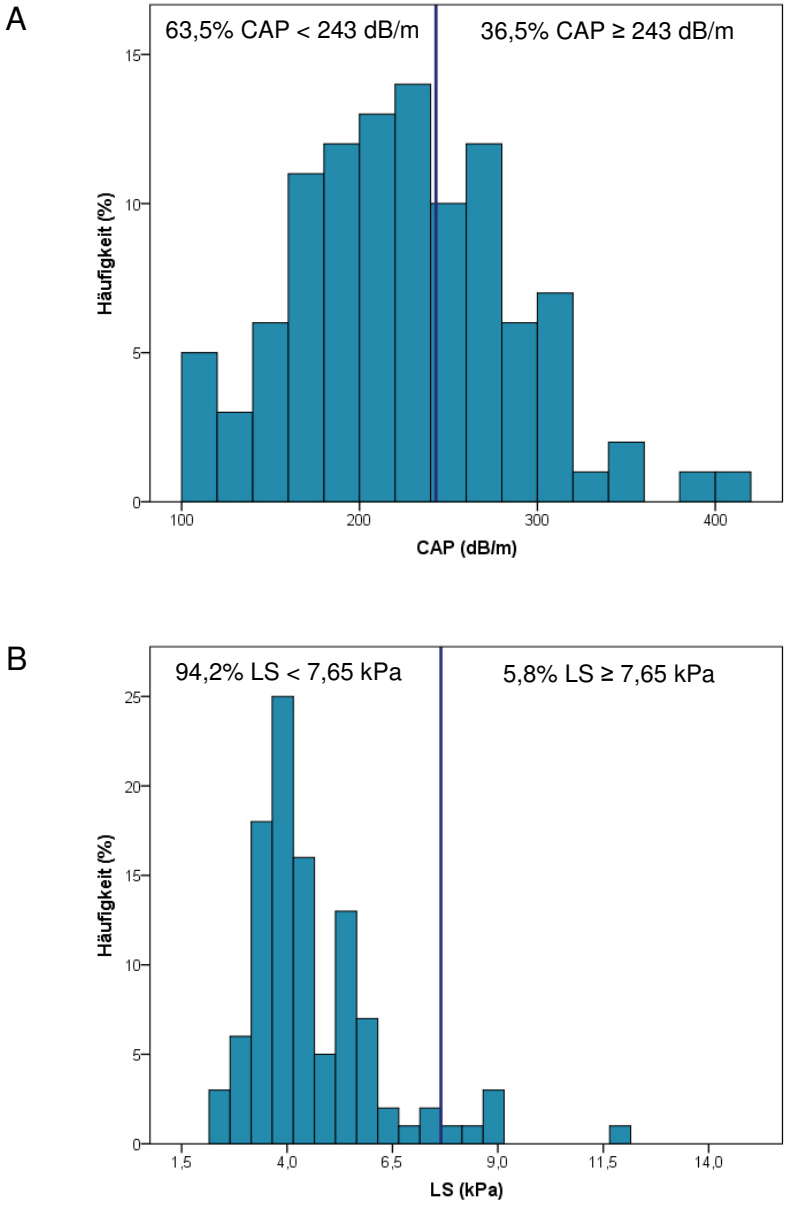

Abb. 2: Häufigkeitsverteilung der (**A**) Leberverfettung (CAP-Werte) und (**B**) Lebersteifigkeit (LS-Werte) sowie Einteilung nach etablierten Grenzwerten.

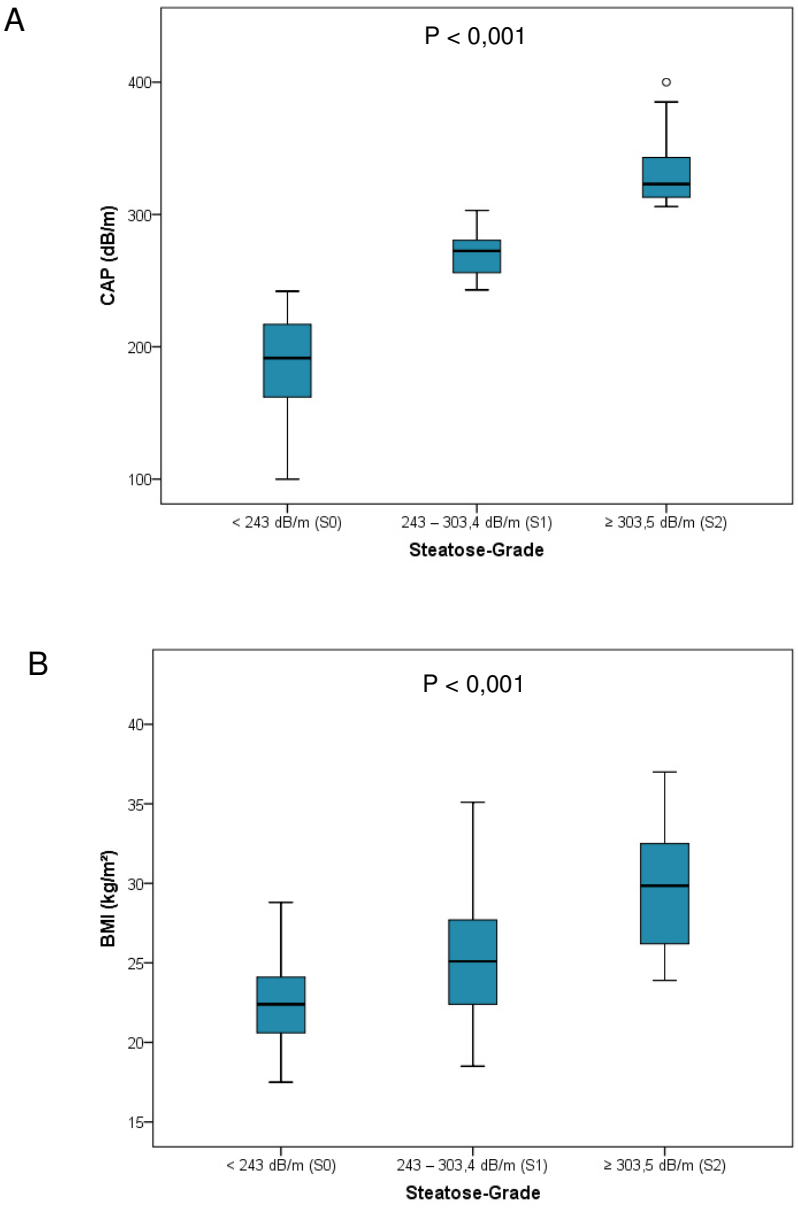

Abb. 3: Verteilung der (**A**) Leberverfettung (CAP-Werte) in dB/m und (**B**) Body Mass Index (BMI) in kg/m² pro Steatose-Grad. S0: 188±37 dB/m und 22,4±2,7 kg/m²; S1: 271±17 dB/m und 25,4±3,9 kg/m²; S2: 336±33 dB/m und 30,1±4,5 kg/m².

Signifikante Zusammenhänge konnten zwischen Laborparametern, die mit der Fettleber assoziiert sind, und dem Grad der Verfettung beobachtet werden: ALT und γ-GT stiegen mit zunehmendem Steatosegrad an ($P = 0,001$ und $P < 0,001$; **Abb. 4a, b** sowie **Tab. 2A**). Auch die Einteilung beider Parameter nach normwertigen bzw. erhöhten ALT und γ-GT ergaben bei Mitarbeitern ohne (S0) und mit Fettleber (≥ S1) signifikante Unterschiede (**Tab. 3**). 6,5% und 4,3% der Mitarbeiter hatten erhöhte ALT und γ-GT bei Vorliegen einer Fettleber (≥ S1).

Interessanterweise lagen bei fast einem Drittel der Fälle mit Fettleber ALT und γ-GT im Normbereich (29,0% und 31,2%; **Tab. 3**).

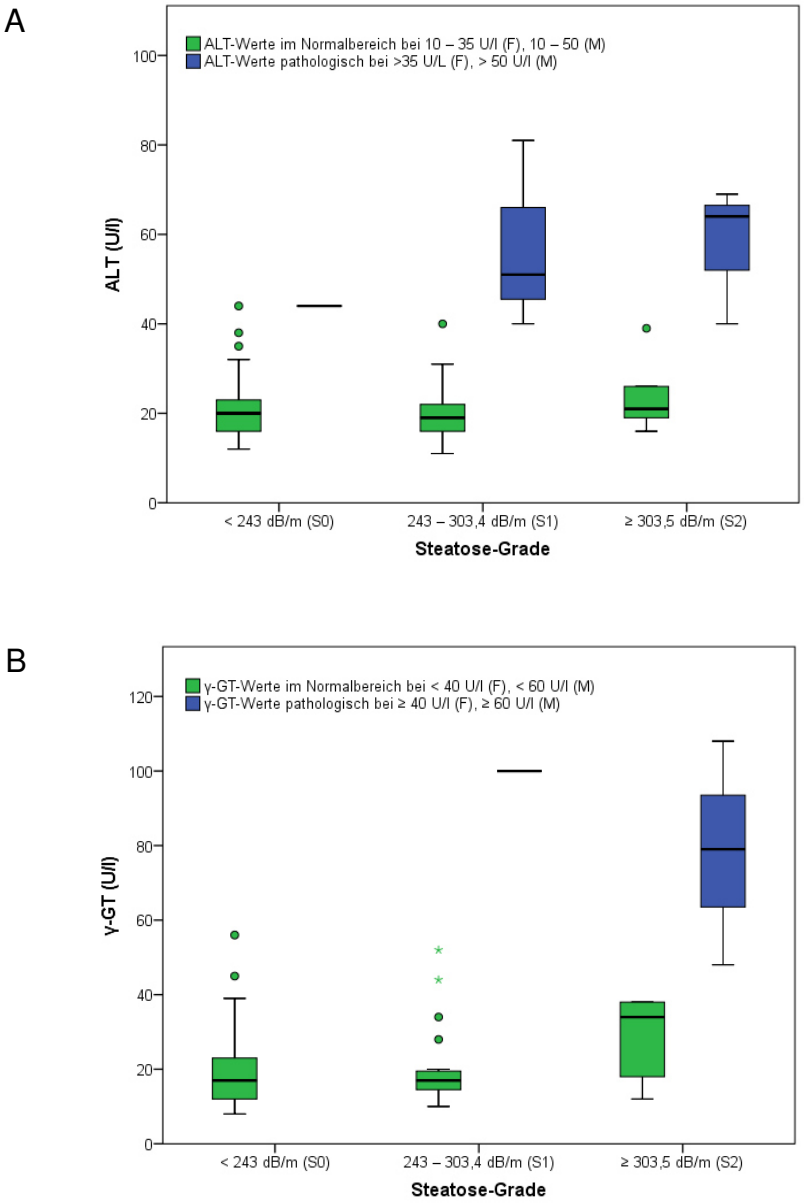

Abb. 4: Verteilung der (**A**) ALT-Werte in U/l und (**B**) γ-GT-Werte in U/l pro Steatose-Grad. S0: 21±7 U/l und 19±9 U/l; S1: 25±15 U/l und 23±19 U/l; S2: 37±20 U/l und 47±32 U/l.

Tabelle 2: Zusammenhang zwischen CAP-Werten, laborchemischen und Parametern der Körperzusammensetzung

	Korrelation mit CAP-Werten	P	S0	S1	S2	P
A – Laborchemische Parameter						
TC (mg/dl)	0,277	**0,008**	183	202	193	n.s.
TG (mg/dl)	0,355	**0,001**	89	107	156	**0,004**
Nüchtern-GLC (mg/dl)	0,370	**0,010**	89	93	96	n.s.
HS (mg/dl)	0,215	**0,044**	4,5	4,7	5,6	n.s.
ALT (U/l)	0,407	**0,001**	21	25	37	**0,001**
AST (U/l)	0,077	n.s.	26	24	27	n.s.
γ-GT (U/l)	0,385	**0,001**	19	23	47	**< 0,001**
B – Parameter der Körperzusammensetzung						
BMI (kg/m²)	0,615	**0,001**	22,4	25,4	30,1	**< 0,001**
TU (cm)	0,684	**0,001**	75	84	96	**< 0,001**
THV	0,406	**< 0,001**	0,85	0,86	0,95	**< 0,001**
KFM (%)	0,515	**0,001**	25,5	33,2	40,3	**< 0,001**
KW (%)	-0,524	**< 0,001**	54,7	49,4	44,5	**< 0,001**
SMM (%)	-0,362	**0,001**	34,7	31,6	28,9	**< 0,001**
VF (l)	0,668	**0,001**	0,8	2,3	-	**0,003**

Abkürzungen: ALT, Alanin-Aminotransferase; AST, Aspartat-Aminotransferase; BMI, Body Mass Index; GLC, Glukose; γ-GT, γ-Glutamyltrans-ferase; F, Frau; HS, Harnsäure; KFM, Körperfettmasse; KW, Körperwasser; M, Mann; P, P-Wert; SMM, Skelettmuskelmasse; TC; Cholesterin; TG, Triglyzeride; THV, Taille-Hüfte-Verhältnis; TU, Taillenumfang; VF, viszerales Fettvolumen

Tabelle 3: Verteilung der absoluten Häufigkeiten von leberassoziierten Laborparametern

	S0 (< 243 dB/m)	≥ S1 (≥ 243 dB/m)	P*
ALT im Normbereich	59 (63,4%)	27 (29,0%)	**0,007**
ALT erhöht	1 (1,1%)	6 (6,5%)	
γ-GT im Normbereich	60 (64,5%)	29 (31,2%)	**0,014**
γ-GT erhöht	0 (0%)	4 (4,3%)	

Abkürzungen: ALT, Alanin-Aminotransferase; γ-GT, γ-Glutamyltransferase; P, P-Wert
* P-Wert mittels Fishers Exact Test ermittelt

Der CAP-Wert nahm mit steigendem BMI zu ($r_p = 0,615$, $P < 0,001$). Der Steatosegrad unterschied sich in charakteristischer Weise zwischen den BMI-Gruppen: S0 war charakteristisch für Normalgewichtige, S1 für Übergewichtige und S2 für adipöse ($P < 0,001$; **Abb. 3B**). Ein ähnliches Muster fand sich bei weiteren Parametern der Körperzusammensetzung, die in **Tab. 2B** aufgelistet sind: Mit zunehmendem CAP-Wert nahmen auch TU, THV, KFM und VF zu und KW und SMM ab (alle $P < 0,001$). Der CAP-Wert wurde durch Alter, BMI, KFM und TU

beeinflusst (alle P < 0,001; univariate lineare Regressionsanalyse). In der multivariaten Analyse zeigte sich, dass die KFM und der TU mit dem CAP-Wert signifikant (P = 0,033 und P < 0,001) um das 1,7-fache und 3,8-fache assoziiert sind (**Tab.4**).

Tabelle 4: Lineare univariate und multivariate Analyse der Leberverfettung

	OR	**95% CI**	**P***
Univariate Analyse			
Alter (Jahre)	1,85	0,98–2,73	**< 0,001**
BMI (kg/m²)	9,30	6,96–11,64	**< 0,001**
Geschlecht	-17,27	-44,15–9,57	n.s.
KFM (%)	3,41	2,25–4,58	**< 0,001**
TU (cm)	3,84	3,02–4,65	**< 0,001**
Multivariate Analyse			
KFM (%)	1,73	0,14–3,32	**0,033**
TU (cm)	3,79	2,00–5,58	**< 0,001**

Abkürzungen: BMI, Body Mass Index; KFM, Körperfettmasse; P, P-Wert; TU, Taillenumfang
* P-Wert mittels Fishers Exact Test ermittelt

Die Fettleber ist mit den Kriterien des metabolischen Syndroms assoziiert

Anhand der vom Arbeitsmediziner angeforderten Labordaten konnten folgende Parameter des metabolischen Syndroms herangezogen werden: TU, TG, Nüchtern-GLC, bekannter Diabetes mellitus Typ 2 und bekannte arterielle Hypertonie. Das HDL-TC wurde nur bei fünf der 104 Mitarbeiter angefordert. Bei 23 Teilnehmern lag ein Kriterium, und bei vier lagen zwei Kriterien für ein metabolisches Syndrom vor; in vier Fällen lagen ≥ 3 Kriterien vor, sodass hier ein metabolisches Syndrom diagnostiziert werden konnte (**Abb. 5A**). Die Verfettung der Leber stieg mit zunehmender Anzahl der Kriterien an (P < 0,001; **Abb. 5B**). Vergleicht man nun Mitarbeiter ohne und mit Fettleber, waren letztere älter (39 Jahre vs. 33 Jahre, P = 0,018), hatten häufiger einen BMI über 25 kg/m^2 (60,5% vs. 16,7%, P < 0,001), eine arterielle Hypertonie (15,5% vs. 0%, P = 0,001), TC-Konzentrationen über 200 mg/dl (46,9% vs. 25,6%, P = 0,031) und TG-Werte im Serum über 150 mg/dl (18,8% vs. 3,6%, P = 0,019; **Tab. 5**).

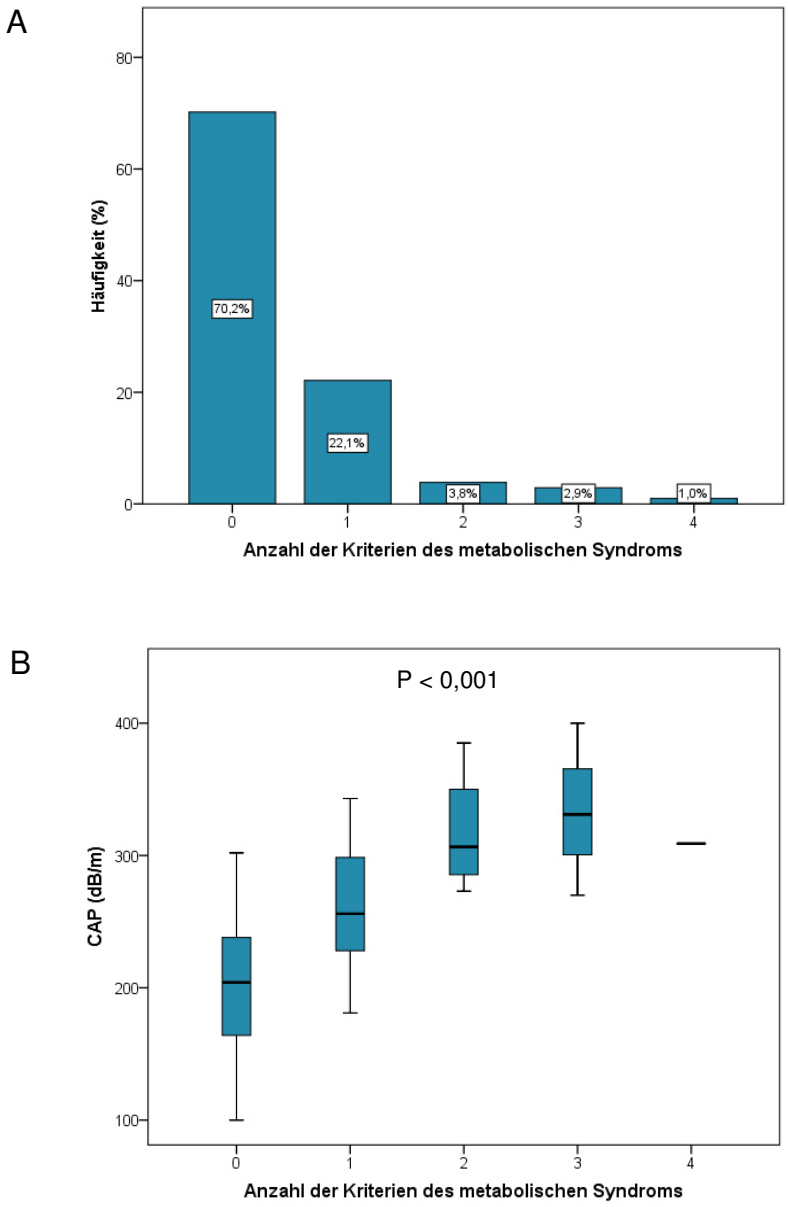

Abb. 5: (**A**) Häufigkeitsverteilung der Anzahl der Kriterien des metabolischen Syndroms und (**B**) die jeweilige Verteilung der Leberverfettung (CAP-Werte) in dB/m. 0: 202±49 dB/m; 1: 259±48 dB/m, 2: 318±48 dB/m: 3: 334±65 dB/m; 4: 309 dB/m.

Tabelle 5: Zusammenhang zwischen CAP-Werten und kardiovaskuläre Risikofaktoren

	Keine Fettleber (CAP < 243 dB/m, N = 66, 63,5%)	**Fettleber (CAP ≥ 243 dB/m, N = 38, 36,5%)**	**P**
Alter (Jahre)	33	39	**0,018**
Arterielle Hypertonie	0%	15,8%	**0,001**
BMI (kg/m²)	22,4	26,7	**< 0,001**
Übergewicht (BMI ≥ 25 kg/m^2)	16,7%	60,5%	**< 0,001**
TC (mg/dl)	183	199	n.s.
TC ≥ 200 mg/dl	25,6%	46,9%	**0,031**
Diabetes mellitus Typ 2	1,5%	5,3%	n.s.
TG (mg/dl)	89	119	**0,012**
TG ≥ 150 mg/dl	3,6%	18,8%	**0,019**

Abkürzungen: BMI, Body Mass Index; CAP, Controlled Attenuation Parameter; P, P-Wert; TC; Cholesterin; TG, Triglyzeride

Quantifizierung der Leberfibrose

Die Lebersteifigkeit als quantitativer Parameter für die Fibrose der Leber lag im Mittel bei 4,6±1,6 kPa (2,4–11,9 kPa). Keiner der Teilnehmer zeigte Werte über 13,0 kPa, die allgemein das Vorliegen einer Leberzirrhose anzeigen (Friedrich-Rust et al., 2008). ALT und γ-GT korrelierten mit zunehmender Lebersteifigkeit (r_p = 0,239, P = 0,004 bzw. r_p = 0,268, P = 0,009). Bei sechs Mitarbeitern (5,8%) lagen Werte ≥ 7,65 kPa vor, entsprechend einem Fibrosestadium ≥ F2 (**Abb. 2B**). Diese Gruppe unterschied sich von der ohne relevante Fibrose (< 7,65 kPa) durch einen höheren BMI (28,9 kg/m² vs. 23,7 kg/m², P = 0,002) und höhere CAP-Werte (287 dB/m vs. 220 dB/m, P = 0,008).

5.7. Diskussion

Erstmals wurde in dieser Studie die nicht-invasive Quantifizierung der Fettleber im Rahmen der betrieblichen Gesundheitsförderung in Deutschland untersucht. Bei 36% der Mitarbeiter des Universitätsklinikums wurde mittels CAP eine Fettleber diagnostiziert, ohne dass diese zuvor davon Kenntnis hatten. Bei einem Drittel dieser Mitarbeiter waren die Leberwerte im Serum normwertig. Die Bestimmung des CAP-Werts ermöglicht die frühzeitige Diagnose eine Steatose, die zur NASH und Fibrose fortschreiten kann und daher eines Monitorings bedarf [15]. Das Sterblichkeitsrisiko bei diesen Patienten ist vor allem aufgrund von kardiovaskulären Erkrankungen erhöht (Than and Newsome, 2015), weshalb sowohl das weitere metabolische aber auch das hepatische Monitoring von Bedeutung sind. Simultan zur Leberverfettung

wurde die Lebersteifigkeit quantifiziert. 6% der Teilnehmer hatten in der Elastographie Hinweise für eine relevante Leberfibrose. Die Fibrose ist ein Prädiktor für leberspezifische Morbidität und Mortalität sowie Gesamtmortalität (Angulo et al., 2015). Bei vier der sechs Patienten waren ALT und γ-GT normwertig, wodurch eine Lebererkrankung ohne bildgebende Verfahren übersehen worden wäre.

Diese Studie bei Mitarbeitern eines Universitätsklinikums zeigt, dass bei Vorliegen einer Fettleber kardiovaskuläre Risikofaktoren gehäuft beobachtet werden (Alter, BMI > 25 kg/m^2, arterielle Hypertonie, Hyperlipoproteinämie). Mit steigendem CAP-Wert nahm die Zahl der Kriterien, die das metabolische Syndrom definieren, zu. Der BMI stieg mit der Leberverfettung an, hatte jedoch keinen unabhängigen Vorhersagewert für die Fettleber. Die am stärksten mit erhöhten CAP-Werten assoziierten Parameter waren die prozentuale Körperfettmasse und der Taillenumfang. Daher sollte die Bestimmung der Körperzusammensetzung durch die Bioelektrische Impedanzanalyse und des Taillenumfangs mittels eines einfachen Maßbandes in der präventionsmedizinischen Praxis einen höheren Stellenwert erhalten. Bei 85 von 578 (15%) chinesischen Mitarbeitern der Dienstleitungsindustrie wurde mittels Ultraschall eine Fettleber diagnostiziert. Der mittlere BMI und der Anteil erhöhter AST-Werte war mit unserer Kohorte vergleichbar (23,0 kg/m² und 5,4% vs. 24,0 kg/m² und 7,6%). Abweichungen gibt es hingegen beim Anteil der Mitarbeiter mit Fettleber, arterieller Hypertonie und erhöhten TG-Werten (Dai et al., 2009). In einer Studie aus Taiwan wurden 1384 Mitarbeiter eines stromproduzierenden Unternehmens mittels Ultraschall untersucht: 27% hatten eine Fettleber bei einer mittlere ALT von 27 U/l. Hierbei wurden Patienten mit chronischer Virushepatitis nicht ausgeschlossen, hingegen Arbeiter mit metabolischem Syndrom schon, wobei sich die verwendete Definition von der des International Diabetes Federation unterscheidet (Lin et al., 2010). Ultraschall als Diagnosemethode ist jedoch fragwürdig bei einer Sensitivität von 43% oder 60% für eine Leberverfettung von > 2% oder > 30% (Hepburn et al., 2005). Es gibt Unterschiede in der Körperzusammensetzung von Asiaten und Europäern: Europäer haben, verglichen mit Asiaten, weniger viszerales Fettgewebe bei gleichem Taillenumfang (Lear et al., 2007) und weniger Körperfett (Kagawa et al., 2007), sodass unterschiedliche anthropometrische Grenzwerte gelten. Die Vergleichbarkeit der Daten aus Asien ist daher nur begrenzt möglich. Weiterhin ist in unserer Studie der Frauenanteil höher

und die körperlichen und die Umwelteinflüsse im Krankenhausumfeld unterscheiden sich von denen der Dienstleistungs- und stromproduzierenden Industrie. Im Jahr 2015 Betrug der Anteil der weiblichen Angestellten am Universitätsklinikum des Saarlandes 73%, sodass die Ergebnisse der UKS-Studie, mit einem Frauenanteil von 74%, repräsentativ sind.

Die transiente Elastographie und die Bioelektrische Impedanzanalyse sind nicht-invasive und patientenfreundliche Methoden mit geringen Kosten, die jederzeit wiederholbar sind, durch Funktionspersonal durchgeführt werden und dadurch unabhängig vom ärztlichem Personal sind. Die Untersuchungsdauer der Leber betrug in dieser Studie durchschnittlich 2,7 Minuten, und das Verfahren kann zurecht als „EKG des Hepatologen“ bezeichnet werden (Arslanow and Lammert, 2016). Die Teilnehmer wurden über auffällige Befunde informiert und auf die Notwendigkeit einer weiteren Abklärung hingewiesen. Bei Teilnehmern, die im Anschluss in der Hochschulambulanz zur Ernährungsberatung vorstellig wurden, wurde der Therapieerlauf mittels transienter Elastographie und Bioelektrischer Impedanzanalyse überwacht.

Die ermittelte Prävalenz für das metabolische Syndrom wird in unserer Kohorte aufgrund der fehlenden HDL-Werte bei einem Großteil der Teilnehmer unterschätzt. Ursächlich hierfür ist das Anforderungsverhalten der Arbeitsmediziner. Im Rahmen der betrieblichen Gesundheitsförderung konnte auch nicht gewährleistet werden, dass alle Teilnehmer mindestens acht Stunden nüchtern waren, sodass auch nicht durchgehend Nüchtern-Glukosewerte zur Verfügung standen.

Die Deutsche Leitlinie zu Fettlebererkrankung empfiehlt hinsichtlich der Therapie aus hepatologischer und kardiologischer Sicht eine Reduktion des Übergewichts und eine Steigerung der körperlichen Aktivität (Roeb et al., 2015). Der Arbeitsmediziner bzw. Betriebsarzt sollte darüber informieren sowie Beratungsgespräche über gesunde Ernährung und Bewegungsprogramme anbieten. Die Lotsenfunktion des Arbeitsmediziners bei aktuell 43 Millionen Beschäftigen hinsichtlich der Prävention von Lebererkrankungen wird durch die transiente Elastographie erleichtert und kann weiteren Beratungs- und Handlungsbedarf an der Schnittstelle zum Haus- und Facharzt anzeigen (Letzel, 2016).

5.8. Fazit für die Praxis

- Die Fettleberkrankheit ist initial meist symptomlos und geht nicht immer mit erhöhten Leberwerten einher, sodass neue Diagnoseverfahren benötigt werden.
- Die transiente Elastographie stellt ein nicht-invasives, risikofreies und schnelles Verfahren zur Beurteilung von Leberverfettung und Leberfibrose im Rahmen der betrieblichen Gesundheitsförderung dar.
- Die prozentuale Körperfettmasse und der Taillenumfang sind die stärksten Prädiktoren für die Leberverfettung.
- Die Befunde der transienten Elastographie und Bioelektrischen Impedanzanalyse sind für den Mitarbeiter leicht zu verstehen und können zur Änderung des Lebensstils motivieren.

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6. Zur Diagnose der Fettleberkrankheit (Diagnosis of fatty liver disease)

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The published manuscript can be found in Appendix 4.

6.1. Zur Diagnose der Fettleberkranheit

Die neue S2k-Leitlinie „Nicht-alkoholische Fettlebererkrankung“ der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten (DGVS) fasst den aktuellen Kenntnisstand zu Epidemiologie, Risikofaktoren, Diagnostik und Therapie zusammen (Roeb et al., 2015). Die Leitlinie definiert: „Die Steatosis hepatis (Fettleber) zeichnet sich durch eine Einlagerung von Fett in den Hepatozyten aus.“ Sie spricht explizit von „Patienten mit nicht-alkoholischer Fettleber (NAFL)“, die im Verlauf eine Steatohepatitis (NASH) und eine Zirrhose entwickeln können (S. 671). Diese Definitionen legen eine bimodale Verteilung, die die Bevölkerung in Patienten mit und Gesunde ohne Fetteber dichotomisiert, nahe. Der Krankheitsbegriff dient aber nicht nur zur Beschreibung, sondern auch dazu, ärztliche Handlungen und Aktionen anzumahnen (Engelhardt, 2012; Maio, 2015). Nur als Randbemerkung sei eingefügt, dass im Englischen zudem streng „Disease“ (wie in NAFLD) von „Illness“ unterschieden wird: Der Mensch kann eine „Krankheit“ haben, ohne „erkrankt“ zu sein, so dass man streng genommen von „Nicht-alkoholischer Fettleberkrankheit“ sprechen müsste.

Die Diagnose der NAFLD wird heute mittels Serummarkern und daraus abgeleiteter Scoring-Systeme, bildgebender Verfahren (Ultraschall, Computertomografie, Magnetresonanz-verfahren) und Leberbiopsie vorgenommen. Vor über 10 Jahren fand die Ultraschall-basierte Elastografie (z. B. Fibroscan®) Einzug in die Hepatologie. Diese nicht-invasive Methode wurde kürzlich durch den simultan ermittelten Controlled Attenuation Parameter (CAP) erweitert (De Ledinghen et al., 2012; Kwok et al., 2016). Dieses Verfahren nutzt die dem Ultraschaller gut vertraute Schallabschwächung bei zunehmender Leberverfettung aus, die häufig zur semiquantitativen Beurteilung des Steatose-Grades herangezogen wird. Beim CAP-Verfahren wird die Schallabschwächung quantifiziert und als CAP-Wert in [dB/m] angegeben. Auch wenn dieses Verfahren keinen „Goldstandard“ darstellt, erlaubt es doch umfangreiche und longitudinale Verlaufsbeurteilungen der Fettleberkrankheit in bisher nicht dagewesenem Umfang.

Am Universitätsklinikum des Saarlandes wurden im Zeitraum von Mai 2012 bis Dezember 2015 insgesamt 6.814 Messungen mit dem Fibroscan® bei ambulanten Patienten der Inneren Medizin II (Schwerpunkte: Gastroenterologie, Hepatologie, Endokrinologie, Diabetologie und Ernährungsmedizin), bei stationären Patienten des gesamten Universitätsklinikums sowie im Rahmen von Studien an Patienten und Gesunden durchgeführt. Nach Abzug von 132 Test- und Trainingsmessungen berichten wir hier von 6.682 Messungen. Bei einer medianen Untersuchungsdauer von 95 sec (Interquartilsabstand (IQR) 69–158 sec) wurde für die Kohorte ein medianer CAP-Wert von

245 dB/m (IQR 202–295 dB/m) dokumentiert; die Lebersteifigkeit als Maß für die Leberfibrose lag bei 7,3 kPa (IQR 5,2–12,2 kPa; **Abb. 1A, B**). Lange Zeit war die Erfassung des CAP-Werts auf den Einsatz der „M-Sonde“ des Fibroscan beschränkt; seit Kurzem kann dieser Parameter auch durch die „XL-Sonde“ erfasst werden, sodass insgesamt für > 90% der Messungen ein CAP-Wert dokumentiert werden konnte. Technisch bedingt werden CAP-Werte zwischen 100 und 400 dB/m ausgedrückt, so dass Patienten mit niedrigen oder hohen Werten in die Randklassen fallen.

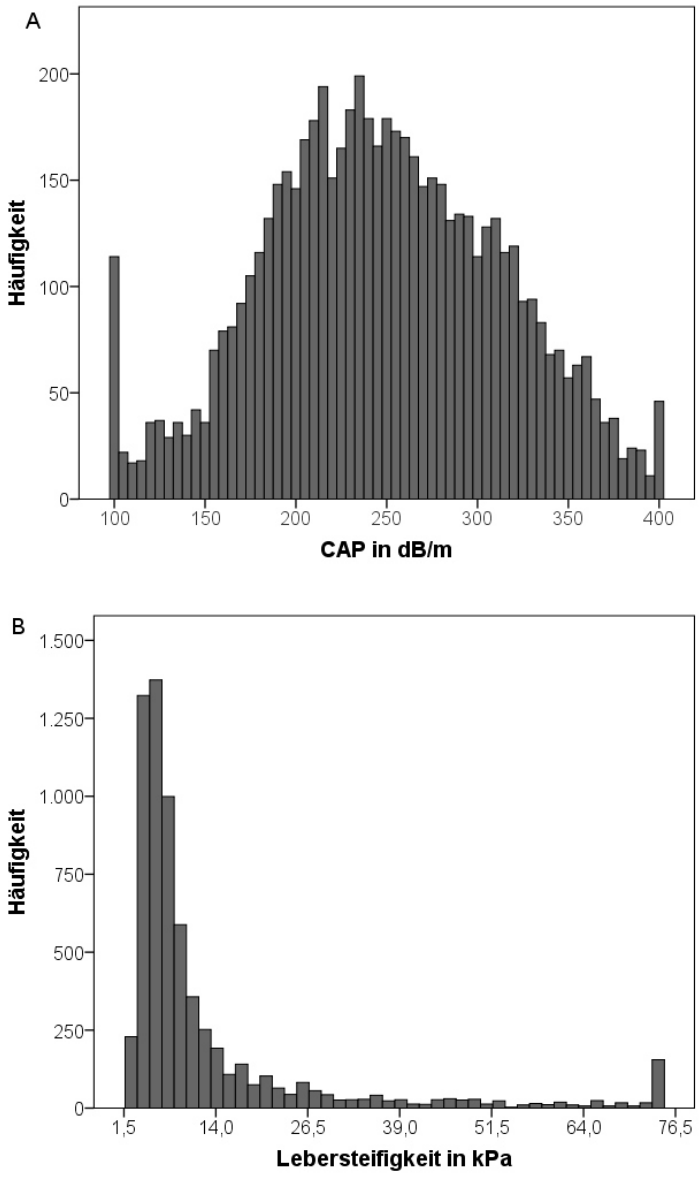

Abb. 1: Häufigkeitsverteilung der **A** CAP-Werte (N = 6.099) und **B** Lebersteifigkeitswerte (N = 6.682) mittels Ultraschall-basierter Elastographie (Fibroscan®) am Universitätsklinikum des Saarlandes.

Aus ärztlicher Sicht wesentlich ist, dass die CAP-Werte keine bimodale Verteilung haben: Es gibt also nicht Menschen mit oder ohne Fettleber, sondern nur Individuen mit mehr oder weniger viel Fett in der Leber. Leberfett lässt sich – wie der Blutdruck – metrisch und nicht nur ordinal messen. Der mediane CAP-Wert von 245 dB/m zeigt nach den publizierten Voruntersuchungen an, dass viele Teilnehmer an der Untersuchung bereits eine „Fettleber" haben (Arslanow et al., 2016). Letztlich ist es eine Frage der Krankheitsdefinition und langfristiger Studien mit harten Endpunkten (Überleben, Zirrhosekomplikationen), welchen Schwellenwert wir der Diagnose der Fettleber zugrunde legen. Interessant ist auch, dass nur wenige Patienten sehr niedrige oder sehr hohe CAP-Werte und demnach eine Leber mit sehr geringer oder sehr starker Fetteinlagerung haben. Die Verteilung insgesamt kann mit der einer Allgemeinbevölkerung verglichen werden, da die Kohorte in ihrer Ätiologie und Krankheitsschwere sehr heterogen aufgebaut ist.

Die S2k-Leitlinie (Roeb et al., 2015) empfiehlt das Screening auf NAFLD nur bei Personen mit Risikofaktoren (Adipositas, Dyslipidämie, Diabetes mellitus, Medikamente). Hinsichtlich der genetischen Prädisposition weist sie darauf hin, dass Patienten mit einer homozygoten Variante im *PNPLA3*-Gen (p. I148 M) eine höhere Prävalenz für die Entstehung einer NAFLD aufweisen. Patienten mit NAFLD sind aber häufig asymptomatisch mit normwertigen Transaminasen. Die CAP-Methode erlaubt nicht nur ein Screening auf NAFLD in der Allgemeinbevölkerung, sondern auch kurzfristige Verlaufskontrollen unter der in der Leitlinie als einzige Therapie bei Fettleber empfohlenen Lebensstilmodifikation (Roeb et al., 2015). Weitere Interventionsstudien mit Monitoring des CAP-Werts werden benötigt, um Behandlungsziele zu definieren und letztlich ein „Treat-to-Target"-Konzept zu implementieren.

Die CAP-Messung zeichnet sich dadurch aus, dass sie patientenfreundlich und nicht invasiv ist. Die Durchführung durch Funktionspersonal, die kurze Messdauer und die geringen variablen Kosten zeichnen die Methode aus – sie kann daher mit Recht als „EKG des Hepatologen" bezeichnet werden.

6.2. Referenzen

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7. Discussion

Evaluation of transient elastography

Transient elastography (TE) provides many benefits compared to biopsy as the "gold standard" for the diagnosis of liver diseases: the area of the liver analyzed by TE is 100 times larger than by biopsy (Sasso et al., 2010) and conversely, sampling errors are more likely in a smaller area (Karlas et al., 2017). Additionally, biopsy carries a risk of bleeding (Terjung et al., 2003), whereas TE does not. The Fibroscan® device can easily be wheeled around to the patient's bedside; therefore it is convenient for patient and examiner. Our studies proved the method's rapidness by measurement times of 95 seconds (interquartile range 69–158 seconds) and 2.7 minutes (±3.0 minutes). Also, there is no need for a physician to carry out the measurement, because it can be performed by trained medical personnel. Interobserver agreement of two examiners is excellent with correlation coefficients of 0.96 and 0.98 for LSM and 0.82 for CAP (Ferraioli et al., 2014b; Fraquelli et al., 2007). The acceptance and consequently patient-friendliness of TE showed high ratings of 95% for convenience and 77% for non-painfulness in a pediatric cohort using a 5-point scale (Chen et al., 2016). Liver stiffness measurements using TE are more cost-effective and patients have shorter waiting times until the examination compared to biopsy, but no data has been published yet for CAP (Van Katwyk et al., 2017; Whitty et al., 2014). In clinical practice and in research, the TE examination can be carried out in frequent check-ups and intervention studies, because there is no limit on the frequency of the measurements.

A non-fasting state of less than two hours, BMI higher than 27.7 kg/m², age above 50 years and the use of a non-appropriate probe have been reported as factors influencing on liver stiffness measurements (Arena et al., 2013; Myers et al., 2012b; Sirli et al., 2013). The reported limitation of elevated BMI is questionable: A large retrospective study of 8,218 patients reported a failure rate of liver stiffness measurements of 29.1%, one reason being BMI > 27.7 kg/m² (Sirli et al., 2013). Although liver stiffness measurements have been available since 2003 (Sandrin et al., 2003), measurements using the XL probe in obese patients has not been available until 2010 (De Ledinghen et al., 2010). Little is known about factors that influence CAP. Recently, food intake prior to the examination was found to increase CAP values, reaching a maximum increase of 7.4 and 9.9% (equal to an absolute increase of 22 dB/m in both cases) after 60 and 120 minutes of a 625 and 1,250 kcal meal. Of note, in this study 83% of the participants presented with alcoholic liver disease, of which 50% had cirrhosis (Kjaergaard et al., 2017), therefore the results need to be confirmed in a cohort of patients with non-alcoholic liver disease. A 2014 study stated a CAP failure rate of 7.7% in 5,323 cases correlated with metabolic syndrome, elevated BMI and female sex (De

Ledinghen et al., 2014). Contrary, CAP measurements with the XL probe parameter have only been introduced last year (Sasso et al., 2016). Importantly, the studies by Sirli (Sirli et al., 2013) and de Ledinghen (De Ledinghen et al., 2014) were carried out before probe improvements took place (i.e. XL probe for obese patients and CAP assessment). Given the potential of the new probe, the reported failure rate may decrease and the results need to be validated in new cohort studies.

CAP has been verified against biopsy, as summarized in a very meta-analysis based on individual data from 2,735 patients. The authors calculated an area under the receiver operating characteristic (AUROC) curve of 0.82 (95% confidence interval 0.81–0.84) and concluded that CAP is a standardized non-invasive method to diagnose steatosis (Karlas et al., 2017). This study confirms findings from a previous meta-analysis with a hierarchical summary receiver operating characteristic (HSROC) curve of 0.85 (95% confidence interval 0.81–0.88) when validated against biopsy (Shi et al., 2014). CAP in relation to magnetic resonance imaging showed a high accuracy of AUROC = 0.93 (95% confidence interval 0.87–0.99) in a single center study (Karlas et al., 2014). For liver stiffness measurements, staging of fibrosis in NAFLD and hepatitis B using TE has been shown to have very good diagnostic accuracy in several meta-analyses (Friedrich-Rust et al., 2008; Li et al., 2016b; Xu et al., 2015).

The definition of cut-off values to determine non-diseased from diseased and further classification of steatosis stages have been challenging as seen in studies with conflicting results. Stern and Castera listed 21 studies published between 2010 and 2016, each of them reporting different thresholds for steatosis grades S1, S2 and S3 (Stern and Castera, 2017). The studies were carried out in patients with different liver diseases or in a general population. Cut-offs for S1 steatosis ranged from as low as 215 dB/m to as high as 283 dB/m, while different definitions were applied (≥ 5%, ≥ 10% and ≥ 11%). The authors specifically pointed out that CAP was only measured with the M probe (Stern and Castera, 2017). The first study using the XL probe was submitted only 9 days later (Chan et al., 2017) and was therefore not included in the review.

Recently, Karlas and colleagues suggested cut-offs for steatosis grades S1, S2 and S3 of 248 dB/m, 268 dB/m and 280 dB/m, respectively (Karlas et al., 2017). The group also recommended that cut-offs should be adjusted based on factors affecting steatosis as follows: CAP -10 dB/m in NAFLD/NASH patients, CAP -10 dB/m in patients with diabetes, CAP +4.4 dB/m per unit when BMI is less than 25 kg/m² to a minimum of 20 kg/m² and CAP -4.4 dB/m per unit when BMI is over 25 kg/m² to a maximum of 30 kg/m² (Karlas et al., 2017).

NAFLD has been phenotyped by proton magnetic resonance imaging in genetic studies with *PNPLA3* and *TM6SF2* (Kozlitina et al., 2014; Romeo et al., 2008), serum surrogate markers (Kozlitina et al., 2014; Romeo et al., 2010), liver biopsy (Krawczyk et al., 2017; Rotman et al., 2010) and by TE only (Krawczyk et al., 2011). We performed the first genetic study to measure hepatic steatosis as assessed by CAP in relation to *PNPLA3* and *TM6SF2*. Here, we reproduced the known steatogenic effect of *PNPLA3.* In our study, we were not able to repeat the fibrogenic effect found previously in 899 CLD patients (Krawczyk et al., 2011), neither did a study in 251 NAFLD patients (Shen et al., 2014). We concluded that *PNPLA3* has a stronger genetic effect on steatosis than on fibrosis, and large cohorts are needed for genetic association studies. For *TM6SF2*, no results regarding an association with steatosis and fibrosis were found, which may be related to the size of the cohort. Liver injury as reflected by elevated serum ALT activities were found in risk carriers of *PNPLA3* and *TM6SF2*. Given the benefits and limitations of the previously discussed methods, TE is suitable for phenotyping steatosis and fibrosis in large genetic studies.

Lifestyle modification as treatment for NAFLD

The most important therapeutic approach for improvement of NAFLD is weight loss, as recommended by international guidelines (Chalasani et al., 2012; European Association for the Study of the Liver et al., 2016; Roeb et al., 2015).

A calorie-reduced diet is mandatory for significant weight loss, irrespective of the dietary composition of carbohydrates, protein and fat, as shown in 811 overweight/obese adults after a follow-up time of two years (Sacks et al., 2009). Specific to the liver, the effect of a 30% energy reduced low-carbohydrate diet compared to a 30% energy reduced low-fat diet was equally positive in both groups regarding weight reduction and intrahepatic lipid contents (Haufe et al., 2011), and improvement of liver status was associated with increased weight reduction (Thoma et al., 2012). In those with a weight loss of ≥ 10%, resulting from a hypocaloric diet, improvement of steatosis, regression of fibrosis and resolution of NASH were observed in 100%, in 45% and in 90% of cases, respectively (Vilar-Gomez et al., 2015). Patients who followed a dietary intervention of six months (Haufe et al., 2011) and terminated the dietary modification, increased body weight after a mean follow-up of 24 months but stayed below the baseline weight, whereas reduction of serum ALT, AST and GGT activities persisted (Haufe et al., 2013).

A meta-analysis of high-protein, low fat diets showed larger reduction of body weight, fat mass and serum triglycerides compared to a standard-protein, low fat diet. Although the effect on the liver was not assessed, the effect on weight loss as the primary therapy for NAFLD was demonstrated (Wycherley et al., 2012). We monitored 60 patients with steatosis

who took part in a dietary program. The program consisted of a hypocaloric high-fiber, high-protein formula diet taken three times a day plus vegetables with a maximum of 100 kcal consumed together with the lunch and dinner formula. The total calorie count was 1,000 kcal per day for a period of 14 days. In our study, the number of meals and therefore calorie intake was limited. The benefit of this regime, compared to five times a day, was confirmed in a standardized hypercaloric diet in 36 healthy men: BMI increased in both groups of three meals a day and five meals a day, as expected, but liver fat contents, subcutaneous and visceral fat only increased in the group that consumed five meals (Koopman et al., 2014). In our study, patients were provided with the amount of formula needed for 14 days. Lists of pre-calculated portions were given as suggestions for the two servings of 100 calories of vegetables. For the course of two weeks, patients only had to take care to purchase vegetables, milk with a fat content of 1.5%, and calorie-free beverages. Not having to calculate calories and prepare meals accordingly could be related to the excellent compliance in our study, where no patient was lost-to-follow up. The observation is in line with results from an Australian study comparing a structured weight loss diet with a meal replacement diet. Both diets were targeting a calorie restriction of 6,000 kJ per day (1,434 calories per day) to achieve weight loss. After six months, both groups had lost the same amount of weight but convenience and compliance were better in the meal replacement group (Noakes et al., 2004). In our study, the daily intake of fiber through the formula diet alone was 21 g plus additional fiber consumed through vegetable intake. Fiber intake is known to increase satiety (Geliebter et al., 2015). This finding suggests that the fiber-enrichment in our study contributed to feeling satiated until the next planned meal and therefore to being compliant.

Dynamic short-term changes of liver fat were observed in as little as 48 hours (Kirk et al., 2009), six days (Sevastianova et al., 2011) and two weeks (Browning et al., 2011). Contrary, excessive caloric intake for three weeks leads to an increase in liver fat content. Sixteen obese patients followed a hypercaloric diet for three weeks and liver fat increased by 27%. The same group was then put on a hypocaloric diet for six months, resulting in a liver fat reduction of 25% (Sevastianova et al., 2012). Unfortunately, there were no additional time points in between to follow liver composition changes. Interestingly, short-term alterations in liver fat were measured using proton magnetic resonance imaging in all four studies.

We monitored the influence of the hypocaloric diet on liver fat and liver stiffness by using transient elastography as a non-invasive method. Overall, CAP decreased significantly by 14.0%, equivalent to an absolute reduction of 47 dB/m. This study is the first one to publish the use of CAP in an intervention study. Clearly, CAP can be used to detect short-term changes in the liver. This finding was confirmed in a study with vitamin D supplementation in

40 patients with hepatic steatosis and vitamin D deficiency. Here, CAP decreased by 5.3% after 4 weeks, 6.0% after three months and 6.4% after 6 months. The intervention consisted of a vitamin D supplementation only without a dietary intervention, and consequently body weight did not change in this cohort (Papapostoli et al., 2016). Both studies demonstrate that CAP can be used successfully to measure short- term changes of liver fat.

We were also interested in whether patients with different *PNPLA3* gene variants reacted differently to the intervention and found that all homozygous carriers of the [M] allele were in the responder group and none was in the non-responder group. Although this distribution was not significant, it suggests that these patients display a better response to dietary intervention. A study with eight homozygous carriers of the *PNPLA3* risk allele [M] found a significant difference in dietary response compared to ten carriers of the homozygous [I] allele (Sevastianova et al., 2011). Both groups, matched by age, sex, BMI and liver fat, received a hypocaloric low carb diet for six days. Reduction of weight was equal (-3.1 kg), but the decrease in liver fat, as assessed by proton magnetic resonance imaging, was 45% in carriers of genotype [MM] as compared to 18% in [II] carriers. The better response rate in homozygous [M] carriers was confirmed in a larger cohort (Shen et al., 2015a). Here, 154 patients with NAFLD, as confirmed by magnetic resonance imaging, were divided in two groups equal in *PNPLA3* genotype frequencies (22/77 [II], 38/77 [IM], 17/77 [MM]). One group took part in an individualized lifestyle modification program instructed by a dietician on a regular basis, and the other group received standard of care. After 12 months, hepatic liver fat was reduced by 6.7% as compared to 2.1%. In the intervention group, the decrease was significantly higher in carriers of one risk allele [M] and highest in careers with two risk alleles (-3.7% versus -6.5% versus -11.3%, respectively). In the control group, that received treatment guideline by a clinician only at baseline, liver fat reduction was also highest in carriers of [MM], but the improvement was not consistent across the genotypes (-2.0% versus -0.8% versus -5.2%, respectively) (Shen et al., 2015a). The data suggest that homozygous carriers of the *PNPLA3* p.148M risk allele have the highest risk for NAFLD but also the best response to treatment.

Individual nutrients, dietary pattern and eating behavior were also shown to have a positive impact on the liver. A reduction of fructose intake by 61% for six months led to a significant reduction in body weight and reduced liver fat content by 36% (Volynets et al., 2013). Zivkovic and colleagues compared 13 different types of diets with regard to changes in body weight, waist circumference, steatosis, insulin sensitivity, de novo lipogenesis, inflammation, and serum lipids. The improvements across these categories were best for the Mediterranean diet (Zivkovic et al., 2007), which consisted particularly of vegetables, fruits,

fish, olive oil, and little red meat. The improvement of hepatic steatosis and insulin sensitivity have been confirmed in a small but well-planned cross-over study of 12 patients with NAFLD, who were non-obese and non-diabetic. The patients were randomized to a Mediterranean diet or a low fat high carbohydrate diet for six weeks, followed by a six week wash-out phase. Both diets resulted in weight reductions of -1.0 and -2.4 kg, respectively, but improvements on liver fat were higher with the Mediterranean diet (39% versus 7%) and insulin resistance improved only in this group (Ryan et al., 2013). In addition, after 5 years the number of cardiovascular events was lower in subjects receiving a Mediterranean diet rich in olive oil or nuts as compared to a control diet (Estruch et al., 2013).

In order to reduce body weight, patients with NAFLD are encouraged to modify the quality and quantity of their food and drinks as well as their eating behavior. A reduction of 4% of body weight was seen in patients after 12 to 18 months of behavioral intervention and was even greater after 24 to 54 months (Leblanc et al., 2011). Behavioral changes include, for instance, identifying the reasons for the current eating habits, setting weight targets, self-monitoring and finding ways to hold on to long-term modifications (Fan and Cao, 2013). For patients with NAFLD, the likelihood to reduce body weight by more than 5% increased with the number of clinic visits, and the effects were more pronounced in those with higher baseline BMI (Dudekula et al., 2014). Brief advice on weight loss was given at each visit. Because the patient data was analyzed retrospectively, the study reflects a standard clinical procedure.

The German, European and American guidelines for the treatment of NAFLD recommend weight loss in combination with physical activity (Chalasani et al., 2012; European Association for the Study of the Liver et al., 2016; Roeb et al., 2015). Specifically, resistance training or moderate physical activity of 150 to 200 minutes per week, divided into three to five sessions, such as stationery cycling or brisk walking (European Association for the Study of the Liver et al., 2016).

In a meta-analysis consisting of 28 interventions, BMI, liver fat content and ALT levels improved due to the exercise. The benefit on liver fat reduction was directly associated with baseline BMI (Orci et al., 2016). A meta-analysis of exercise as a therapeutic option for NAFLD states that physical activity, in particular aerobic exercise and/or progressive resistance training, has low or no impact on body weight but reduces liver fat (Keating et al., 2012). In detail, aerobic training as compared to resistance training three times per week for four months resulted in small BMI reduction (-0.7 kg/m^2 versus -0.55 kg/m^2) but improvements of HbA1c, HDL-cholesterol and serum triglycerides in both groups. A marked reduction of liver fat contents and the resolution of steatosis in 23% of cases were found (Bacchi et al., 2013). This type of physical activity may benefit those who do not have to lose

weight but want to reduce liver fat content. Recently, the influence of exercise on resolution of fatty liver and the progression to fatty liver disease has been studied in almost 170,000 Korean adults by means of a physical activity questionnaire and ultrasound. After five years, 22.5% were newly diagnosed and 34.1% resolved fatty liver, while BMI decreased sparsely by 0.5 kg/m². The benefit in preventing and resolving fatty liver was highest when a minimum of ten minutes per session at least five times a week was achieved through moderate or vigorous activity (Sung et al., 2016).

Lifestyle factors also contribute to liver health. Almost 140,000 Korean adults were screened to find an effect of a sedentary lifestyle on NAFLD. Increased sitting time and reduced physical activity were independent risk factors for NAFLD. Interestingly, participants who spent more than ten hours daily sitting were younger, male, had higher BMI, higher education and no history of NAFLD-related co-morbidities, indicating that these young men are at risk and need to be monitored (Ryu et al., 2015). According to a recent meta-analysis, the risk of NAFLD increased by 20% for a sleep duration of ≤ 6 hours. The authors suggested three underlying mechanism driven by the lack of sleep: induction of inflammatory tumor necrosis factor alpha and interleukin-6, induction of appetite and consequently obesity induced by reduced serum leptin and elevated ghrelin concentrations and the production of stress hormones that are linked to insulin resistance, i.e. cortisol and adrenocorticotropic hormone (Wijarnpreecha et al., 2016).

Occurrence of NAFLD in the absence of obesity

Although elevated BMI is a strong predictor, NAFLD can also occur in those with normal weight, defined as BMI 18.5–24.9 kg/m² (World Health Organization, 2000). This phenotype was described for the first time in a study conducted in 2001 in Asia (Kim et al., 2004) and later referred to as “lean NASH” (Das and Chowdhury, 2013). The authors proposed that the difference between lean and obese NASH begins in early life. Patients with lean NASH can experience previous in-utero malnutrition, low birth weight and childhood under-nutrition, whereas obese NASH may be linked to maternal adiposity and childhood over-nutrition. The resulting energy imbalance in both phenotypes contributes to the development of NAFLD (Das and Chowdhury, 2013).

Diverse prevalence rates of lean NAFLD in the general population have been reported: starting at 2.2% in Austria (Feldman et al., 2017), 7.4% in the US (Younossi et al., 2012), 15.2% in Japan (Nishioji et al., 2015) and reaching 19.3% in China (Wei et al., 2015). Based on these numbers, lean NAFLD is more common in Asian countries. For all four studies, the term lean was defined as BMI < 25 kg/m^2. In 2004, the WHO expert consultation group stated that the BMI threshold of 25 kg/m² for obesity should be adjusted for the Asian

population (WHO Expert Consultation, 2004), This is justified by the increasing number of type 2 diabetes in non-obese patients and the larger proportion of body fat. The 2014 guideline on identification, assessment, and management of overweight and obesity by the National Institute for Health and Care Excellence in the UK specifically discusses the interpretation of BMI values of the Asian population (Stegenga et al., 2014). The recommendation is based on a previous guideline on BMI thresholds in black, Asian and other minority ethnic groups in 2013. Here, an updated BMI cut-off of 23 kg/m² is recommended for the prevention of type 2 diabetes (National Institute for Health and Care Excellence, 2013). Therefore, the prevalence of lean NASH in the Japanese and Chinese study (Nishioji et al., 2015; Wei et al., 2015) may also include non-lean NASH and should be interpreted with caution. Based on a multicenter study from Germany, one out of two non-obese non-diabetic NAFLD patients developed the disease due to carrying the *PNPLA3* p.148M risk allele (population attributable fraction of 0.498)(Feldman et al., 2017).

Use of body impedance analysis in NAFLD studies

In liver disease, body impedance analysis has mainly been used to measure muscle mass and phase angle as markers for malnutrition in chronic hepatitis B and C, cirrhosis, and HCC (Nishikawa et al., 2017; Peres et al., 2012; Schutte et al., 2015). For the intervention study and the UKS-study, we measured body height, waist and hip circumference as anthropometric parameters. We also availed of body impedance analysis (BIA) as an additional non-invasive method to measure body composition. We were especially interested in body and visceral fat, which are associated with NAFLD (Karlas et al., 2014). Body impedance analysis is the measurement of impedance of cell membranes, intra- and extracellular fluid when an electrical current is passing through these tissues at low voltage and high frequency The method is rapid. The overall time expense taking into account the time required to assess body height, weight and waist circumference and is two minutes per patient. Alternative methods to assess body composition are dual-energy x-ray absorptiometry, magnetic resonance imaging, or computer tomography (Baracos et al., 2012). All three methods are expensive and non-portable and therefore not practical for large NAFLD studies.

Using body impedance analysis, we were able to obtain additional information about the patients at baseline and the effect of the intervention after two weeks in the intervention study. The weight reduction of -4.6% consisted of 62% loss in body fat mass, and the remainder was loss of total body water and body fat free mass. Visceral fat mass, as declared by the visceral fat index, was also monitored, and an improvement was noted. The UKS-study provided data on body composition parameters and steatosis. Here, CAP

showed a positive correlation with body fat, body water, and visceral fat mass. These findings have also been reported for CAP and visceral fat mass (Karlas et al., 2014). For anthropometric measurements, waist circumference was also associated with steatosis and even represented an independent predictor for steatosis. No data on body impedance analysis were documented for the 174 CLD patients at the time of ultrasound and transient elastography because body impedance analysis was not part of the diagnostic work-up at the time. However, BMI was correlated with CAP in all three studies. Therefore, we conclude that assessing steatosis by means of CAP serves as a validation of previous data on BMI and steatosis (Li et al., 2016a).

Assessment of steatosis and fibrosis in the general population

In the UKS-study, three out of 105 employees of the Saarland university hospital reported an already known liver disease, leaving 102 as perceived hepatologically healthy. Of those, serum ALT and GGT activities were elevated and characteristic for the presence of steatosis in 6.5% and 4.3%, respectively. When considering CAP as a marker, 35.6% presented with steatosis. Elevated ALT and GGT levels were associated with elevated CAP in 29% and 31% of cases, respectively, leaving about two-thirds that would not have been diagnosed based on liver enzymes alone.

Our data is consistent with results from Spain, where randomly selected subjects listed in the public health registry were invited for liver stiffness measurements. Out of 495 study participants, 28 (5.7%) presented with fibrosis when using 6.8 kPa as cut-off (Fabrellas et al., 2013). Kim and colleagues (Kim et al., 2015) examined 280 subjects from Korea as part of a health check-up program. CAP ≥ 238 dB/m identified 57% with steatosis and was superior of ultrasonography (43%). Kaya and colleagues (Kaya et al., 2016) included 112 medical students from Turkey with an alcohol consumption of less than 20 grams per day and reported a prevalence of 23.2% for CAP ≥ 238 dB/m. Both studies used the same CAP cut-off to differentiate between non-steatosis and steatosis. Interestingly, the study from Turkey had a lower prevalence when excluding extensive alcohol intake as compared to the Korean study that tolerated any alcohol intake. The authors of a study from Portugal found that the prevalence of steatosis when including alcohol consumption and viral hepatitis, was higher as compared to patients without (38.4% versus 23.6%) (Carvalhana et al., 2014). The difference of reported prevalence rates was probably not caused by different BMI values; it was more likely the inclusion of alcohol consumption and viral hepatitis.

A recent systematic review states that the diagnosis of NAFLD based on aminotransferases alone does not identify all patients. Here, 19 studies from the general population (e.g. health check-up, community screening, primary care setting) were included. Non-invasive

assessment of NAFLD was carried out by transient elastography or a combination of different serum parameters. Here, 40% to 75% of the subjects were identified with a liver disease while serum ALT activities were within the normal range (Harris et al., 2017).

8. Conclusion and outlook

The presented data demonstrates that transient elastography is a valid method for assessing and monitoring liver steatosis and fibrosis. Transient elastography has been proven to be useful in NAFLD and a wide range of other chronic liver diseases in adults as well as in children.

The non-invasive measurement of liver stiffness as marker for fibrosis is widely accepted as reflected in guidelines and clinical practice. It can be carried out by transient elastography as a stand-alone machine as well as in integrated ultrasonography systems The non-invasive measurement of liver steatosis is limited to magnetic resonance imaging or computer tomography, which are not easily accessible for a large number of patients. Ultrasound is semi-quantitative and therefore not an ideal choice. The controlled attenuation parameter (CAP) represents an additional method with several benefits. Besides the simultaneous assessment of fibrosis and steatosis, it is rapid, patient-friendly, can be carried out by trained medical personnel, and allows frequent measurements for therapy follow-up and monitoring of disease progression. It can also be used for prevention in the general population and in clinical trials.

Obesity is a risk factor for diabetes, chronic kidney diseases and cardiovascular diseases. These comorbidities represent extrahepatic complications of the metabolic syndrome; therefore these patients should also be screened for NAFLD in general, and particularly for steatosis. The German Diabetes guideline acknowledges this association but no recommendation is given towards screening of liver parameters or imaging, except when liver-associated side effects of drugs are suspected (Bundesärztekammer (BÄK) et al., 2014). Interestingly, research is one step ahead by studying CAP in diabetics using transient elastography (Chon et al., 2016; Kwok et al., 2016). No recommendation can be found for patients with chronic kidney diseases or cardiovascular diseases regarding the identification or monitoring of NAFLD. Fortunately, research observational studies using CAP in these patients have been carried out, although the number of studies is still small (Friedrich-Rust et al., 2017; Mikolasevic et al., 2013; Yoon et al., 2017).

Physicians in the United States may be reimbursed for transient elastography. To date, it is not refundable by social health insurance in Germany, although the liver stiffness measurement is widely accepted and recommended by guidelines. The number of validation studies of CAP in patients with NAFLD and for prevention in the general population are increasing.

The predicted increase in obesity rates worldwide will go hand in hand with a higher prevalence of NAFLD. This will cause a medical as well as a financial burden. Here, early

diagnosis and continuous monitoring of NAFLD are key. In addition, the ongoing pharmacological studies are anticipated to identify additional treatments for NAFLD. Most importantly, obesity as the main causal factor for the expected NAFLD rise needs to be tackled in order to kill two birds with one stone. Here, the key to success might be long-term lifestyle modification, consisting of diet, physical activity and behavioral changes, for individual patients as well as the entire population.

9. References

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ORIGINAL ARTICLE

The common *PNPLA3* variant p.I148M is associated with liver fat contents as quantified by controlled attenuation parameter (CAP)

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is becoming the most prevalent liver disorder. The *PNPLA3* (adiponutrin) variant p.I148M has been identified as common genetic modifier of NAFLD. Our aim was to assess the relationships between genetic risk and non-invasively measured liver fat content. *Methods:* Hepatic steatosis was quantified by transient elastography, using the controlled attenuation parameter (CAP) in 174 patients with chronic liver diseases (50% women, age 18–77 years). In addition, a cohort of 174 gender-matched healthy controls (50% women, age 32–77 years) was recruited. The *PNPLA3* mutation as well as the novel NAFLD-predisposing genetic variant (*TM6SF2* p.E167K) were genotyped with allele-specific probes. *Results:* The *PNPLA3* genotype correlated significantly ($P = 0.001$) with hepatic CAP measurements. The p.148M risk allele increased the odds of developing liver steatosis (OR = 2.39, $P = 0.023$). In multivariate models, BMI and *PNPLA3* mutation were both independently associated with CAP values ($P < 0.001$ and $P = 0.007$, respectively). Carriers of the *TM6SF2* risk allele presented with increased aminotransferase activities (ALT: $P = 0.007$, AST: $P = 0.004$), but the presence of this variant did not affect CAP values. *Conclusions:* The *PNPLA3* p.I148M variant represents the most important prosteatotic genetic risk factor. NAFLD carriers of this variant should be followed up carefully, with elastography and CAP being ideally suited for this purpose.

Keywords
adiponutrin – elastography – non-alcoholic fatty liver disease – *TM6SF2*

Non-alcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease (CLD) in Western countries and its prevalence continues to increase. NAFLD prevalence varies among ethnic groups in Europe and may reach 50% of the general population (1). Fatty liver is defined by the abnormal accumulation

Abbreviations
ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CI, confidence interval; CLD, chronic liver disease; E, glutamic acid; HWE, Hardy–Weinberg equilibrium; I, isoleucine; INR, International Normalized Ratio; IQR, interquartile range; K, lysine; LSM, liver stiffness measurement; M, methionine; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; PASH, PNPLA3-associated steatohepatitis; PNPLA3, patatin-like phospholipase domain containing 3; *P*, *P*-value; TM6SF2, transmembrane 6 superfamily member 2; VCTE, vibration-controlled transient elastography; γ-GT, gamma-glutamyl transferase.

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Key points

- The common *PNPLA3* p.I148M variant is associated with increased CAP levels in patients with chronic liver diseases.
- CAP represents a novel non-invasive method to characterize NAFLD patients and measure liver fat in genetic studies.
- The *PNPLA3* and *TM6SF2* variants are associated with serum surrogate markers of liver injury.
- *PNPLA3* remains the most clinically significant genetic marker of hepatic steatosis.

of fat in hepatocytes, which can progress to steatohepatitis, fibrosis and cirrhosis (2, 3). The diagnosis and assessment of hepatic steatosis has been until recently based primarily on abdominal ultrasonography and liver biopsy. Ultrasonography is widely available (4), but is only semiquantitative, strongly depends on operator experience and is associated with intra- and interoperator variability (5). Liver biopsy is generally considered to represent the 'gold standard' for the detection and assessment of steatosis, although it represents only about 1/50 000th of the liver volume and is affected by sampling errors (6, 7). In addition, biopsy is costly, carries specific risks such as bleeding and does not allow regular follow-up measurements. Magnetic resonance imaging (MRI) and computed tomography (CT) as non-invasive steatosis imaging techniques are limited by availability and costs.

The controlled attenuation parameter (CAP) represents a novel quantitative parameter based on the attenuation of the ultrasound signal by liver fat (3) determined during non-invasive transient elastography. It is a non-invasive, reliable and patient-friendly tool for the quantification of liver fat contents (8) and can be readily integrated into routine work-up and follow-up of patients with CLD. Recently, CAP measurements have been evaluated in patients with NAFLD, non-alcoholic steatohepatitis as well as chronic hepatitis B and C virus infections (8–13).

The occurrence of NAFLD is generally attributed to different interacting factors, in particular insulin resistance, diabetes and obesity but may also occur without these obvious environmental challenges (14, 15). In a genome-wide association study (16), the variant p.I148M rs738409 of the triglyceride hydrolase patatin-like phospholipase domain containing 3 (*PNPLA3*) was demonstrated to increase the susceptibility for hepatic steatosis, steatohepatitis, fibrosis and cirrhosis. For example, Valenti *et al.* (17) assessed the influence of this risk variant on liver histopathology and reported that it was associated with the grade of steatosis in 574 adults, conferring an odds ratio (OR) of 1.35. Our group availed of non-invasive assessment of liver fibrosis by transient elastography, demonstrating that the *PNPLA3* variant increases liver stiffness and represents a risk factor for developing cirrhosis (OR = 1.56) in 899 patients with various CLDs (18). This finding is supported by a recent meta-analysis reporting that the [M] allele results in an elevated cirrhosis risk as compared to the [I] allele (OR = 1.86) (19). As demonstrated in *Pnpla3* p.148M knock-in mice, the mutation leads to the accumulation of PNPLA3 on lipid droplets and an increase in triglyceride concentrations in liver in the presence of a dietary overload (20). In fact, the gene variant may confer a substantial risk for hepatic steatosis and consecutive inflammation in humans consuming Westernized diets, as highlighted by the term *PNPLA3*-associated steatohepatitis (PASH) (21). Very recently, the novel but less common gene variant *TM6SF2* p.E167K has been reported to increase NAFLD risk (OR = 1.37) (22).

To date, no study has been carried out to show the association between non-invasively quantified hepatic fat content assessed by CAP and the steatogenic risk variants of the *PNPLA3* and *TM6SF2* genes. Hence, the specific aim of this study was to determine if these variants influence elastography-based phenotypes such as hepatic steatosis and fibrosis and to analyse the correlation between these novel phenotypes, risk genotypes and conventional non-invasive surrogate markers of liver injury.

Patients and methods

Patient cohorts

This observational study recruited 174 patients admitted to the Department of Internal Medicine II at Saarland University Medical Center (Homburg, Germany), a tertiary referral centre, between September 2010 and September 2014 for diagnostic work-up and treatment of CLD. We excluded patients who presented with viral and cholestatic liver diseases (primary biliary cirrhosis, primary sclerosing cholangitis) or liver tumors. Table 1 summarizes the clinical characteristics of the cohort. The cohort comprised 50% women, median age was 50 years (range 18–77 years) and median body mass index (BMI) was 28.4 kg/m² (range 17.2–47.3 kg/m²). Overall, 70.7% of the patients were diagnosed with NAFLD, 21.8% presented with cryptogenic CLD and 7.5% suffered from alcohol-induced liver injury. In addition, we studied 174 individuals matched by gender (50% women; median age 59 years, range 32–77 years), who underwent colonoscopy without abnormal findings at our unit and served as the control group for the comparison of *PNPLA3* genotype frequencies in the population.

All patients underwent a clinical examination, and blood samples were drawn in the fasted state. Liver function tests and other analyses were performed with standard clinical chemical assays in the certified central laboratory of our university hospital. Informed consent was obtained from each patient included in this study. The protocol of this study conforms to the ethical

Table 1. Clinical characteristics of individuals with chronic liver diseases

Variables	Patient characteristics (n = 174)
n men/women	87 (50%)/87 (50%)
Age (years)	50 (18–77)
BMI (kg/m²)	28.4 (17.2–47.3)
Underweight BMI < 18.5	2 (1.2%)
Normal weight 18.5 ≤ BMI < 25	15 (8.6%)
Overweight 25 ≤ BMI < 30	50 (28.7%)
Obese BMI ≥ 30	50 (28.7%)
BMI data unavailable	57 (32.8%)
Primary liver disease	
NAFLD	123 (70.7%)
Cryptogenic CLD	38 (21.8%)
Alcoholic liver disease	13 (7.5%)
Transient elastography	
CAP (dB/m)	285 (100–398)
LSM (kPa)	6.1 (1.6–69.1)
Ultrasonography	
Grade 0	62 (35.6%)
Grade 1	55 (31.6%)
Grade 2	42 (24.2%)
Grade 3	15 (8.6%)
Liver function tests	
ALT (U/L)	47 (9–272)
AST (U/L)	35 (17–179)
AST/ALT ratio	0.73 (0.21–3.56)
γ-GT (U/L)	79 (11–1287)
AP (U/L)	78 (33–345)
Bilirubin (mg/dl)	0.6 (0.2–9.4)
Albumin (g/L)	46 (22–53)
Glucose (mg/dl)	98 (75–375)
Creatinine (mg/dl)	0.85 (0.47–1.95)
Platelets ($\times 10^9$/L)	232 (39–443)
INR	0.99 (0.84–2.61)
Lipid metabolism	
Total cholesterol (mg/dl)	207 (95–403)
Triglycerides (mg/dl)	125 (39–1618)
LDL cholesterol (mg/dl)	124 (17–313)
HDL cholesterol (mg/dl)	55 (16–153)

Values are displayed as medians (ranges). ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CLD, chronic liver disease; γ-GT, gamma-glutamyl transferase; INR, International Normalized Ratio; LSM, liver stiffness measurement; NAFLD, non-alcoholic fatty liver disease.

guidelines of the Declaration of Helsinki as reflected in *a priori* approval by the institution's human research ethics committee (Ärztekammer des Saarlandes; ref 62/09 and 271/11).

Non-invasive phenotyping of liver steatosis and fibrosis

Hepatic steatosis and liver stiffness were assessed non-invasively by vibration-controlled transient elastography (VCTE) (FibroScan®, Echosens, Paris) and expressed as CAP and liver stiffness measurement (LSM) respectively. During elastography, a shear-wave is being generated by a mechanical impulse of the transducer, which is in contact with the patient's skin. The dispersion of the shear-wave through the liver is monitored by ultrasound signals sent by the probe. As the ultrasound waves propagate through the liver, the alteration of the amplitude is determined at 3.5 MHz (M-probe) or 2.5 MHz (XL-probe for obese patients). A decrease in amplitude secondary to an increase in liver fat content is measured and expressed as CAP value in dB/m, currently only available with the M-probe. In addition, the propagation speed of the shear-wave, which increases with liver fibrosis, is determined and used for LSM calculation (expressed in kPa) using both, the M- and XL-probes.

Patients were placed in the dorsal decubitus position with the right arm stretched behind their head. All measurements were carried out in fasted patients. The probe was placed on the skin between the ribs at the midaxillary line vertical to the xyphoid process. At least 10 measurements were performed per patient. In order to include a patient's result in the study analysis, a success rate of ≥60% based on at least 10 valid measurements and an interquartile range (IQR)/median LSM ≤ 30% were required (23). According to a recent study, the IQR/LSM ratio was not taken into account in patients with median LSM < 7.1 kPa (24).

For this study, we used the CAP cut-off values reported by Sasso *et al.* (8), as follows: Hepatic steatosis grade S1 (corresponding to 11–32% liver fat) is defined by CAP 238–258 dB/m, grade S2 (33–65% liver fat) corresponds to CAP 259–291 dB/m, and grade S3 (≥66% liver fat) is indicated by CAP ≥ 292 dB/m.

For comparison, we assessed hepatic steatosis by conventional ultrasonography. Abdominal ultrasound examinations were carried out by experienced physicians on the Hitachi EUB-8500 system (Hitachi Medical Systems, Wiesbaden, Germany). The semiquantitative assessment of hepatic steatosis was based on the visual impression of the liver echogenicity and expressed as grades S0 to S3: grade S0 indicates the absence of steatosis, grade S1 corresponds to minimal, grade S2 to moderate and grade S3 to severe steatosis (25).

Genotyping of risk variants

Genomic DNA was isolated from the EDTA-anticoagulated blood samples using the membrane-based QIAamp DNA extraction protocol (Qiagen, Hilden, Germany). Genotyping of *PNPLA3* rs738409 and *TM6SF2* rs58542926 polymorphisms was performed using PCR-based assays with 5'-nuclease and fluorescence detection (TaqMan®, Life Technologies, Darmstadt, Germany; rs738409: C__7241_10; rs58542926: C__89463510_10).

Statistical analyses

All statistical analyses were performed with SPSS 20.0 (SPSS, Munich, Germany) or GRAPHPAD PRISM 5.0

(GraphPad Software Inc., San Diego, CA, USA), unless stated otherwise. Two-sided P-values < 0.05 were regarded as significant. Kolmogorov–Smirnov tests were used to determine whether data were normally distributed. Quantitative data were expressed as median and ranges. Correlations were tested using Spearman's rank correlation tests. Quantitative traits were assessed using Mann–Whitney U or Kruskal–Wallis tests, as apropriate. The associations of categorical variables were tested in contingency tables. The genotype frequencies of all polymorphisms were tested for consistency with Hardy–Weinberg equilibrium using exact tests (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). De Finetti diagrams with Hardy–Weinberg parabola were generated using the online software tool (https://finetti.meb.unibonn.de). Allele and genotype frequency differences were assessed by chi-squared and Armitage's trend tests, respectively. Genetic associations were tested by univariate logistic and linear regression analyses; herein, significant parameters underwent further evaluation in multivariate models.

Results

Liver steatosis assessed by CAP correlates with semiquantitative ultrasonography scores

In total, we performed elastography measurements in 174 CLD patients. Eighteen cases (10.3%) were excluded as a result of an invalid elastography measurement. An LSM value was determined for all of the other patients and a CAP value for 148 patients, because in eight patients (5.1%), the XL-probe was used, for which no CAP measurement has been implemented. Median liver stiffness was 6.1 kPa (range 1.6–69.1 kPa) and median CAP was 285 dB/m, ranging from 100 to 398 dB/m. Figure 1a illustrates the distribution of CAP values in our cohort.

Based on conventional ultrasonography, 62 cases with CLD (35.6%) showed no pronounced liver steatosis. Steatosis grade S1 was observed in 55 patients (31.6%), steatosis was classified as grade S2 in 42 cases (24.2%) and 15 patients (8.7%) presented with steatosis grade S3.

Overall, the prevalence of hepatic steatosis in our cohort as assessed by CAP (≥238 dB/m) or ultrasonography (≥S1) was 74.3% and 64.4%, respectively. Cases were identified by both methods as positive in 67.8% and as negative in 18.6%; conflicting results were obtained in 13.6% of the patients. Figure 1b illustrates the significant positive correlation of CAP values and steatosis grades ($r = 0.442$, $P < 0.001$): Median CAP values were 279 dB/m for patients with steatosis grade S1, 317 dB/m for grade S2 and 335 dB/m for grade S3. Moreover, CAP was positively correlated with BMI and serum ALT activities (both $P \leq 0.001$) as well as serum glucose and triglyceride concentrations and LSM (all $P < 0.05$).

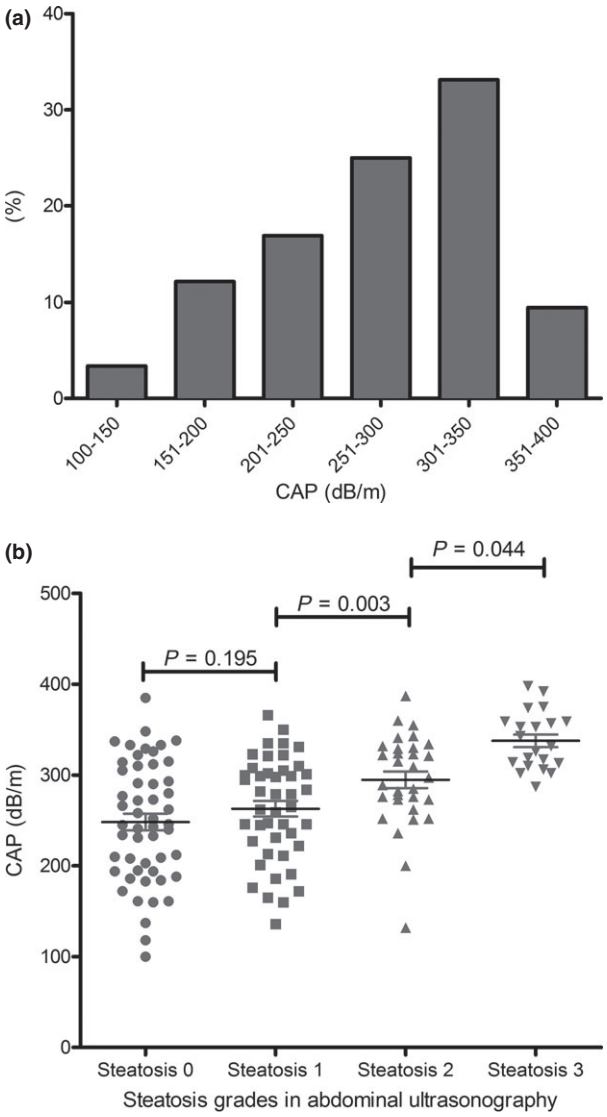

Fig. 1. (a) Hepatic fat distribution in 148 CLD patients. Liver steatosis was quantified using controlled attenuation parameter (CAP) during transient elastography. Values range from 100 to 398 dB/m with a median CAP of 285 dB/m. (b) Non-invasive assessment of hepatic steatosis. Controlled attenuation parameter (CAP) correlates positively with the steatosis grade in liver ultrasonography (grade 0–3). Symbols represent the value for each patient.

Liver fat contents measured by CAP are associated with the *PNPLA3* risk variant

In this cohort, the frequencies of wild-type, heterozygous and homozygous genotypes were [II]: 87, 50.0%; [IM]: 67, 38.5%; [MM]: 20, 11.5% for the *PNPLA3* variant and [EE]: 139, 79.9%; [EK]: 33, 19.0%; [KK]: 2, 1.1% for the *TM6SF2* variant. None of the genotype distributions deviated from Hardy–Weinberg equilibrium ($P > 0.05$, exact tests; Fig. S1a,b), and all were consistent with the frequencies published in the *Entrez* database.

Overall, CAP values showed a positive correlation with *PNPLA3* p.I148M genotypes ($r = 0.302$, $P = 0.001$). Figure 2a illustrates the significant differences in median CAP measurements across the carriers of distinct *PNPLA3* genotypes, which were 261 dB/m (100–392 dB/m), 308 dB/m (137–398 dB/m) and 318 dB/m (208–359 dB/m) in patients with genotpyes [II] ($n = 74$), [IM] ($n = 58$) and [MM] ($n = 16$), respectively. The data are consistent with a dominant model of inheritance with respect to the liver steatosis phenotype, as the differences in CAP between carriers of *PNPLA3* genotypes [II] and [MM] as well as genotypes [II] and [IM] were significant ($P = 0.004$ and $P = 0.003$, respectively), whereas CAP did not differ between heterozgyous and homozygous carriers of the risk allele p.148M ($P > 0.05$). The same associations were observed for men in a subgroup analysis, however, for women, significant CAP values were only detected between carriers of genotypes [II] and [MM] (data not shown).

Figure 2b depicts the distributions of *PNPLA3* genotypes in relation to CAP levels. Of note, the frequency of individuals carrying the *PNPLA3* risk allele [M] increased at higher CAP values. Table S1A summarizes the results of the univariate linear regression analysis with CAP being significantly related to BMI and *PNPLA3* genotypes ($P < 0.001$ and $P = 0.023$) but not with age, gender or LSM. In the multivariate model (Table S1B), CAP remained associated with BMI and *PNPLA3* genotypes ($P < 0.001$ and $P = 0.007$).

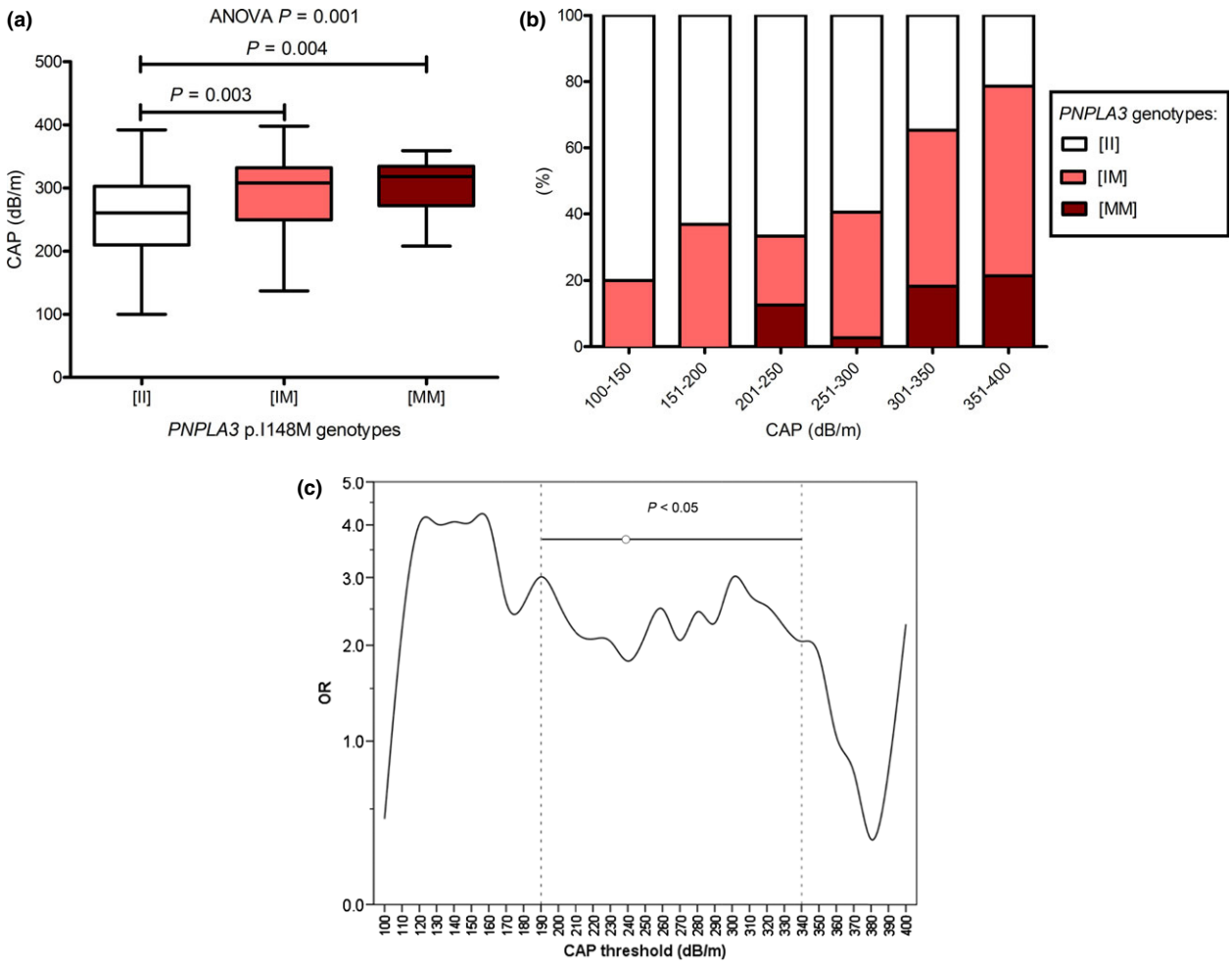

Fig. 2. (a) Relationship between controlled attenuation parameter (CAP) values among carriers of different *PNPLA3* p.I148M genotypes: [II] ($n = 74$), [IM] ($n = 58$) and [MM] ($n = 16$). (b) Distribution of *PNPLA3* genotypes [II], [IM] and [MM] in relation to CAP classes. (c) Sensitivity analysis of odds ratios (OR) for developing hepatic steatosis in carriers of the *PNPLA3* p.148M risk allele using CAP thresholds between 100 and 400 dB/m in increments of 10 dB/m. OR is given on a log scale. The [M] allele is a significant ($P < 0.05$) risk factor for hepatic steatosis for CAP thresholds between 190 and 340 dB/m (except for CAP = 240 dB/m, $P = 0.054$ denoted by ○) with OR for this window ranging from 3.0 to 2.1. Of note, the association with the CAP threshold of 238 dB/m used in this study based on previous publications (8) is also significant ($P = 0.040$).

The *PNPLA3* risk allele increases the risk of liver steatosis determined by CAP

Table 2A summarizes that in patients with hepatic steatosis, as defined by CAP ≥ 238 dB/m, and in patients with CAP < 238 dB/m, the frequencies of *PNPLA3* p.I148M [II], [IM] and [MM] genotypes were 44.6% vs. 65.8%, 43.6% vs. 26.3%, and 11.8% vs. 7.9%, respectively. Overall, the presence of the *PNPLA3* risk allele, p.148M, significantly increased the odds of developing hepatic steatosis (odds ratio = 1.90, 95% confidence interval = 1.02–3.53, $P = 0.040$; Table 2A). Of the 174 patients, 25 (14.4%) had a diagnosis of diabetes mellitus type 2 and these patients had a significantly higher median CAP value as compared to the non-diabetics, (314 vs. 279 dB/m, $P = 0.034$). The groups of patients with and without steatosis did not differ in age and gender, but the difference in BMI (28.8 vs. 25.0 kg/m²) was significant ($P < 0.001$). In the logistic univariate analysis (Table S2A), both *PNPLA3* mutation and BMI ($P = 0.042$ and $P < 0.001$, respectively), but not age, gender or LSM (all $P > 0.05$) were associated with CAP ≥ 238 dB/m. In the multivariate model (Table S2B), both the *PNPLA3* mutation and BMI proved to be independently associated with increased CAP values ($P = 0.041$ and $P < 0.001$, respectively).

Based on the CAP values ranging from 100 to 400 dB/m, we performed a sensitivity analysis using different CAP thresholds at increments of 10 dB/m (Fig. 2c, Table S3). The *PNPLA3* p.148M allele was a significant ($P < 0.05$) risk factor for hepatic steatosis for all CAP thresholds between 190 and 340 dB/m, apart from 240 dB/m where we calculated marginal significance ($P = 0.054$). In this window, odds ratios (OR) for steatosis range from 3.0 to 2.1 (Table S3).

The frequencies of the genotypes [EE], [EK] and [KK] in the *TM6SF2* gene were 80.9%, 17.3% and 1.8% vs. 76.3%, 23.7%, 0% and 80.0% in patients with CAP values ≥238 dB/m vs. <238 dB/m, respectively. Overall, this variant did not significantly influence the risk of developing hepatic steatosis ($P > 0.05$).

Carriers of the *PNPLA3 and TM6SF2* risk alleles present with increased serum aminotransferase activities

Overall, we detected significantly increased serum activities of ALT (53 vs. 42 U/L, $P = 0.014$; Fig. 3a) and AST (37 vs. 33 U/L, $P = 0.008$) as well as HDL concentrations (57 vs. 50 mg/dl, $P = 0.014$) in carriers of the *PNPLA3* p.148M risk allele. In carriers of the *TM6SF2* genotypes, serum ALT (57 vs. 44 U/L, $P = 0.007$; Fig. 3b) and AST activities (48 vs. 33 U/L, $P = 0.004$) were increased.

As expected, a positive association between hepatic steatosis and fibrosis, assessed using CAP and LSM, was observed ($r = 0.174$, $P = 0.035$). Table S1C demonstrates that LSM values were affected by patients' age ($P < 0.001$) but not by gender or BMI (both $P > 0.05$). For carriers of distinct *PNPLA3* genotpyes [II] ($n = 78$), [IM] ($n = 62$) and [MM] ($n = 16$), median values were 6.2 kPa (1.6–46.4 kPa), 6.4 kPa (2.5–69.1 kPa) and 5.0 kPa (3.0–12.8 kPa) but did not differ significantly between genotypes ($P > 0.05$). No association between the *TM6SF2* variant and CAP or LSM results were detected (Table S4).

Table 2. Distribution of *PNPLA3* p.I148M alleles and genotypes in (A) patients with and without hepatic steatosis; (B) CLD patients and healthy controls

(A)	Patients with CAP ≥ 238 dB/m (2N = 220)	Patients with CAP < 238 dB/m (2N = 76)
Counts of alleles/genotypes (%)		
[I]	146 (66.4)	60 (79.0)
[M]	74 (33.6)	16 (21.0)
[II]	49 (44.6)	25 (65.8)
[IM]	48 (43.6)	10 (26.3)
[MM]	13 (11.8)	3 (7.9)
Association tests	χ^2	***P***
Allele frequency difference test	4.23	0.040
Armitage's trend test	3.94	0.050
OR statistics	**OR**	**95% CI**
[M] ↔ [I]	1.90	1.02–3.53
[MM] ↔ [IM]	2.45	1.06–5.64
[MM] ↔ [II]	2.21	0.58–8.48
[MM+IM] ↔ [II]	2.39	1.11–5.16
[MM] ↔ [IM+II]	1.56	0.42–5.82

(B)	CLD patients (2N = 348)	Healthy controls (2N = 348)
Counts of alleles/genotypes (%)		
[I]	241 (69.3)	270 (77.6)
[M]	107 (30.7)	78 (22.4)
[II]	87 (50.0)	104 (59.8)
[IM]	67 (38.5)	62 (35.6)
[MM]	20 (11.5)	8 (4.6)
Association tests	χ^2	***P***
Allele frequency difference test	6.19	0.013
Armitage's trend test	5.90	0.015
OR statistic	**OR**	**95% CI**
[M] ↔ [I]	1.54	1.09–2.16
[MM] ↔ [IM]	1.29	0.83–2.02
[MM] ↔ [II]	2.99	1.26–7.12
[MM+IM] ↔ [II]	1.49	0.97–2.27
[MM] ↔ [IM+II]	2.70	1.15–6.30

CAP, controlled attenuation parameter; CI, confidence interval; CLD, chronic liver disease; I, isoleucine; M, methionine; OR, odds ratio; *P*, p-value; *PNPLA3*, patatin-like phospholipase domain containing 3.
The [M] allele of the *PNPLA3* variant represents the risk allele for hepatic steatosis (16).

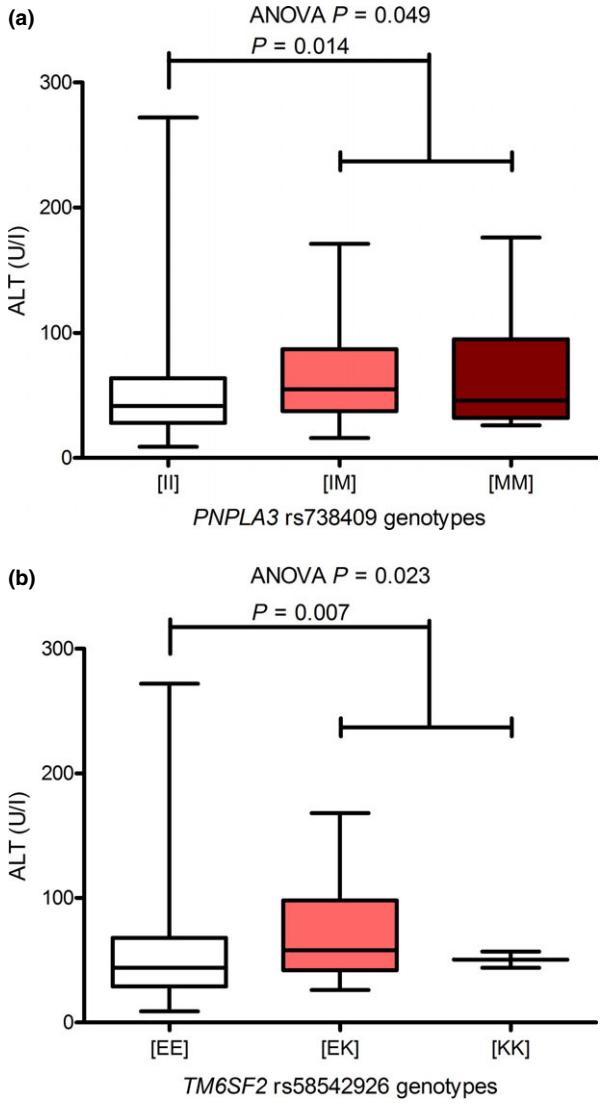

Fig. 3. Serum ALT activities in relation to (a) *PNPLA3* and (b) *TM6SF2* genotypes in 174 CLD patients.

Carriers of the *PNPLA3* risk allele are at risk of presenting with CLD

Patients referred to our liver clinic displayed significantly higher frequencies of the *PNPLA3* risk allele p.148M as compared to healthy controls ($P < 0.05$). Table 2B summarizes the distribution of alleles and genotypes in these two groups. The *PNPLA3* risk allele conferred a 1.54-fold increased risk of presenting with CLD as compared to the controls (95% confidence interval = 1.26–7.12, $P = 0.013$). Overall, individuals carrying the prosteatotic *PNPLA3* genotypes were more likely to develop hepatic phenotypes that led to referral to a tertiary reference centre for further work-up.

Discussion

In previous genetic studies, liver steatosis and fibrosis were mostly quantified using liver biopsy, CT and MRI, or circulating serum surrogate markers (21). In the current report we availed of the non-invasive VCTE-based approach including the recently developed CAP methodology. This allowed us, firstly, to quantify the effects of the *PNPLA3* p.I148M mutation on hepatic fat contents. Secondly, we incorporated the newly detected genetic risk factor *TM6SF2* for NAFLD in our analysis to further assess the landscape of inherited predisposition to liver steatosis.

Although CAP is a relatively new tool, a recent meta-analysis of nine CAP-based studies has underscored its specificity and sensitivity for the assessment of hepatic steatosis (26). VCTE with CAP is a rapid, patient-friendly method providing immediate results concerning not only the degree of steatosis but also liver stiffness. In addition to these advantages, our current study demonstrates that CAP can be used for a timely recruitment of CLD patients for genetic analyses of liver steatosis. In our previous elastography-based studies in different cohorts, we demonstrated that the *PNPLA3* (18) and *SREBP1c* (27) variants as well as polymorphisms in the vitamin D pathway (28) are associated with hepatic injury. Of note, all these studies were performed without CAP, which was not available at the time. Herein, we extend these observations by demonstrating a strong association between the *PNPLA3* risk allele and increased CAP values independent from other non-genetic triggers.

CAP is a novel tool for quantifying hepatic steatosis and so far no clear cut-off values for NAFLD have been developed. Indeed, previous studies claimed that CAP results ranging from 215 dB/m (11) to 300 dB/m (29) might be regarded as thresholds for diagnosing steatosis. Based on these uncertainties, we performed a sensitivity analysis demonstrating that the *PNPLA3* mutation is associated with an increased risk of higher CAP values using thresholds between 190 and 340 dB/m. Of note, the ORs for steatosis influenced by *PNPLA3* tend to decrease with higher CAP thresholds, taking into account that values at the extremes are unreliable owing to the small number of cases. This observation indicates stronger genetic effects in patients with moderately elevated CAP but multifactorial effects in severe NAFLD. Hence, this unique analysis, which is possible only in the setting of VCTE-based data acquisition, further supports the role of variant *PNPLA3* as a common risk factor for increased hepatic fat accumulation.

The inclusion of both CAP and LSM in this study gave us the opportunity to investigate the role of *PNPLA3* p.I148M variant in modulating steatosis and fibrosis in CLD. Interestingly, carriers of the *PNPLA3* risk allele, p.148M, showed increased CAP levels but this variant did not significantly affect LSM. This observation contrasts with our previous study in 899 CLD patients (18)

but is consistent with a report by Shen *et al.* (30), who did not detect a significant association between the *PNPLA3* variant and LSM in 251 NAFLD patients either. The negative LSM results are most likely related to the smaller sample and indicate that the genetic effects on CAP might be stronger than the more indirect effects on liver fibrogenesis. We speculate that the VCTE-based results indirectly demonstrate that the *PNPLA3* risk allele might have more pronounced effects on hepatic steatosis as compared to liver fibrosis. This underpins a functional analysis performed in *Pnpla3* 148M knock-in mice (20). This study has demonstrated that a high-sucrose diet leads to the accumulation of PNPLA3 on the surface of intrahepatic lipid droplets, which are, in turn, larger in size and contribute to hepatic steatosis (20). The molecular mechanisms that lead to fatty liver and fibrosis, and whether they are secondary to (extra)hepatic fat accumulation or if other *PNPLA3*-related pathways could be involved, have not been elucidated thus far. Recently *PNPLA3* has been identified to play a role as lipase in retinol metabolism in hepatic stellate cells, which are critical for fibrogenesis (31). On the other hand, the exact function of *PNPLA3* is to be defined (32) and it remains unclear if the enzyme represents a triglyceride hydrolase (33) and/or lysophosphatidic acid acyltransferase (34) *in vivo*. Studies in mouse models do not clearly support either gain- or loss-of-function effects of the p.I148M variant (32). Hence, further studies are needed to delineate the role of *PNPLA3* and to elucidate the mechanisms that lead to increased hepatic fat accumulation in carriers of the risk allele p.148M (35).

In contrast to previous genetic analyses concerning hepatic steatosis and fibrosis, in the current report not only do we use VCTE to measure liver injury but we also extend the number of genotyped variants by including the *TM6SF2* polymorphisms. The increased serum aminotransferase activities in carriers of the risk variant are consistent with previous studies (22,36–38). As a result of its low frequency (minor allele frequency around 0.1% in European populations), the *TM6SF2* variant rs58542926 was first identified in a cohort comprising more than 80 000 individuals (36). This low frequency of the risk allele, resulting in decreased power of the analysis, is also a potential explanation for the lack of association between this variant and CAP. Similar to *PNPLA3*, *TM6SF2* could be involved in the remodelling of lipid droplets (39) but also play a role in VLDL secretion (36). Although it remains unclear whether the *TM6SF2* variant is associated with both liver steatosis and fibrosis (22,37), our results underscore the genetic burden in carriers of the minor allele who are at risk of progressive liver injury in the setting of chronic liver diseases.

In our study we did not perform invasive assessment of liver phenotypes by liver biopsy. On the other hand, we used three non-invasive methods, namely CAP, VCTE and abdominal ultrasonography, to assess liver status in the recruited patients. Although we had a 10% failure rate in obtaining reliable LSM and results (mostly because of obesity), the comparison of CAP values and steatosis as quantified by abdominal ultrasonography confirmed the good correlation between these two methods. Moreover, as we detected increased CAP values in carriers of the prosteatotic *PNPLA3* variant, our results further validate CAP as a reliable method of quantifying liver fat. Comparable results were recently obtained by Karlas *et al.* (40) in a study performed in transplanted patients, which demonstrated that graft recipients bearing the *PNPLA3* p.148M allele are at risk of increased VTCE-quantified liver steatosis and stiffness.

Mounting evidence suggests that the *PNPLA3* p.I148M variant represents the major genetic determinant of hepatic steatosis (32). Our study expands on these observations by identifying CAP as a new tool to measure liver fat in genetic studies and by showing its value in the carriers of the *PNPLA3* mutation. Given the low frequencies of the *TM6SF2* risk genotypes in the general population, variant *PNPLA3* p.I148M remains, in our view, the most clinically important prosteatotic genetic risk factor. Individuals carrying this mutation should be included in prospective studies with repeat measurements of steatosis and fibrosis progression, which could be accomplished in a timely and non-invasive manner using elastography with CAP.

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Conflict of interest: The authors do not have any disclosures to report.

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Supporting information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1111/liv.12937/suppinfo

Appendix 2. Arslanow et al. Clinical and Translational Gastroenterology 2016

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Short-Term Hypocaloric High-Fiber and High-Protein Diet Improves Hepatic Steatosis Assessed by Controlled Attenuation Parameter

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OBJECTIVES: Non-alcoholic fatty liver disease is one of the most prevalent liver diseases and increases the risk of fibrosis and cirrhosis. Current standard treatment focuses on lifestyle interventions. The primary aim of this study was to assess the effects of a short-term low-calorie diet on hepatic steatosis, using the controlled attenuation parameter (CAP) as quantitative tool.
METHODS: In this prospective observational study, 60 patients with hepatic steatosis were monitored during a hypocaloric high-fiber, high-protein diet containing 1,000 kcal/day. At baseline and after 14 days, we measured hepatic fat contents using CAP during transient elastography, body composition with bioelectrical impedance analysis, and serum liver function tests and lipid profiles using standard clinical–chemical assays.
RESULTS: The median age was 56 years (25–78 years); 51.7% were women and median body mass index was 31.9 kg/m^2 (22.4–44.8 kg/m^2). After 14 days, a significant CAP reduction (14.0%; $P<0.001$) was observed from 295 dB/m (216–400 dB/m) to 266 dB/m (100–353 dB/m). In parallel, body weight decreased by 4.6% ($P<0.001$), of which 61.9% was body fat. In addition, liver stiffness ($P=0.002$), γ-GT activities, and serum lipid concentrations decreased (all $P<0.001$).
CONCLUSIONS: This study shows for the first time that non-invasive elastography can be used to monitor rapid effects of dietary treatment for hepatic steatosis. CAP improvements occur after only 14 days on short-term low-calorie diet, together with reductions of body composition parameters, serum lipids, and liver enzymes, pointing to the dynamics of hepatic lipid turnover.
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Subject Category: Liver

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of progressive liver conditions in the absence of significant alcohol consumption. Bland steatosis occurs when intrahepatic triglycerides accumulate in hepatocytes, which may progress to non-alcoholic steatohepatitis (NASH) if accompanied by inflammation.[1] In 10–25% of patients, steatosis advances to hepatic fibrosis, cirrhosis, and end-stage liver disease. In addition, the likelihood of cardiovascular disease is increased when NAFLD occurs, and consequently, these patients have an increased risk of overall and liver-specific mortality.[2,3] NAFLD has emerged as one of the most widespread liver diseases in western societies, with prevalence estimates ranging up to 50% and even higher in diabetics.[4] This variation is based on differences in screening and detection strategies as well as genetic and environmental risk factors.[5,6] For instance, overweight, type 2 diabetes[7] as well as genetic predisposition, such as the patatin-like phospholipase domain containing 3 (*PNPLA3)* variant p.I148M, are all implicated in fatty liver manifestation.[8,9] Specifically, carriers of the *PNPLA3* risk allele carry a more than twofold increased steatosis risk[10] as well as an increased likelihood of developing fibrosis, cirrhosis, and hepatocellular carcinoma.[11,12]

Currently, treatment options for NAFLD are limited and no accepted standard pharmacotherapy exists. According to AASLD guidelines, reduction of body weight of at least 3–5% through a hypocaloric diet alone or together with increased physical activity has been recommended to reduce steatosis.[1] Lifestyle intervention studies, specifically diet alone (such as, low-fat or low-carbohydrate diets) or combined with physical activity, have shown potential in ameliorating hepatic steatosis.[13,14] Specifically, preliminary data suggests that a protein-enriched dietary intervention reduces hepatic steatosis in obese patients.[15] However, the majority of studies have employed serum surrogate markers,[16] semiquantitative ultrasonography,[17] elaborate computer tomography, or magnetic resonance imaging.[18] Only few studies have used liver biopsy, the "gold standard" for assessing histological changes in hepatic steatosis,[19,20] which carries a risk of bleeding and is affected by sampling errors.[21,22] The use of non-invasive and risk-free techniques to diagnose and monitor hepatic steatosis is highly sought after.[23] As such, non-invasive techniques are increasingly being evaluated. During ultrasound-based vibration-controlled transient elastography (VCTE), the attenuation of low-frequency ultrasound waves by liver tissue can be measured. The controlled attenuation parameter (CAP) quantifies liver fat (while simultaneously detecting liver stiffness).[24] VCTE is the most widely validated technique for the detection of liver fibrosis, as documented by the new European Association for the Study of the Liver (EASL)

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recommendations on non-invasive tests for the evaluation of liver diseases.[25]

Until now, no study has compared CAP at baseline and at follow-up in combination with a dietary intervention. The aim of this study was to monitor patients with hepatic steatosis receiving a short-term hypocaloric high-fiber, high-protein diet with the primary outcome of improving liver fat, as quantified with CAP. We hypothesized that this 14-day low-calorie diet would significantly reduce CAP and therefore hepatic steatosis.

PATIENTS AND METHODS

Study design. This prospective observational pilot study followed patients with fatty liver taking part in a dietary program at four nutrition centers between September 2013 and April 2014 in the Saarland and Palatinate region in Southwest Germany. Specifically, patients received a 14-day hypocaloric high-fiber, high-protein liquid formula diet (HEPA-FAST) containing three shakes per day with a total of 786 kcal (41% protein, 29% carbohydrate, 24% fat, and 6% fiber). The formula alone consists of 14.2 g soluble fiber (soluble to insoluble fiber ratio of 3:2) and provides 21 g of total fiber daily. Table 1 summarizes the full nutrient composition. In addition, one to two portions of non-starchy vegetables were recommended daily, bringing the total energy intake to 1,000 kcal/day. To support digestion of the fiber-enriched product, patients were advised to drink at least 2 l of calorie-free beverages per day. Group meetings were offered at baseline and after seven and 14 days to provide background information and to support compliance with the diet. No other specific dietary or physical activity targets were given. The patients were asked to maintain their habitual level of physical activity.

The primary outcome of this study was the effect of the dietary intervention on liver fat contents as measured by CAP.

Table 1 HEPAFAST nutrient composition

	Per 100 g of powdered product	Per 30 g of powdered product in 350 ml milk (fat content 1.5%)
Energy and nutrient content		
Energy	324 kcal (1,359 kJ)	262 kcal (1,100 kJ)
Fat	4.8 g	7.0 g
Saturated fat	1.5 g	3.6 g
Total carbohydrate	5.8 g	19.0 g
Sugar	3.5 g	18.0 g
Fiber	24.0 g	7.0 g
Protein	51.0 g	27.0 g
Sodium	101 mg	195 mg
Key ingredients		
L-carnitine	2,000 mg	600 mg
Taurine	2,000 mg	600 mg
Omega-3-fatty acids	1,140 mg	342 mg
Choline	550 mg	165 mg
Oatmeal	20.0 g	6.0 g
β-glucan	5.6 g	1.7 g
Inulin	7.3 g	2.2 g
Oat fiber	5.0 g	1.5 g

Secondary outcomes included changes in body composition, liver stiffness measurements (LSM), serum lipid concentrations, and cardiovascular risk profile. At the Department of Medicine II of Saarland University Medical Center (Homburg, Germany), we quantified CAP and LSM, and determined body composition. Patients were included in the study if they had a CAP $\geq$ 215 dB/m at baseline and were excluded from observation if they had any of the following: harmful alcohol intake based on the AUDIT questionnaire,[26] histologically defined liver cirrhosis or LSM $\geq$ 13 kPa,[27] pregnancy, cardiac pacemaker, or stage IV or V chronic kidney disease.[28]

Written informed consent was obtained from each participant. The study protocol complies with the ethical guidelines of the Declaration of Helsinki as reflected in *a priori* approval by the Saarland Ethics Committee (Ärztekammer des Saarlandes, ref. 271/11).

Anthropometric, clinical and biochemical assessments. After an 8-h overnight fast, the following parameters were measured: height was recorded using a stadiometer (seca 217; Seca, Hamburg, Germany), weight and body composition (body mass index, BMI; body fat mass, BFM; body fat free mass, BFFM; total body water, TBW; visceral fat index, VFI) were assessed using a segmental bioelectrical impedance analyzer (Tanita BC-418MA; Tanita Europe, Sindelfingen, Germany), and waist circumference (WC) was measured using a tape measure aligned at the lowest border of the rib cage in an exhaled and relaxed position. Office systolic and diastolic blood pressure was taken using a digital blood pressure monitor (Visomat; UEBE Medical, Wertheim, Germany). Medical history and current medication were documented. At baseline, 16 patients stated that they took no medication, and 44 listed their current medication, including antihypertensives ($N=28$), antidiabetics ($N=14$) and lipid-lowering agents ($N=11$). At the end of the intervention, antihypertensives were discontinued in five and metformin in three cases; of note, no new medication was started during the intervention.

Fasted blood samples were collected for routine analysis of liver function tests and serum lipids: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl transferase (γ-GT), pseudocholinesterase (PChE), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol as well as uric acid, glucose, HbA1c, and kidney function tests. Fatty liver index (FLI), which is based on BMI, WC, γ-GT, and TG, was calculated according to Bedogni *et al.*:[29]

$$\mathrm{FLI} = \frac{e^{0.953\times \log e(\mathrm{TG})+0.139\times \mathrm{BMI}+0.718\times \log e(\gamma-\mathrm{GT})+0.053\times \mathrm{WC}-15.745}}{1+e^{0.953\times \log e(\mathrm{TG})+0.139\times \mathrm{BMI}+0.718\times \log e(\gamma-\mathrm{GT})+0.053\times \mathrm{WC}-15.745}}\times 100$$

Non-invasive assessment of hepatic steatosis and fibrosis. Hepatic steatosis and liver stiffness were assessed using VCTE (FibroScan, Echosens, Paris). At 3.5 MHz, the M-probe provides CAP values (100–400 dB/m) and LSM values (1.5–75.0 kPa), as described in our previous study.[10] All elastrography measurements were carried out in fasted patients by the same experienced operator (AA, who has

carried out >600 measurements). According to the 2015 EASL-ALEH Clinical Practice Guidelines for non-invasive tests for evaluation of liver disease severity and prognosis,[25] results were only included if they fulfilled the criteria for a valid transient elastography measurement: at least 10 valid shots, a success rate of ≥60%, and an interquartile range (IQR)/liver stiffness (LSM) of ≤30%.

Genotyping of the *PNPLA3* variant p.I148M. DNA was isolated from EDTA blood according to membrane-based QIAamp DNA extraction protocol (Qiagen, Hilden, Germany). Genotyping of the *PNPLA3* single nucleotide polymorphism rs738409 (c.617G>C, resulting in the amino acid substitution p.I148M) was conducted using a PCR-based assay with 5′-nuclease and fluorescence detection (TaqMan, Life Technologies, Darmstadt, Germany; rs738409: C__7241_10) as decribed.[11]

Statistical analysis. All statistical analyses were performed with SPSS 20.0 (SPSS, Munich, Germany) and GraphPad Prism 5.0 (GraphPad Software, CA, USA). A two-sided P value<0.05 was regarded as significant. Most of the variables were non-parametric, as assessed using the Kolmogorov–Smirnov tests. Results are presented as medians and ranges, unless stated otherwise, or as frequencies and percentages.

Variables were tested for correlation using the Spearman's rank coefficient r_s. Contingency tables were used to assess for associations between categorical variables. Parameter changes after the dietary intervention are reported as absolute and relative frequencies. Comparisons between two and three unpaired groups were conducted using the Mann–Whitney *U* and Kruskal–Wallis tests, respectively. The Wilcoxon-signed ranks tests were used for comparisons between two paired groups. Both linear univariate and multivariate regression analysis were employed to detect the influence of baseline variables on absolute CAP changes. We also carried out subgroup analyses assessing for sex-specific differences, patients with and without type 2 diabetes, and comparing CAP responders (defined by CAP reduction) with CAP non-responders (defined by CAP increase).

RESULTS

Patient characteristics. A total of 84 patients were screened for this open study. As depicted in the flow chart (Figure 1), 24 patients were excluded, mainly due to invalid transient elastography ($N=8$) or harmful alcohol use ($N=6$), and none of the 60 patients was lost to follow-up. Table 2 summarizes their clinical characteristics. The cohort comprised 31 (51.7%) women and had a median age of 56 years (25–78 years). Median CAP was 295 dB/m (216–400 dB/m). As stated in Methods, the presence of steatosis (steatosis grade ≥S1) was confirmed based on a CAP≥215 dB/m.[30] This cut-off has been validated by de Lédinghen *et al.*[30] with an area under receiver operating characteristic curve of 0.84 (95% confidence interval; 0.76–0.92) in reference to liver biopsy. Alternatively, fatty liver can be defined using the FLI, with values ≥60 indicating steatosis.[29] At baseline,

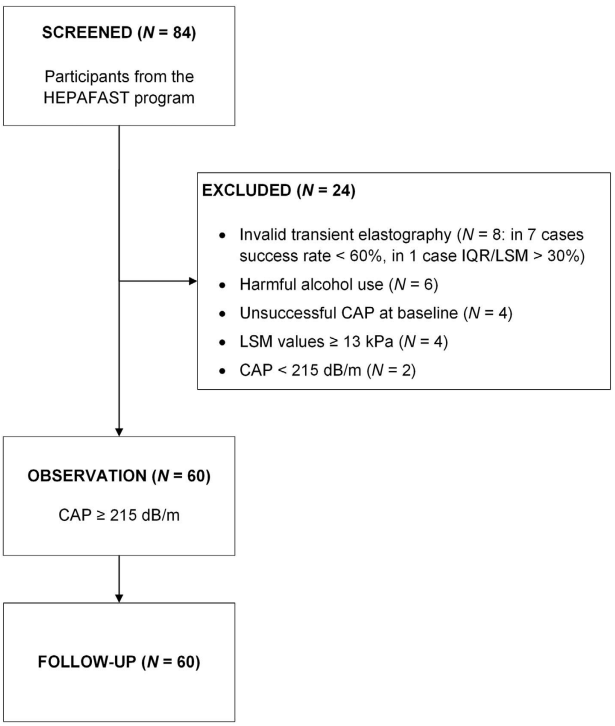

Figure 1 Flow chart of study recruitment and participation.

CAP≥215 dB/m and FLI≥60 were simultaneously detected in 50 (83.3%) patients, and only three cases presented with FLI<30 ($r_s=0.465$, $P<0.001$). Three patients had normal weight (BMI 18.5–24.9 kg/m^2), 19 (31.7%) were overweight (BMI 25.0–29.9 kg/m^2), and 38 patients (63.3%) were obese (BMI≥30.0 kg/m^2). In addition, 55 (91.6%) patients had elevated BFM. In total, 57 patients (95.0%) were above the European WC thresholds of 94 cm for men and 80 cm for women.[31] Median HbA1c was 5.7% (4.9–10.4%), and type 2 diabetes was present in 14 (23.3%) patients. Overall, 26 patients (43.3%) presented with the metabolic syndrome, defined as elevated WC, TG, blood pressure, fasting plasma glucose, diagnosis of type 2 diabetes and reduced HDL cholesterol, as outlined by the International Diabetes Federation.[31] Baseline activity level was monitored through a self-report questionnaire, specifically type, frequency and duration of physical activity. At follow-up, an increase in activity level was reported in a single patient only.

The dietary intervention has a positive impact on CAP. Overall, the median CAP decreased significantly by 14.0% (47 dB/m, $P<0.001$, Figure 2a) from 295 dB/m (216–400 dB/m) to 266 dB/m (100–353 dB/m) (Table 2). Figure 2b displays all CAP values of individual patients.

Of 60 patients, 49 (81.7%) demonstrated a decrease in median CAP of 15.9% (50 dB/m, $P<0.001$) and were defined as CAP responders (see Statistical analysis). Of these, 10 patients (20.4%) showed resolution of steatosis, i.e., they presented with CA$P<215$ dB/m after the dietary intervention. Interestingly, 11 demonstrated a median CAP increase of 3.4% (9 dB/m, $P=0.003$) after the 14-day intervention despite

improvements in body composition, thus we classified them as CAP non-responders. Compared to responders, non-responders were mostly women (72.7 vs. 46.9%, $P>0.05$), younger (51 vs. 56 years, $P>0.05$), had a higher baseline BMI (32.2 vs. 31.2 kg/m^2, $P>0.05$) and a lower baseline CAP (262 vs. 308 dB/m, $P=0.001$). When comparing genetic variation,

Table 2 Clinical characteristics of the study cohort

	At baseline	At follow-up	Relative reduction (%)	*P*
Sociodemographic characteristics				
N (men/women)	60 (29/31)			
Age (years)	56 (25–78)			
Body composition				
Body weight (kg)	95.1 (60.7–125.6)	90.5 (58.2–120.1)	– 4.6 (–8.0– – 0.7)	**<0.001**
BMI (kg/m^2)	31.9 (22.4–44.8)	30.6 (21.3–43.5)	– 4.7 (–8.1– – 0.6)	**<0.001**
BFM (kg)	34.5 (16.8–63.4)	31.8 (13.4–59.5)	– 6.9 (–27.0–4.6)	**<0.001**
BFFM (kg)	58.2 (39.5–84.9)	55.3 (39.3–81.9)	– 3.3 (–9.1–4.2)	**<0.001**
TBW (kg)	42.6 (28.9–62.2)	40.5 (28.8–60.0)	– 3.3 (–9.1–4.1)	**<0.001**
WC (cm)	107 (78–127)	103 (76–128)	– 4.1 (–9.2–2.2)	**<0.001**
VFI	13 (5–24)	12 (4–21)	– 7.1 (–20.0–11.1)	**<0.001**
Liver markers				
CAP (dB/m)	295 (216–400)	266 (100–353)	– 14.0 (–68.6–38.2)	**<0.001**
FLI	83 (7–99)	63 (4–98)	– 21.3 (–74.0–0.0)	**<0.001**
LSM (kPa)	6.2 (1.5–11.9)	5.3 (1.5–12.0)	– 11.7 (–70.5–43.6)	**0.002**
ALT (U/l)	38 (12–118)	36 (14–150)	0 (–73.1–122.2)	>0.05
AST (U/l)	25 (10–121)	24 (8–141)	0 (–80.2–464.0)	>0.05
AP (U/l)	74 (37–159)	64 (32–144)	– 11.5 (–43.0–24.1)	**<0.001**
γ-GT (U/l)	37 (7–335)	26 (7–113)	– 26.7 (–77.3–50.0)	**<0.001**
PChE (kU/l)	10.7 (6.6–17.0)	10.4 (6.7–15.3)	– 3.8 (–22.6–19.2)	**0.006**
Metabolic markers				
Glucose (mg/dl)	89 (63–232)	84 (60–126)	– 7.1 (–50.4–52.4)	**<0.001**
TG (mg/dl)	128 (60–419)	83 (48–183)	– 34.1 (–84.0–35.9)	**<0.001**
TC (mg/dl)	214 (147–303)	163 (95–249)	– 23.5 (–45.6–10.9)	**<0.001**
LDL cholesterol (mg/dl)	142 (78–226)	96 (45–193)	– 25.3 (–53.1–41.0)	**<0.001**
HDL cholesterol (mg/dl)	50 (29–110)	45 (28–77)	– 13.0 (–66.4–28.9)	**<0.001**
Uric acid (mg/dl)	6.1 (2.9–8.6)	5.6 (3.1–10.0)	– 7.6 (–40.9–43.5)	**0.024**
SBP (mm Hg)	138 (110–175)	130 (104–184)	– 5.6 (–28.6–40.5)	**<0.001**
DBP (mm Hg)	92 (74–125)	87 (72–120)	– 4.5 (–34.2–18.8)	**0.001**

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BFFM, body fat free mass; BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; FLI, fatty liver index; γ-GT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LSM, liver stiffness measurement; *N*, number; PChE, pseudocholinesterase; SBP, systolic blood pressure; TBW, total body water; TC, total cholesterol; TG, triglycerides; VFI, visceral fat index; WC, waist circumference.
Significant *P* values are highlighted in bold.

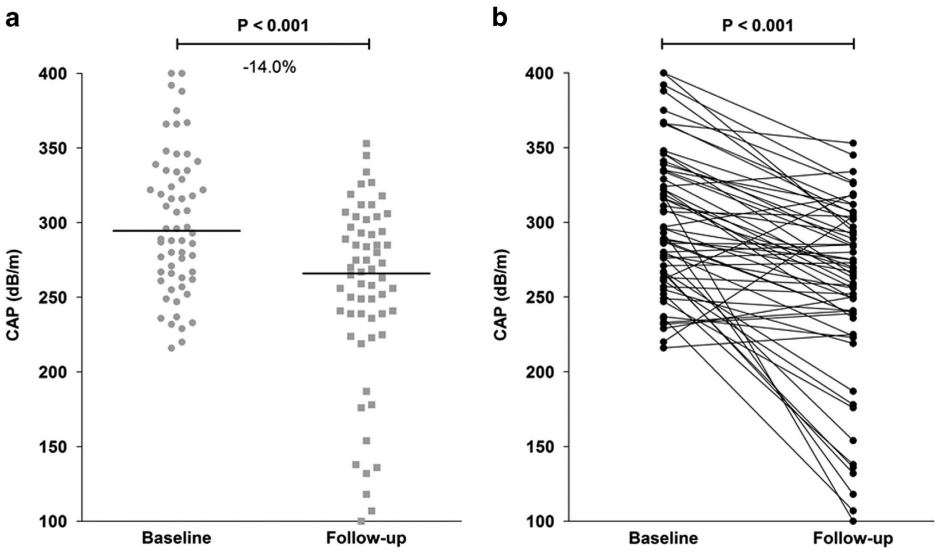

Figure 2 Changes of CAP in all 60 patients. (**a**) Median and individual CAP at baseline and follow-up at the end of the dietary intervention. The median CAP reduction was 47 dB/m in the entire cohort, corresponding to a relative median reduction of 14.0% ($P<0.001$). (**b**) Absolute CAP in the individual patients during the dietary intervention.

Table 3 Distribution of CAP values across *PNPLA3* genotypes

***PNPLA3* variant p.I148M**	**[II]**	**[IM]**	**[MM]**
Frequency (%)	60.0	31.7	8.3
N	36	19	5
CAP at baseline (dB/m)	291 (216–400)	296 (232–392)	339 (267–367)
CAP at follow-up (dB/m)	269 (100–353)	256 (154–334)	292 (132–306)
Absolute median CAP reduction (dB/m)	– 48 (–218–84)	– 39 (–123–57)	– 49 (–135– – 22)
Relative median CAP reduction (%)	– 13.8 (–68.6–38.2)	– 14.1 (–44.4–21.8)	– 14.4 (–50.6– – 7.6)

CAP, controlled attenuation parameter; I, isoleucine; M, methionine; *N*, number; *PNPLA3*, patatin-like phospholipase domain containing 3 (adiponutrin).
The distribution of CAP values and CAP reduction across *PNPLA3* p.I148M genotypes does not differ (all *P* values > 0.05).

Table 4 Univariate and multivariate analysis of determinants of CAP reduction

	β coefficient	***P***
Univariate analysis		
Age	0.042	> 0.05
BFM at baseline	0.126	> 0.05
BMI at baseline	0.142	> 0.05
CAP at baseline	– 0.273	**0.035**
HDL cholesterol at baseline	– 0.335	**0.009**
Sex	0.178	> 0.05
TC at baseline	– 0.062	> 0.05
TG at baseline	0.206	> 0.05
Multivariate analysis		
CAP at baseline	– 0.285	**0.020**
HDL cholesterol at baseline	– 0.345	**0.005**

BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; HDL, high-density lipoprotein; TC, total cholesterol; TG, triglycerides.
Significant *P* values are highlighted in bold.

all five homozygous *PNPLA3* mutation carriers with two prosteatogenic p.148M alleles were responders.

The dietary response might be influenced by *PNPLA3*. In this cohort, the *PNPLA3* p.148M frequencies for wild-type, heterozygous, and homozygous genotypes were [II]: $N = 36$, 60.0%; [IM]: $N = 19$, 31.7%; and [MM]: $N = 5$, 8.3%. With a minor allele frequency of 0.24 for the risk allele [M], this result is comparable to 0.23 reported in the first genome-wide association study.[8] Homozygous carriers of the prosteatogenic [M] allele had a markedly higher baseline CAP (339 dB/m) as compared to wild-type (291 dB/m) and heterozygous (296 dB/m) patients and as shown in Table 3, they had a slightly larger decrease in CAP at 14 days ($P > 0.05$).

Factors associated with CAP. CAP at baseline was associated with BMI ($r_s = 0.330$, $P = 0.010$), WC ($r_s = 0.414$, $P = 0.001$), LSM ($r_s = 0.460$, $P < 0.001$), ALT ($r_s = 0.455$, $P < 0.001$), AST ($r_s = 0.412$, $P = 0.001$), and glucose concentrations ($r_s = 0.366$, $P = 0.004$), but not with TG or γ-GT. Table 4 summarizes the results of univariate regression analysis with absolute reduction of CAP as dependent variable. Age, baseline BFM, BMI, sex, TC, and TG were not correlated, whereas both baseline CAP and HDL cholesterol showed a significant inverse association ($P = 0.035$ and $P = 0.009$, respectively). In multivariate regression analysis, these two variables were independent predictors of absolute CAP reduction ($P = 0.020$ and $P = 0.005$, respectively). As expected, CAP at follow-up correlated with FLI ($r_s = 0.502$, $P < 0.001$).

Changes of liver stiffness and liver enzymes. Patients presented with a median LSM of 6.2 kPa (1.5–11.9 kPa) at baseline and 5.3 kPa (1.5–12.0 kPa) after the intervention (Table 2). Overall, median LSM decreased by 11.7% (0.8 kPa, $P = 0.002$). With respect to liver function tests, baseline γ-GT activity was elevated in 35% of cases compared to 20% after the intervention. Significant reductions of 26.7 and 11.5% were observed for γ-GT and AP activities, respectively (both $P < 0.001$; Table 2). Although no overall reduction in ALT and AST activities occurred, nonsignificant ($P > 0.05$) improvements in patients with elevated baseline activities were detected.

Effects on body composition. All 60 patients were compliant with the program when using weight loss as a marker. Overall, the parameters related to body composition decreased significantly, as summarized in Table 2 (all $P < 0.001$). A median weight reduction of 4.6% (4.2 kg, $P < 0.001$) occurred. A total of 17 (28.3%) patients were reclassified into a lower BMI category after 14 days, whereas 43 (71.7%) remained within their initial category. Table 2 shows that BFM decreased by 6.9% (2.6 kg, $P < 0.001$). Overall, 61.9% of the weight reduction was based on loss of BFM. The absolute BFM reduction did not correlate with the reduction of CAP ($r_s = -0.04$, $P > 0.05$).

Reductions in CAP between patients with a weight reduction ≥ 5 and < 5% were similar (14.9 and 11.4%, both $P < 0.001$). Table 5 summarizes within and between group changes.

Overall, 14 (23.3%) patients had a previous diagnosis of type 2 diabetes. When compared to non-diabetics, these patients were older, had higher serum glucose concentrations and higher baseline CAP, but CAP reductions did not differ between the two groups (Table 6).

Metabolic effects. At baseline, over half of the cohort (60.0%) presented with TC concentrations ≥ 200 mg/dl. LDL cholesterol concentrations were above 130 mg/dl in 35 (58.6%) patients, and 22 (36.7%) patients had increased TG levels ≥ 150 mg/dl. After the dietary intervention, all lipid parameters decreased by one-third to a quarter apart from HDL cholesterol, which reduced by 13.0% (all $P < 0.001$; Table 2). Specifically, a reduction of 17.1% for HDL

Table 5 Comparison of patients with weight loss ≥5 and <5%

	Weight loss ≥5%		Weight loss <5%		
	At baseline	**At follow-up**	**At baseline**	**At follow-up**	***P***
Sociodemographic characteristics					
N (men/women)	26 (20/6)		34 (9/25)		***
Age (years)	50 (25–66)		58 (33–78)		**
Body composition					
Body weight (kg)	92.8 (61.9–125.6)	86.7 (58.6–115.7)$^{###}$	95.6 (60.7–125.5)	92.1 (58.2–120.1)$^{###}$	***
BMI (kg/m^2)	29.6 (22.4–41.5)	27.8 (21.3–38.5)$^{###}$	32.4 (25.3–44.8)	30.9 (24.2–43.5)$^{###}$	***
BFM (kg)	29.2 (17.0–49.5)	26.0 (13.4–44.1)$^{###}$	36.7 (16.8–63.4)	34.8 (15.4–59.5)$^{###}$	***
BFFM (kg)	68.4 (45.8–84.9)	66.6 (44.4–81.9)$^{###}$	53.2 (39.5–77.1)	51.8 (39.3–78.9)$^{###}$	***
TBW (kg)	50.1 (33.5–62.2)	48.8 (32.5–60.0)$^{###}$	38.9 (28.9–56.4)	37.9 (28.8–57.8)$^{###}$	***
WC (cm)	103 (82–124)	98 (77–120)$^{###}$	107 (78–127)	104 (76–128)$^{###}$	***
VFI	12 (5–24)	12 (5–21)$^{###}$	14 (5–21)	13 (4–20)$^{###}$	**
Liver markers					
CAP (dB/m)	295 (216–400)	251 (100–345)$^{###}$	298 (220–392)	283 (136–353)$^{###}$	n.s.
FLI	82 (30–99)	42 (14–93)$^{###}$	83 (7–99)	71 (4–98)$^{###}$	***
LSM (kPa)	5.9 (1.5–11.9)	5.0 (1.5–12.0)$^{##}$	6.2 (3.8–9.7)	5.4 (3.1–10.0)$^{#}$	n.s.
ALT (U/l)	38 (12–118)	31 (14–150)$^{n.s.}$	37 (18–108)	40 (14–105)$^{n.s.}$	n.s.
AST (U/l)	26 (10–121)	22 (11–141)$^{n.s.}$	24 (10–46)	24 (8–46)$^{n.s.}$	n.s.
γ-GT (U/l)	40 (12–335)	26 (7–99)$^{###}$	36 (7–93)	25 (7–113)$^{###}$	**
Metabolic markers					
Glucose (mg/dl)	87 (71–232)	74 (60–115)$^{###}$	91 (63–164)	85 (72–126)$^{##}$	**
TG (mg/dl)	138 (60–419)	72 (48–148)$^{###}$	119 (63–340)	102 (49–183)$^{###}$	***
TC (mg/dl)	219 (147–303)	161 (95–229)$^{###}$	209 (149–302)	166 (103–249)$^{###}$	*
LDL cholesterol (mg/dl)	143 (84–201)	98 (45–172)$^{###}$	140 (78–226)	95 (47–193)$^{###}$	n.s.
HDL cholesterol (mg/dl)	46 (33–110)	42 (28–75)$^{##}$	54 (29–82)	46 (30–77)$^{###}$	n.s.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BFFM, body fat free mass; BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; FLI, fatty liver index; γ-GT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LSM, liver stiffness measurement; N, number; TBW, total body water; TC, total cholesterol; TG, triglycerides; VFI, visceral fat index; WC, waist circumference.
P value between baseline and follow-up determined with the Wilcoxon-signed rank test: $^{###}P \leq 0.001$, $^{##}P \leq 0.01$, $^{#}P \leq 0.05$, $^{n.s.}P > 0.05$. *P* value between relative difference of baseline and follow-up value between both groups (weight loss ≥5% and weight loss <5%) determined with the Mann–Whitney *U* test: $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, $^{n.s.}P > 0.05$.

cholesterol was noted in 46 patients, whereas 13 patients improved by 9.1% and one case remained stable. Most importantly, LDL cholesterol levels decreased by 25.3%, which corresponds to an absolute change of 32.5 mg/dl. The reduction occurred in 54 patients (90.0%).

Overall, glucose levels decreased significantly by 7.1% ($P<0.001$). A positive change in systolic and diastolic blood pressure of −5.6 and −4.5% (both $P \leq 0.001$) was observed. The median reductions of key parameters assessed during the study are summarized in Figure 3.

DISCUSSION

The aims herein were to simultaneously and non-invasively monitor liver and body fat composition in patients with NAFLD participating in a short-term dietary program, and to avail of CAP to assess dynamic changes in liver fat. After a 14-day hypocaloric high-fiber, high-protein diet, an absolute CAP reduction of 47 dB/m and a relative decrease of 14.0% were achieved. This was accompanied by a significant weight loss of 4.6%, of which almost two-thirds (61.9%) was loss of body fat mass. To our knowledge, this is the first study to monitor rapid changes in hepatic steatosis using CAP, which represents a patient-friendly non-invasive tool.

The results are in line with previous smaller studies using other methodologies to assess changes of liver phenotypes after dietary interventions. According to the AASLD guidelines, a reduction of body weight of at least 3–5% through a hypocaloric diet alone or together with increased physical activity is recommended for ameliorating steatosis.[1] Colles *et al.*[32] studied a very low-calorie diet (680 kcal/day) for 12 weeks in 32 morbidly obese patients. Body weight was reduced by 10.6% (14.8 kg), and liver volume assessed by computer tomography decreased by 18.7% (0.56 l). Interestingly, after 2 weeks, 80% of the overall decrease in liver volume was detected, whereas body weight improved steadily throughout.[32] We did not observe a significant association between the extent of weight loss and improvement of liver phenotypes, as reported by others,[18,33] which might be related to less drastic changes in body fat mass (Figure 3). Some of the previously reported studies compared overall weight loss and BMI reductions only rather than compartmental changes of body composition. As the interventions were significantly longer, they may have resulted in greater changes of fat mass and corresponding liver-specific effects.[18,33]

In addition to CAP reductions and weight loss, the dietary modification assessed herein also resulted in metabolic improvements, particularly those related to the metabolic syndrome. Besides the expected reduction in triglycerides, the reduction of LDL cholesterol levels by 33 mg/dl after 14 days is remarkable, especially in comparison to a meta-analysis of 174,000 patients, which reported a decrease of 42.5 mg/dl after 1 year of statin therapy.[34] Another meta-analysis on dietary fiber reported a reduction of LDL cholesterol of

Table 6 Clinical baseline characteristics stratified according to the presence of diabetes

	Patients with diabetes	Patients without diabetes	*P*
Sociodemographic characteristics			
N (men/women)	14 (6/8)	46 (23/23)	>0.05
Age (years)	61 (36–74)	54 (25–78)	**0.038**
Body composition			
Body weight (kg)	95.3 (74.5–119.8)	94.5 (60.7–125.5)	>0.05
BMI (kg/m^2)	31.9 (27.0–44.8)	31.9 (22.4–43.5)	>0.05
BFM (kg)	34.2 (18.9–53.4)	34.5 (16.8–63.4)	>0.05
BFFM (kg)	55.4 (46.3–80.9)	61.6 (39.5–84.9)	>0.05
TBW (kg)	40.6 (33.9–59.2)	45.1 (28.9–62.2)	>0.05
WC (cm)	109 (96–127)	103 (78–124)	>0.05
VFI	15 (11–21)	13 (5–24)	>0.05
Liver markers			
CAP (dB/m)	337 (255–375)	288 (216–400)	**0.025**
FLI	86 (30–99)	76 (7–99)	>0.05
LSM (kPa)	6.7 (1.5–9.9)	5.9 (3.3–11.9)	>0.05
ALT (U/l)	42 (12–118)	38 (16–84)	>0.05
AST (U/l)	26 (10–68)	24 (10–121)	>0.05
AP (U/l)	75 (48–106)	74 (37–159)	>0.05
γ-GT (U/l)	47 (17–93)	32 (7–335)	>0.05
PChE (kU/l)	11.3 (7.9–17.0)	10.4 (6.6–15.0)	>0.05
Metabolic markers			
Glucose (mg/dl)	115 (63–232)	86 (68–156)	<**0.001**
TG (mg/dl)	152 (60–273)	122 (65–419)	>0.05
TC (mg/dl)	210 (149–258)	220 (147–303)	>0.05
LDL cholesterol (mg/dl)	139 (78–184)	142 (84–226)	>0.05
HDL cholesterol (mg/dl)	50 (29–82)	51 (33–110)	>0.05
Uric acid (mg/dl)	6.4 (4.6–8.5)	6.0 (2.9–8.6)	>0.05
SBP (mm Hg)	131 (110–175)	138 (110–175)	>0.05
DBP (mm Hg)	87 (79–175)	93 (74–125)	>0.05

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BFFM, body fat free mass; BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; FLI, fatty liver index; γ-GT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LSM, liver stiffness measurement; N, number; PChE, pseudocholinesterase; SBP, systolic blood pressure; TBW, total body water; TC, total cholesterol; TG, triglycerides; VFI, visceral fat index; WC, waist circumference.
Significant *P* values are highlighted in bold.

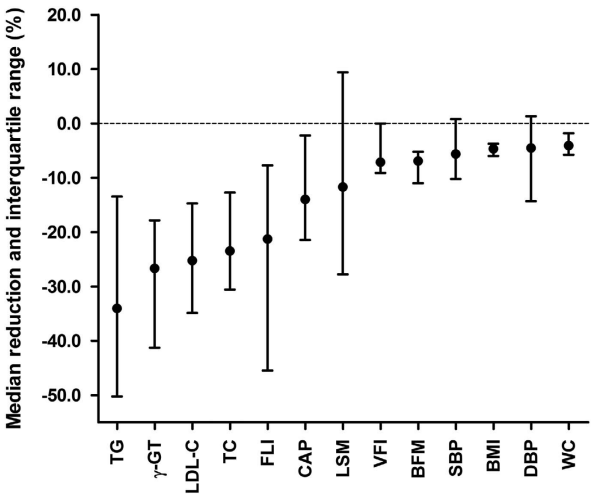

Figure 3 Summary of significant reductions of key parameters assessed during the dietary intervention. The values are displayed as medians, interquartile range, and ordered based on the extent of reduction. BFM, body fat mass; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; FLI, fatty liver index; γ-GT, gamma-glutamyl transferase; LDL-C, low-density lipoprotein cholesterol; LSM, liver stiffness measurement; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; VFI, visceral fat index; WC, waist circumference.

2.21 mg/dl for each 1 g of soluble fiber per day,[35] which is reflected in our study with a daily intake of 14 g soluble fiber. HDL cholesterol levels decreased, which has previously been reported during acute weight loss, however HDL subsequently increased in the weight maintenance phase.[36–38] Brinton *et al.*[39] suggested that the reduction in HDL cholesterol might be associated with lower HDL apolipoprotein transport rates, and Aminian *et al.*[40] observed a decrease in lipoprotein lipase activity by up to 50% during caloric restriction. Once weight stabilized, HDL cholesterol metabolism reversed and led to increased HDL cholesterol concentrations above the pre-intervention levels.[40]

The diet given to patients as part of a specifically designed weight loss program contains ingredients known to be potentially beneficial for the liver, including omega-3 poly-unsaturated fatty acids,[41] L-carnitine,[42] choline,[43] β-glucan,[44] inulin,[45] and taurine.[46]

A limitation of this study is that we were unable to determine if, and to what extent, these ingredients contributed to the overall effects of liver fat reduction, as compared to the overall effects of the caloric restriction and concomitant weight reduction. Moreover, the significant improvements observed after 14 days do not afford the opportunity to evaluate the liver-specific long-term effects of the diet. Although several key parameters of the metabolic syndrome improved,

we did not specifically assess the effect of the diet on insulin resistance.

Shen *et al.*[47] studied the effect of a lifestyle modification program in NAFLD patients and observed that patients who carry the *PNPLA3* mutation p.I148M showed a better response as compared to patients with wild-type alleles.[47] Although, the current data on genetic associations in our study are hampered by sample size, we also note that hepatic response was observed in all homozygous carriers of the *PNPLA3* risk allele, which should be further evaluated as personalized biomarker for a response to the dietary regimen.

Recent recommendations from a joint AASLD–FDA workshop pointed out that the use of elastography in subjects with NASH has not been explored in great detail, and that non-invasive measures should be included as secondary or exploratory endpoints in current trials.[48] Our study results illustrate that CAP might represent a reliable alternative for monitoring hepatic steatosis in research and clinical settings.[23,49]

In conclusion, the 14-day hypocaloric high-fiber, high-protein diet reduced CAP, and hence hepatic steatosis simultaneously to improvements in parameters of the metabolic syndrome. We demonstrated that improvements in hepatic fat contents can be observed after a couple of weeks only, which highlights the possibility for dynamic short-term modulation of liver fat. Whether such a program provides long-term benefits for these patients should be substantiated, but extent and rate of liver fat reduction set the benchmark for pharmacological treatment. Regardless, CAP provides a convenient and patient-friendly method to assess lipid turnover during lifestyle and dietary interventions to combat NAFLD.

CONFLICT OF INTEREST

Guarantor of the article: F. Lammert, MD, PhD.
Specific author contributions: After designing the study by A. Arslanow, F. Lammert, and C. S. Stokes; A. Arslanow, M. Teutsch, and H. Walle recruited patients; A. Arslanow collected the data and together with F. Lammert and C. S. Stokes analyzed the data and drafted the manuscript, which was then critically revised by all authors. The final draft submitted has been approved by all authors.
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Potential competing interests: None.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Non-alcoholic fatty liver disease (NAFLD) is a global rapidly growing health problem.
- ✓ Non-inasive methods are increasingly being used to evaluate hepatic steatosis.

WHAT IS NEW HERE

- ✓ Profound reduction of hepatic steatosis can be detected after only 14 days of dietary intervention using the controlled attenuation parameter.
- ✓ Calorie reduced high-fiber and high-protein diet causes dynamic short-term changes of hepatic and systemic lipids.
- ✓ These can be simultaneously and non-invasively assessed by the combination of transient elastography and bioelectrical impedance analysis.

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Appendix 3. Arslanow et al. Zentralblatt für Arbeitsmedizin, Arbeitsschutz und Ergonomie 2017

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Nichtinvasive Früherkennung von Lebererkrankungen im Rahmen der betrieblichen Gesundheitsförderung

Einführung

Patienten mit einer Fettleber sind meist symptomlos, und die Diagnose wird oftmals nur als Zufallsbefund gestellt, wobei es unerkannt zur Progression der Erkrankung bis hin zur Zirrhose und zur Entwicklung eines Leberzellkarzinoms kommen kann. Bisher hat sich keine Methode durchgesetzt, die in der Fläche zur frühzeitigen Erkennung eingesetzt werden kann. Die betriebsärztliche Vorsorgeuntersuchung bietet die Möglichkeit, die Gesundheit der Mitarbeiter regelmäßig zu überprüfen, hierbei auch Lebererkrankungen früh zu erkennen und so das Risiko einer Krankheitsprogression zu senken.

Hintergrund und Fragestellung

Bis zu 50 % der europäischen Bevölkerung haben eine Fettleber, die durch eine Akkumulation von Triglyzeriden über 5 % in den Hepatozyten definiert ist [6]. Die Fettleber kann zu Steatohepatitis und Zirrhose voranschreiten [19]. Diese 3 Entitäten werden als Fettlebererkrankung zusammengefasst [22]. Ein Viertel der Erwachsenen in Europa sind vom metabolischen Syndrom betroffen, und die Fettleber wird als hepatische Manifestationen des metabolischen Syndroms verstanden [24]. Das Sterblichkeitsrisiko für die meisten dieser Patienten ist insbesondere durch kardiovaskuläre Erkrankungen erhöht [30].

Der Goldstandard zur Beurteilung einer Lebererkrankung ist die (perkutane) Leberbiopsie. Diese Methode trägt ein Blutungsrisiko [29], kann eine beträchtliche Variabilität aufweisen [23] und sollte nur bei einem begründeten Verdacht eingesetzt werden. Im klinischen Alltag hat sich die Bestimmung der Serumaktivitäten von *Leberenzymen* wie der Alanin-Aminotransferase (ALT) und der γ-Glutamyltransferase (γ-GT) als einfach durchzuführende Methode etabliert, obgleich diese aber auch bei einer bereits bestehenden Lebererkrankung normwertig sein können. Unter 386 und 458 Fällen mit histologisch nachgewiesener NAFLD lagen die Raten der Patienten mit normwertiger ALT bei 13 und 14 %, von denen in der Biopsie 6 bzw. 8 Fälle mit Zirrhose diagnostiziert wurden [10, 20]. Patienten mit Leberzellkarzinom auf dem Boden einer NAFLD können normwertige ALT-Aktivitäten aufweisen [28].

Arbeitsmediziner beschäftigen sich mit den Wechselwirkungen von Arbeit und Gesundheit, geregelt durch die Verordnung zur arbeitsmedizinischen Vorsorge (ArbMedVV). Die Untersuchungsabstände finden in einem regelmäßigen Turnus statt. 2016 ist in Deutschland das Gesetz zur Stärkung der Gesundheitsförderung und der Prävention, kurz Präventionsgesetz (PrävG), in Kraft getreten, das die Rolle des Betriebsarztes durch Leistungen zur Gesundheitsförderung in Betrieben (betriebliche Gesundheitsförderung) fördert. Das PrävG bezieht die Primär-, Sekundär- und Quartärprävention in die (arbeits)medizinische Prävention ein [16]. Charakteristisch für

Abkürzungen

ALT	Alanin-Aminotransferase
AST	Aspartat-Aminotransferase
BMI	Body-Mass-Index
CAP	Controlled Attenuation Parameter
CI	Konfidenzintervall
GLC	Glukose
γ-GT	γ-Glutamyltransferase
F	Frau
HS	Harnsäure
HU	Hüftumfang
KFM	Körperfettmasse
KW	Körperwasser
LS	Lebersteifigkeit
M	Mann
OR	Odds ratio
P	*p*-Wert
PrävG	Präventionsgesetz
r_p	Pearson-Korrelationskoeffizient
SMM	Skelettmuskelmasse
TC	Cholesterin
TG	Triglyzeride
THV	Taille-Hüfte-Verhältnis
TU	Taillenumfang
UKS	Universitätsklinikum des Saarlandes
VF	Viszerales Fettvolumen

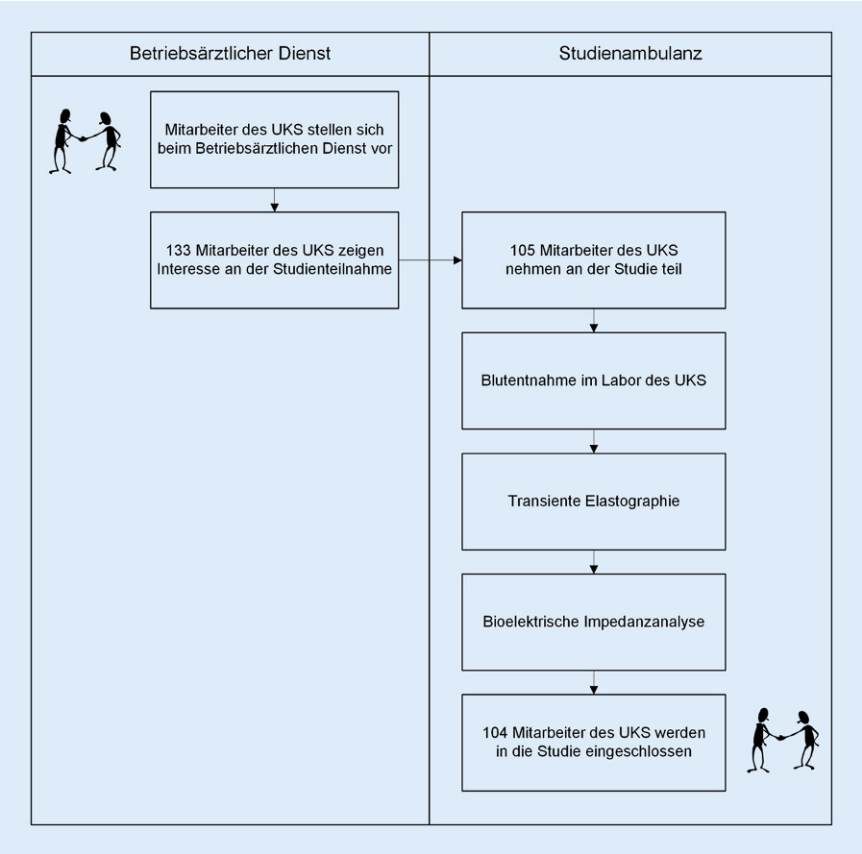

Abb. 1 ▲ Graphischer Ablauf der Studie. *UKS* Universitätsklinikum des Saarlandes

die Sekundärprävention sind das Durchführen von Screenings zur Erkennung von Risikofaktoren und die Früherkennung von Krankheiten. Die neue Rolles des Betriebsarztes kommt vor allem denjenigen zugute, die nicht an den Präventionsprogrammen der Krankenkassen teilnehmen und aufgrund von ausbleibenden Krankheitssymptomen keinen Arzt aufsuchen [16].

Zur Untersuchung der Leber im Rahmen der betrieblichen Gesundheitsförderung ist ein invasiver Eingriff wie die Biopsie weder durchführbar noch gerechtfertigt. Die ebenfalls mögliche Untersuchung mittels Magnetresonanztomographie ist nichtinvasiv, jedoch zeit- und ressourcenintensiv und stellt damit auch keine geeignete Methode dar. Bisher gibt es nur wenige Berichte darüber, dass Mitarbeiter im Rahmen der betriebsärztlichen Untersuchungen mittels bildgebender Methoden auf eine Fettlebererkrankung hin untersucht werden. Einzelne Studien zur Untersuchung mittels Ultraschall wurden aus China und Taiwan berichtet [9, 17]. Nachteilig bei der Sonographie sind jedoch die nur semiquantitative Beurteilung der Leberverfettung und die unzureichende Beurteilung der Leberfibrose.

Die Fettleber ist häufig mit einer Adipositas und einer erhöhten Körperfett- und Viszeralfettmasse assoziiert [27]. Mit zunehmendem Body-Mass-Index (BMI) der gesamten Bevölkerung wird mit einer steigenden Prävalenz der Fettleber gerechnet [21]. Die Progression der Fettleber, bei normwertigen wie auch pathologischen Leberenzymen, ist von Umweltfaktoren, aber auch von genetischen Faktoren abhängig. Es konnte gezeigt werden, dass insbesondere Träger einer häufigen Genvariante des Gens der Triglyceridhydrolase PNPLA3 häufiger eine Fettleber [5], eine Zirrhose [14] und ein Leberzellkarzinom [18] entwickeln.

Mittels transienter Elastographie können nichtinvasiv und innerhalb kurzer Zeit simultan die Leberverfettung und die Lebersteifigkeit zur Abschätzung einer Leberfibrose quantifiziert werden [5, 26].

Mit dieser Studie soll primär untersucht werden, ob dieses Verfahren hinsichtlich der frühzeitigen Diagnose einer Fettleber einen Mehrwert im Vergleich zu Laborparametern bietet. Die UKS-Studie ist die erste Studie, bei der die Lebergesundheit in der betrieblichen Gesundheitsförderung bei Krankenhausmitarbeitern in Deutschland unter Verwendung der transienten Elastographie untersucht wird. Zusätzlich sollte untersucht werden, wie hoch die Prävalenz der Fibrose bei nach eigenen Angaben gesunden Klinikumsmitarbeitern ist und welche Risikofaktoren für die Entstehung einer Fettleber in dieser Kohorte vorliegen.

Studiendesign und Untersuchungsmethoden

Studiendesign

Im Rahmen der betrieblichen Gesundheitsförderung durch den Betriebsärztlichen Dienst des Universitätsklinikums des Saarlandes (UKS) wurde Mitarbeitern im Zeitraum von Mai 2014 bis Dezember 2015 die freiwillige Teilnahme an der Studie angeboten. Hierzu wurde ein Studieninformationszettel mit wesentlichen Informationen zur Studie ausgehändigt. Schwangere und Träger eines Herzschrittmachers waren von der Studienteilnahme ausgeschlossen. Insgesamt erhielten 133 interessierte Mitarbeiter vom Arbeitsmediziner einen Studiencode zur pseudonymisierten Weiterverwendung der Daten. Davon meldeten sich 105 Mitarbeiter (79 %) persönlich zur Teilnahme an der Studie in der Studienzentrale.

Nach der Deklaration von Helsinki in ihrer aktuellen Fassung wurde von jedem Teilnehmer im Anschluss an die schriftliche und mündliche Erläuterung der Studie persönlich eine Einwilligungserklärung unterzeichnet. Unter Verwendung eines Studiencodes wurden anschließend alle Untersuchungen pseudonymisiert durchgeführt. Die Durchführung der transienten Elastographie bei Gesunden wurde durch die Ethik-Kommission des Saarlandes genehmigt (Referenznummer 07/11). Zur Studienteilnahme wurde eine Flüssigkeitsbeschränkung von maximal 200 ml

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Nichtinvasive Früherkennung von Lebererkrankungen im Rahmen der betrieblichen Gesundheitsförderung

Zusammenfassung

Hintergrund. Bis zu 50 % der europäischen Bevölkerung haben eine Fettlebererkrankung, die häufig zunächst symptomlos ist, aber ein erhöhtes metabolisches und kardiovaskuläres Risiko anzeigt. Im Rahmen betriebsärztlicher Untersuchungen werden gesundheitliche Risiken bei Mitarbeitern in einem regelmäßigen Turnus erfasst.
Ziel der Arbeit. Es sollte untersucht werden, ob die nichtinvasive transiente Elastographie im Rahmen der betrieblichen Gesundheitsförderung im Vergleich zu Laborparametern einen Mehrwert hinsichtlich der Diagnose einer Fettlebererkrankung bietet.
Material und Methoden. In dieser prospektiv geplanten Querschnittsstudie wurden 133 Mitarbeitern eines Universitätsklinikums beim Betriebsarzt zusätzliche nichtinvasive Untersuchungen angeboten: transiente Elastographie zur Quantifizierung der Leberverfettung (CAP-Wert) und Lebersteifigkeit (LS-Wert), Ermittlung der Körperzusammensetzung mittels bioelektrischer Impedanzanalyse sowie die Erhebung von anthropometrischen Parametern. Laborparameter wurden mit klinisch-chemischen Standardtests bestimmt.
Ergebnisse. Insgesamt 104 Mitarbeiter (74 % Frauen, mittleres Alter 35 Jahre) wurden in die Studie aufgenommen. Bei 36 % der Mitarbeiter wurde eine Fettleber (CAP-Wert ≥243 dB/m) neu diagnostiziert. In einem Drittel dieser Fälle waren ALT- und γ-GT-Aktivitäten normwertig, sodass eine Lebererkrankung durch die Serumparameter alleine nicht erfasst worden wäre. Der CAP-Wert ist mit steigendem BMI assoziiert (r_p = 0,615; p < 0,001). Die stärksten Prädiktoren für eine Verfettung der Leber waren Körperfettmasse und Taillenumfang (Odds-Ratios 1,7 bzw. 3,8; p = 0,033 bzw. p < 0,001).
Diskussion. Diese Studie belegt, dass die transiente Elastographie im Vergleich zu Leberenzymen mehr Patienten mit einer Fettlebererkrankung identifiziert und dass die Fettlebererkrankung bei Mitarbeitern im Gesundheitswesen unterdiagnostiziert ist.

Schlüsselwörter

Bioelektrische Impedanzanalyse · Fettleber · Fibrose · Prävention · Transiente Elastographie

Noninvasive early detection of liver diseases as part of occupational health check-ups

Abstract

Background. Up to 50% of the European population develops fatty liver disease, which although often asymptomatic, is associated with increased metabolic and cardiovascular risk. Thus, regular occupational health check-ups for employees might help timely identification of this liver disease.
Objectives. The specific aim of the study was to determine whether measuring liver fat noninvasively using transient elastography is more effective for diagnosing fatty liver disease compared to serum parameters obtained during occupational health check-ups.
Materials and methods. In this prospective cross-sectional study, 133 employees of a university hospital were offered additional noninvasive tests during occupational health check-ups: transient elastography to quantify hepatic steatosis (CAP value) and liver stiffness (LS value), bioelectric impedance analysis to determine body composition, assessment of additional anthropometric parameters, and measurement of serum markers by standard clinical chemical tests.
Results. In total, 104 employees (74% women, mean age 35 years) were included. Fatty liver (as defined by CAP value ≥243 dB/m) was newly diagnosed in 36% of employees. In one third of these cases, ALT and γ-GT activities were within the normal range; therefore liver disease was undetected when using serum parameters alone. The CAP value was associated with increased body mass index (r_p = 0.615, p < 0.001). The strongest predictors for accumulation of hepatic fat were body fat mass and waist circumference (odds ratios 1.7 and 3.8, p = 0.033 and p < 0.001, respectively).
Conclusion. This study shows that transient elastography provides additional value in the detection of fatty liver as compared to serum parameters in occupational health check-ups. Furthermore, the study provides evidence that fatty liver disease is underdiagnosed among health care workers.

Keywords

Bioelectrical impedance analysis · Fatty liver · Fibrosis · Prevention · Transient elastography

Wasser oder Kaffee und eine Nahrungskarenz von 120 min vorausgesetzt [3], wobei 51,4 % der Mitarbeiter zum Zeitpunkt der betrieblichen Gesundheitsförderung länger als 8 h nüchtern waren. Mittels eines standardisierten Fragebogens wurden Alter, Geschlecht und Vorhandensein bekannter Erkrankungen (Lebererkrankungen, Diabetes mellitus Typ 2, arterielle Hypertonie) erfragt. Die Einnahme von Medikamenten, Alkoholkonsum und Rauchverhalten wurde nicht dokumentiert, um die Akzeptanz der Studie durch die Mitarbeiter zu erhöhen. Der Zeitaufwand für Aufklärung, Befragung, Durchführung der transienten Elastographie und der bioelektrischen Impedanzanalyse betrug pro Mitarbeiter ca. 10 min. Das Flowchart in **Abb. 1** fasst den Ablauf der Studie zusammen.

Laborchemische Untersuchungen

Vom Arbeitsmediziner angeordnet, erfolgte eine Blutentnahme im Labor, die anschließend im Zentrallabor des UKS mittels klinisch-chemischer Standardtests analysiert wurde. Die Anforderung der Parameter unterlag allein dem Arbeitsmediziner anhand der medizinischen Notwendigkeit. Von den bestimmten Parametern wurden folgende in dieser Studie ausgewertet: Cholesterin (TC), Triglyzeride (TG), Glukose (GLC), Harnsäure (HS), Alanin-Aminotransferase (ALT), Aspartat-Aminotransferase (AST) und γ-Glutamyltransferase (γ-GT).

Tab. 1 Laborchemische Parameter und Parameter der Körperzusammensetzung der Studienkohorte

	Mittelwert ± Standardabweichung (Spannweite)	Referenzbereich	Außerhalb des Referenzbereiches (%)
A – Laborchemische Parameter			
TC (mg/dl)	189 ± 38 (115–313)	<200	32,6
TG (mg/dl)	100 ± 56 (28–363)	<150	7,7
Nüchtern-GLC (mg/dl)	91 ± 9 (68–109)	60–100	14,9
HS (mg/dl)	4,6 ± 1,3 (1,9–9,6)	2,4–5,7 (F), 3,4–7,0 (M)	10,2
ALT (U/l)	23 ± 12 (11–81)	10–35 (F), 10–50 (M)	7,5
AST (U/l)	25 ± 9 (12–69)	10–35 (F), 10–50 (M)	7,6
γ-GT (U/l)	23 ± 17 (8–108)	<40 (F), <60 (M)	4,3
B – Parameter der Körperzusammensetzung			
BMI (kg/m^2)	24,0 ± 4,0 (17,5–37,0)	–	–
TU (cm)	80 ± 11 (61–119)	≤80 (F), ≤94 (M)	26,5
THV	0,87 ± 0,07 (0,74–1,09)	<0,85 (F), <0,90 (M)	49,0
KFM (%)	28,8 ± 9,2 (7,9–51,1)	–	–
KW (%)	52,4 ± 6,3 (37,0–67,7)	–	–
SMM (%)	33,4 ± 5,0 (23,1–45,6)	–	–
VF (l)	1,4 ± 1,3 (0,2–4,8)	–	–

ALT Alanin-Aminotransferase, *AST* Aspartat-Aminotransferase, *BMI* Body-Mass-Index, *GLC* Glukose, *γ-GT* γ-Glutamyltransferase, *F* Frau, *HS* Harnsäure, *KFM* Körperfettmasse, *KW* Körperwasser, *M* Mann, *SMM* Skelettmuskelmasse, *TC* Cholesterin, *TG* Triglyzeride, *THV* Taille-Hüfte-Verhältnis, *TU* Taillenumfang, *VF* viszerales Fettvolumen

Untersuchungen in der Studienambulanz

Bioelektrische Impedanzanalyse und Anthropometrie

Die bioelektrische Impedanzanalyse mit 8 Elektroden (seca mBCA515, Seca, Hamburg) ermittelt, an unbedeckten Händen und Füßen auf einer Wiegeplattform stehend, folgende Parameter: Gewicht, BMI, prozentuale Körperfettmasse (KFM), prozentuales Körperwasser (KW), prozentuale Skelettmuskelmasse (SMM) und viszerales Fettvolumen (VF). Die Körperlänge wurde mittels Stadiometer (seca217, Seca, Hamburg) ermittelt. Taillenumfang (TU) und Hüftumfang (HU) wurden mittels Maßband im stehenden und ausgeatmeten Zustand auf Höhe des letzten Rippenbogens und der Beckenknochen gemessen. Das Taille-Hüfte-Verhältnis (THV) berechnet sich als Quotient aus TU und HU. Als Referenzwerte für Europäer gelten gemäß WHO ein TU ≤80 cm bei Frauen und ≤94 cm bei Männern sowie ein THV von <0,85 bei Frauen und <0,90 bei Männern [31].

Transiente Elastographie

Mittels Ultraschall-basierter transienter Elastographie (FibroScan®, Echosens, Paris), einem seit über 10 Jahren bewährten Verfahren zur Untersuchung der Lebergesundheit, werden simultan die Leberverfettung und die Lebersteifigkeit quantifiziert. Hierfür wird in Rückenlage im Zwischenrippenraum des rechten Leberlappens an der Sonde ein niedrig-frequenter Ultraschall mittels Scherwelle ausgelöst. Die Verfettung der Leber wird durch die zunehmende Schallabschwächung als Controlled Attenuation Parameter (CAP) quantifiziert und kann Werte von 100 bis 400 dB/m annehmen [26]. In einer Studie der Allgemeinbevölkerung wurden Messungen ≥243 dB/m als Fettleber gewertet (entspricht Steatosegrad ≥S1), und CAP-Werte ≥303,5 dB/m wurden als moderate Fettleber definiert (Steatosegrad ≥S2). Die Performance des CAP-Wertes für S1 und S2 wurde mit einem AUROC von 0,94 und 0,95 bestimmt [8].

Die Ausbreitungsgeschwindigkeit des Schalls in der Leber wird als Lebersteifigkeit (LS) in kPa ausgegeben; sie nimmt Werte von 1,5 bis 75,0 kPa an und steigt mit zunehmender Fibrosierung [25]. Werte ≥7,65 kPa sprechen für ein Fibrosestadium ≥F2, und Werte ≥13,0 kPa zeigen eine Leberzirrhose (F4) an [11]. Als Gültigkeitskriterium für die Messungen gelten ein Interquartilsabstand zum Median des LS-Wertes ≤30 oder >30 % bei einem LS-Wert <7,1 kPa [7]. Zum Zeitpunkt der Messung standen 2 Sonden mit unterschiedlichen Eigenschaften zur Verfügung. Die M-Sonde misst simultan den CAP und LS-Wert. Die XL-Sonde, die bei adipösen Menschen eingesetzt wird, ermittelt nur den LS-Wert. Zur Auswertung der Fettleber mittels CAP-Wert konnten demnach nur Messungen mit der M-Sonde berücksichtigt werden.

Metabolisches Syndrom

Für diese Studie wurden zur Definition des metabolischen Syndroms die Kriterien der International Diabetes Federation verwendet. Diese sind ein erhöhter TU und mindestens zwei der folgenden Kriterien: erhöhte Serum-TG-Konzentrationen, Nüchtern-GLC, Diabetes mellitus Typ 2, arterielle Hypertonie oder vermindertes Serum-HDL-TC [1].

Statistik

Die Auswertung der Daten erfolgte mittels SPSS 20.0 (SPSS, München). Aufgrund der Normalverteilung nach Kolmogorow-Smirnow-Test werden metrische Daten als Mittelwert ± Standardabweichung angegeben und durch die Angabe der Spannweite ergänzt. Für unabhängige Stichproben aus 2 Gruppen wurden die Mittelwerte mittels t-Tests, bei mehr als 2 Gruppen mittels einfaktorieller ANOVA berechnet. Der Zusammenhang zwischen 2 Parametern wird mittels Pearson-Korrelationskoeffizient (r_p) dargestellt. Die Verteilung der Häufigkeiten zweier nominal verteilter Parameter wurde mittels χ^2-Test oder Fishers Exact Test (wie in **Tab. 3 und 4** angegeben) berechnet. Parameter, die den CAP-Wert beeinflussen, wurden mittels univariater linearer Regressionsanalyse ermittelt, und signifikante Faktoren wurden in multivariaten Analysen erneut überprüft. Als

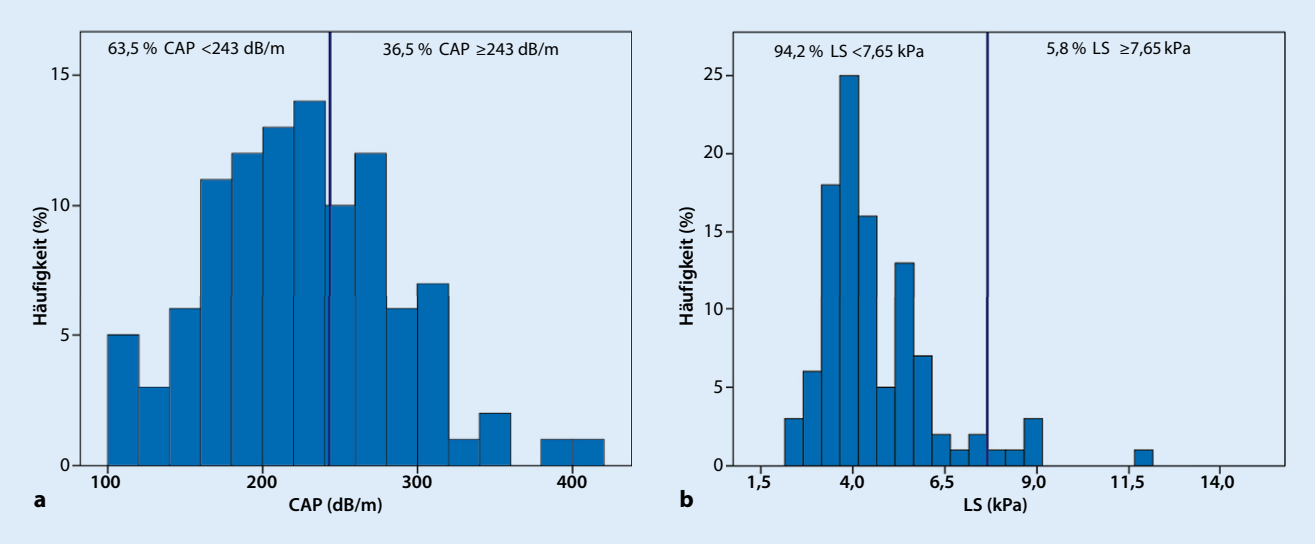


Abb. 2 ▲ Häufigkeitsverteilung der **a** Leberverfettung (CAP-Werte) und **b** Lebersteifigkeit (LS-Werte) sowie Einteilung nach etablierten Grenzwerten

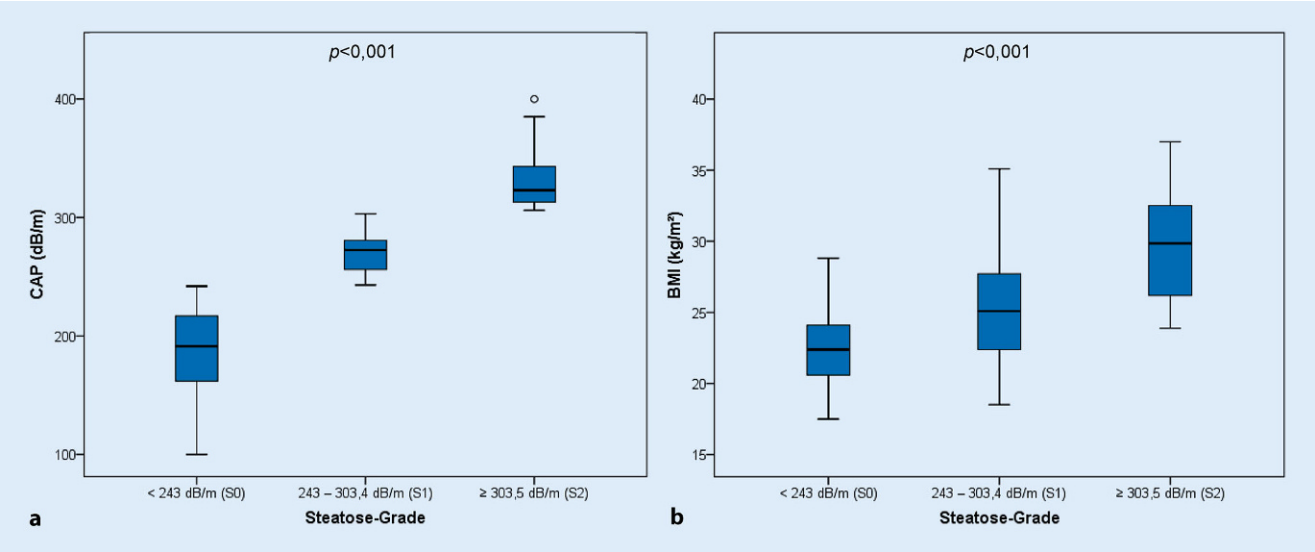


Abb. 3 ▲ Verteilung der **a** Leberverfettung (CAP-Werte) in dB/m und **b** Body-Mass-Index (BMI) in kg/m^2 pro Steatose-Grad. S0: 188 ± 37 dB/m und 22,4 ± 2,7 kg/m^2; S1: 271 ± 17 dB/m und 25,4 ± 3,9 kg/m^2; S2: 336 ± 33 dB/m und 30,1 ± 4,5 kg/m^2

signifikant wurden *p*-Werte <0,05 definiert.

Ergebnisse

Studienkollektiv

An der UKS-Studie nahmen 104 Mitarbeiter, darunter 77 Frauen (74 %), im Alter von 21–63 Jahren (mittleres Alter 35,1 ± 12,5 Jahre), teil. Bei allen konnten die bioelektrische Impedanzanalyse und die transiente Elastographie durchgeführt werden. In einem Fall konnte nur die XL-Sonde eingesetzt werden, sodass der fehlende CAP-Wert zum Ausschluss eines Mitarbeiters aus der Studie führte. Der durchschnittliche BMI lag bei 24,0 ± 4,0 kg/m^2 (17,5–37,0 kg/m^2), wobei 67 % der Teilnehmer einen BMI <25 kg/m^2, 24 % einen BMI zwischen 25 und 29,9 kg/m^2 und 9 % einen BMI ≥30 kg/m^2 hatten. Initial gaben 3 Teilnehmer (3 %) vor den Untersuchungen an, dass eine Lebererkrankung bekannt sei (Fettleber bei *N* = 1, chronische Hepatitis-B-Virus-Infektion bei *N* = 2). Weiterhin hatten 3 Teilnehmer (3 %) einen Diabetes mellitus Typ 2 und 6 (6 %) eine arterielle Hypertonie.

Laborchemische Parameter und Parameter der Körperzusammensetzung

Im Rahmen der betrieblichen Gesundheitsförderung waren bei 32,6 und 7,7 % der Mitarbeiter die TC- bzw. die TG-Konzentrationen im Serum erhöht. Nüchtern-GLC-Werte (*N* = 47) lagen bei 14,9 % der Studienteilnehmer oberhalb von 100 mg/dl. Der HS-Wert war

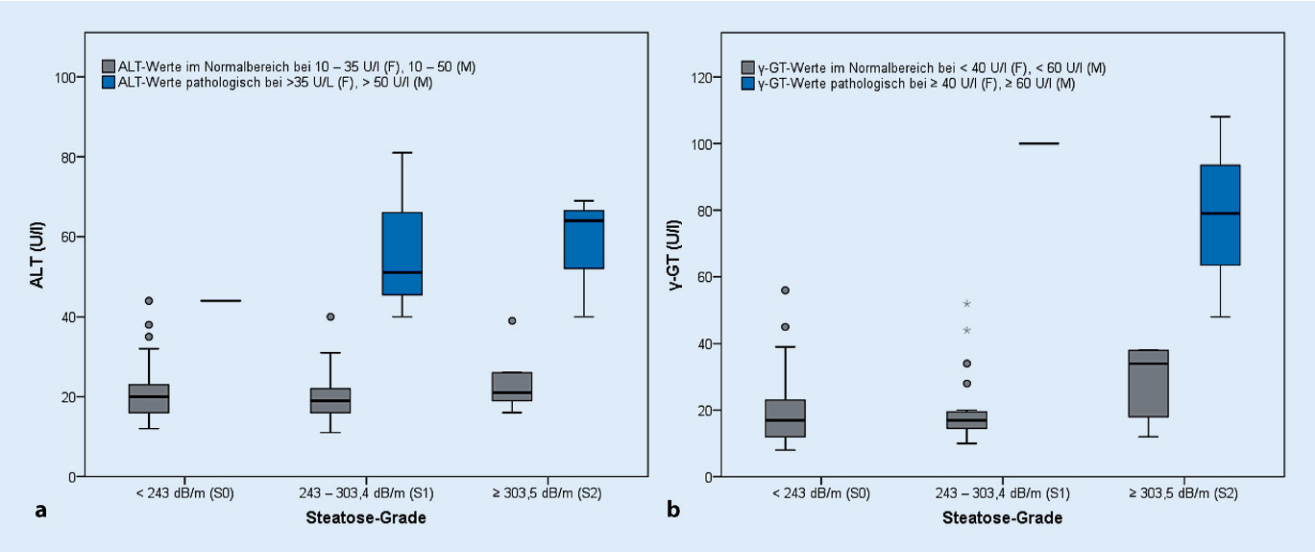


Abb. 4 ▲ Verteilung der **a** ALT-Werte in U/l und **b** γ-GT-Werte in U/l pro Steatose-Grad. S0: 21 ± 7 U/l und 19 ± 9 U/l; S1: 25 ± 15 U/l und 23 ± 19 U/l; S2: 37 ± 20 U/l und 47 ± 32 U/l

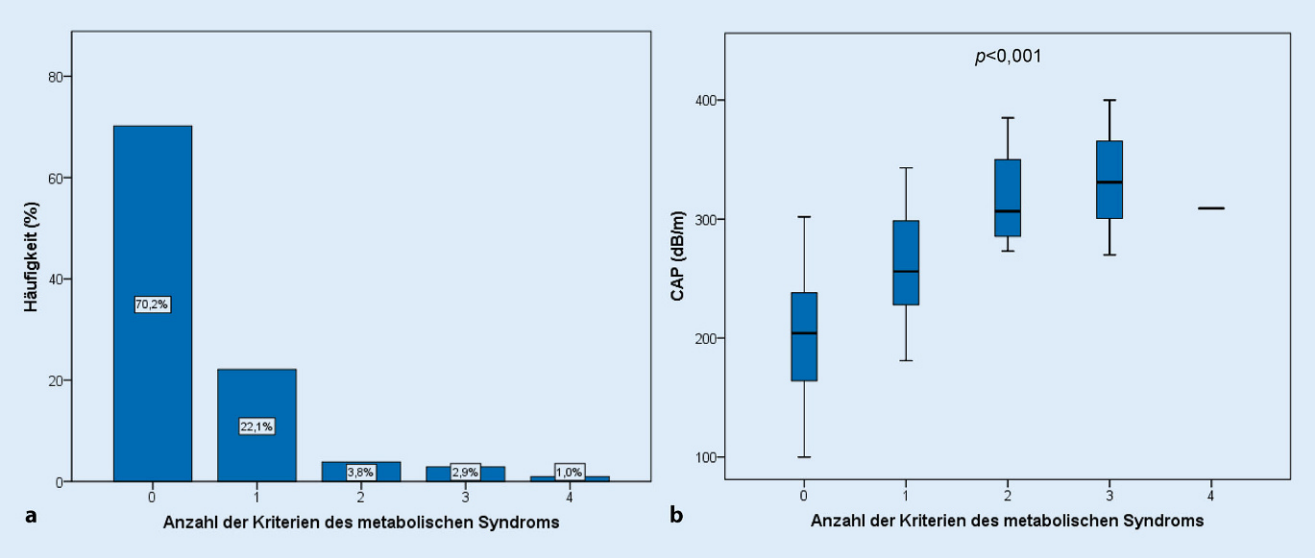


Abb. 5 ▲ **a** Häufigkeitsverteilung der Anzahl der Kriterien des metabolischen Syndroms und **b** die jeweilige Verteilung der Leberverfettung (CAP-Werte) in dB/m. 0: 202 ± 49 dB/m; 1: 259 ± 48 dB/m, 2: 318 ± 48 dB/m: 3: 334 ± 65 dB/m; 4: 309 dB/m

bei 10,2 % der Mitarbeiter erhöht. Die Parameter zur Bestimmung der Lebergesundheit (ALT, AST und γ-GT) waren bei 7,5, 7,6 und 4,3 % der Teilnehmer auffällig (**Tab. 1**A).

Bei 26,5 % der Mitarbeiter lag der TU über dem WHO-Grenzwert, wobei er bei 14,7 % erhöht (>80 cm bei Frauen und >94 cm bei Männern) und bei 11,8 % stark erhöht (>88 cm bei Frauen und >102 cm bei Männern) war; das THV lag bei 49,0 % der Mitarbeiter oberhalb der Norm. Mittels bioelektrischer Impedanzanalyse wurden bei den Mitarbeitern folgende Parameter ermittelt: 28,8 % KFM, 52,4 % KW, 33,4 % SMM und 1,4 l VF (**Tab. 1**B).

Fettleberdiagnostik mittels CAP-Messung

Mittels transienter Elastographie konnte bei 104 Mitarbeitern mit einer durchschnittlichen Messdauer von 2,7 ± 3,0 min eine Messung mit der M-Sonde durchgeführt werden (s. Methoden). Der mittlere CAP betrug 224 ± 61 dB/m (100–400). In 38 Fällen (36,5 %) lag eine Fettleber vor (CAP ≥243 dB/m; **Abb. 2a**), wovon 26,9 und 9,6 % auf die Steatosegrade S1 und S2 entfielen (**Abb. 3a**). Unter Ausschluss der 3 Teilnehmer mit bekannten Leberkrankheiten betrug die Häufigkeit einer neu diagnostizierten Fettleber 35,6 %.

Signifikante Zusammenhänge konnten zwischen Laborparametern, die mit der Fettleber assoziiert sind, und dem Grad der Verfettung beobachtet werden: ALT und γ-GT stiegen mit zunehmendem Steatosegrad an ($p = 0{,}001$ und $p < 0{,}001$; **Abb. 4a, b** sowie **Tab. 2**A).

Tab. 2 Zusammenhang zwischen CAP-Werten, Laborparametern und Parametern der Körperzusammensetzung

	Korrelation mit CAP-Werten	*p*-Wert	S0	S1	S2	*p*-Wert
A – Laborchemische Parameter						
TC (mg/dl)	0,277	*0,008*	183	202	193	n. s.
TG (mg/dl)	0,355	*0,001*	89	107	156	*0,004*
Nüchtern-GLC (mg/dl)	0,370	*0,010*	89	93	96	n. s.
HS (mg/dl)	0,215	*0,044*	4,5	4,7	5,6	n. s.
ALT (U/l)	0,407	*0,001*	21	25	37	*0,001*
AST (U/l)	0,077	n. s.	26	24	27	n. s.
γ-GT (U/l)	0,385	*0,001*	19	23	47	*<0,001*
B – Parameter der Körperzusammensetzung						
BMI (kg/m^2)	0,615	*0,001*	22,4	25,4	30,1	*<0,001*
TU (cm)	0,684	*0,001*	75	84	96	*<0,001*
THV	0,406	*<0,001*	0,85	0,86	0,95	*<0,001*
KFM (%)	0,515	*0,001*	25,5	33,2	40,3	*<0,001*
KW (%)	–0,524	*<0,001*	54,7	49,4	44,5	*<0,001*
SMM (%)	–0,362	*0,001*	34,7	31,6	28,9	*<0,001*
VF (l)	0,668	*0,001*	0,8	2,3	–	*0,003*

ALT Alanin-Aminotransferase, *AST* Aspartat-Aminotransferase, *BMI* Body-Mass-Index, *GLC* Glukose, *γ-GT* γ-Glutamyltransferase, *F* Frau, *HS* Harnsäure, *KFM* Körperfettmasse, *KW* Körperwasser, *M* Mann, *P p*-Wert, *SMM* Skelettmuskelmasse, *TC* Cholesterin, *TG* Triglyzeride, *THV* Taille-Hüfte-Verhältnis, *TU* Taillenumfang, *VF* viszerales Fettvolumen

Tab. 3 Verteilung der absoluten Häufigkeiten von leberassoziierten Laborparametern

	S0 (<243 dB/m)	≥S1 (≥243 dB/m)	*p*-Wert[a]
ALT im Normbereich	59 (63,4 %)	27 (29,0 %)	*0,007*
ALT erhöht	1 (1,1 %)	6 (6,5 %)	
γ-GT im Normbereich	60 (64,5 %)	29 (31,2 %)	*0,014*
γ-GT erhöht	0 (0 %)	4 (4,3 %)	

ALT Alanin-Aminotransferase, *γ-GT* γ-Glutamyltransferase, [a] *p*-Wert mittels Fishers Exact Test ermittelt

Auch die Einteilung beider Parameter nach normwertigen bzw. erhöhten ALT und γ-GT ergaben bei Mitarbeitern ohne (S0) und mit Fettleber (≥S1) signifikante Unterschiede (**Tab. 3**). 6,5 und 4,3 % der Mitarbeiter hatten erhöhte ALT und γ-GT bei Vorliegen einer Fettleber (≥S1). Interessanterweise lagen bei fast einem Drittel der Fälle mit Fettleber ALT und γ-GT im Normbereich (29,0 und 31,2 %; **Tab. 3**).

Der CAP-Wert nahm mit steigendem BMI zu (r_p = 0,615; p < 0,001). Der Steatosegrad unterschied sich in charakteristischer Weise zwischen den BMI-Gruppen: S0 war charakteristisch für Normalgewichtige, S1 für Übergewichtige und S2 für adipöse (p < 0,001; **Abb. 3b**). Ein ähnliches Muster fand sich bei weiteren Parametern der Körperzusammensetzung, die in **Tab. 2**B aufgelistet sind: Mit zunehmendem CAP-Wert nahmen auch TU, THV, KFM und VF zu und KW und SMM ab (alle p < 0,001). Der CAP-Wert wurde durch Alter, BMI, KFM und TU beeinflusst (alle p < 0,001; univariate lineare Regressionsanalyse). In der multivariaten Analyse zeigte sich, dass die KFM und der TU mit dem CAP-Wert signifikant (p = 0,033 und p < 0,001) um das 1,7-fache und 3,8-fache assoziiert sind (**Tab. 4**).

Assoziation von Fettleber und metabolischem Syndrom

Anhand der vom Arbeitsmediziner angeforderten Labordaten konnten folgende Parameter des metabolischen Syndroms herangezogen werden: TU, TG, Nüchtern-GLC, bekannter Diabetes mellitus Typ 2 und bekannte arterielle Hypertonie. Das HDL-TC wurde nur bei 5 der 104 Mitarbeiter angefordert. Bei 23 Teilnehmern lag ein Kriterium, und bei 4 lagen 2 Kriterien für ein metabolisches Syndrom vor; in 4 Fällen lagen ≥3 Kriterien vor, sodass hier ein metabolisches Syndrom diagnostiziert werden konnte (**Abb. 5a**). Die Verfettung der Leber stieg mit zunehmender Anzahl der Kriterien an (p < 0,001; **Abb. 5b**). Vergleicht man nun Mitarbeiter ohne und mit Fettleber, waren letztere älter (39 *vs.* 33 Jahre; p = 0,018), hatten häufiger einen BMI über 25 kg/m^2 (60,5 % *vs.* 16,7 %; p < 0,001), eine arterielle Hypertonie (15,5 % *vs.* 0 %; p = 0,001), TC-Konzentrationen über 200 mg/dl (46,9 % *vs.* 25,6 %; p = 0,031) und TG-Werte im Serum über 150 mg/dl (18,8 % *vs.* 3,6 %; p = 0,019; **Tab. 5**).

Quantifizierung der Leberfibrose

Die Lebersteifigkeit als quantitativer Parameter für die Fibrose der Leber lag im Mittel bei 4,6 ± 1,6 kPa (2,4–11,9). Keiner der Teilnehmer zeigte Werte über 13,0 kPa, die allgemein das Vorliegen einer Leberzirrhose anzeigen [11]. ALT und γ-GT korrelierten mit zunehmender Lebersteifigkeit (r_p = 0,239; p = 0,004 bzw. r_p = 0,268; p = 0,009). Bei 6 Mitarbeitern (5,8 %) lagen Werte ≥7,65 kPa vor, entsprechend einem Fibrosestadium ≥F2 (**Abb. 2b**). Diese Gruppe unterschied sich von der ohne relevante Fibrose (<7,65 kPa) durch einen höheren BMI (28,9 *vs.* 23,7 kg/m^2; p = 0,002) und höhere CAP-Werte (287 *vs.* 220 dB/m; p = 0,008).

Diskussion

Erstmals wurde in dieser Studie die nichtinvasive Quantifizierung der Fettleber im Rahmen der betrieblichen Gesundheitsförderung in Deutschland untersucht. Bei 36 % der Mitarbeiter des Universitätsklinikums wurde mittels CAP eine Fettleber diagnostiziert, ohne dass diese zuvor davon Kenntnis hatten. Bei einem Drittel dieser Mitarbeiter waren die Leberwerte im Serum normwertig. Die Bestimmung des CAP-Werts

Tab. 4 Lineare univariate und multivariate Analyse der Leberverfettung

	OR	95 % CI	*p*-Wert[a]
Univariate Analyse			
Alter (Jahre)	1,85	0,98–2,73	*<0,001*
BMI (kg/m^2)	9,30	6,96–11,64	*<0,001*
Geschlecht	–17,27	–44,15–9,57	n. s.
KFM (%)	3,41	2,25–4,58	*<0,001*
TU (cm)	3,84	3,02–4,65	*<0,001*
Multivariate Analyse			
KFM (%)	1,73	0,14–3,32	*0,033*
TU (cm)	3,79	2,00–5,58	*<0,001*

BMI Body Mass Index, *CI* Konfidenzintervall, *KFM* Körperfettmasse, *OR* Odds Ratio, *TU* Taillenumfang
[a]*p*-Wert mittels Fishers Exact Test ermittelt

Tab. 5 Zusammenhang zwischen CAP-Werten und kardiovaskulären Risikofaktoren

	Keine Fettleber (CAP <243 dB/m, *N* = 66, 63,5 %)	Fettleber (CAP ≥243 dB/m, *N* = 38, 36,5 %)	*p*-Wert
Alter (Jahre)	33	39	*0,018*
Arterielle Hypertonie	0 %	15,8 %	*0,001*
BMI (kg/m^2)	22,4	26,7	*<0,001*
Übergewicht (BMI ≥25 kg/m^2)	16,7 %	60,5 %	*<0,001*
TC (mg/dl)	183	199	n. s.
TC ≥200 mg/dl	25,6 %	46,9 %	*0,031*
Diabetes mellitus Typ 2	1,5 %	5,3 %	n. s.
TG (mg/dl)	89	119	*0,012*
TG ≥150 mg/dl	3,6 %	18,8 %	*0,019*

BMI Body-Mass-Index, *CAP* Controlled Attenuation Parameter, *TC* Cholesterin, *TG* Triglyzeride

ermöglicht die frühzeitige Diagnose eine Steatose, die zur NASH und Fibrose fortschreiten kann und daher eines Monitorings bedarf [15]. Das Sterblichkeitsrisiko bei diesen Patienten ist vor allem aufgrund von kardiovaskulären Erkrankungen erhöht [30], weshalb sowohl das weitere metabolische aber auch das hepatische Monitoring von Bedeutung sind. Simultan zur Leberverfettung wurde die Lebersteifigkeit quantifiziert. 6 % der Teilnehmer hatten in der Elastographie Hinweise für eine relevante Leberfibrose. Die Fibrose ist ein Prädiktor für leberspezifische Morbidität und Mortalität sowie Gesamtmortalität [2]. Bei 4 der 6 Patienten waren ALT und γ-GT normwertig, wodurch eine Lebererkrankung ohne bildgebende Verfahren übersehen worden wäre.

Diese Studie bei Mitarbeitern eines Universitätsklinikums zeigt, dass bei Vorliegen einer Fettleber kardiovaskuläre Risikofaktoren gehäuft beobachtet werden (Alter, BMI >25 kg/m^2, arterielle Hypertonie, Hyperlipoproteinämie). Mit steigendem CAP-Wert nahm die Zahl der Kriterien, die das metabolische Syndrom definieren, zu. Der BMI stieg mit der Leberverfettung an, hatte jedoch keinen unabhängigen Vorhersagewert für die Fettleber. Die am stärksten mit erhöhten CAP-Werten assoziierten Parameter waren die prozentuale Körperfettmasse und der Taillenumfang. Daher sollte die Bestimmung der Körperzusammensetzung durch die bioelektrische Impedanzanalyse und des Taillenumfangs mittels eines einfachen Maßbandes in der präventionsmedizinischen Praxis einen höheren Stellenwert erhalten. Bei 85 von 578 (15 %) chinesischen Mitarbeitern der Dienstleitungsindustrie wurde mittels Ultraschall eine Fettleber diagnostiziert. Der mittlere BMI und der Anteil erhöhter AST-Werte war mit unserer Kohorte vergleichbar (23,0 kg/m^2 und 5,4 % *vs.* 24,0 kg/m^2 und 7,6 %). Abweichungen gibt es hingegen beim Anteil der Mitarbeiter mit Fettleber, arterieller Hypertonie und erhöhten TG-Werten [9]. In einer Studie aus Taiwan wurden 1384 Mitarbeiter eines stromproduzierenden Unternehmens mittels Ultraschall untersucht: 27 % hatten eine Fettleber bei einer mittlere ALT von 27 U/l. Hierbei wurden Patienten mit chronischer Virushepatitis nicht ausgeschlossen, hingegen Arbeiter mit metabolischem Syndrom schon, wobei sich die verwendete Definition von der des International Diabetes Federation unterscheidet [17]. Ultraschall als Diagnosemethode ist jedoch fragwürdig bei einer Sensitivität von 43 oder 60 % für eine Leberverfettung von >2 oder >30 % [12]. Es gibt Unterschiede in der Körperzusammensetzung von Asiaten und Europäern: Europäer haben, verglichen mit Asiaten, weniger viszerales Fettgewebe bei gleichem Taillenumfang [15] und weniger Körperfett [13], sodass unterschiedliche anthropometrische Grenzwerte gelten. Die Vergleichbarkeit der Daten aus Asien ist daher nur begrenzt möglich. Weiterhin ist in unserer Studie der Frauenanteil höher und die körperlichen und die Umwelteinflüsse im Krankenhausumfeld unterscheiden sich von denen der Dienstleistungs- und stromproduzierenden Industrie. Im Jahr 2015 Betrug der Anteil der weiblichen Angestellten am Universitätsklinikum des Saarlandes 73 %, sodass die Ergebnisse der UKS-Studie, mit einem Frauenanteil von 74 %, repräsentativ sind.

Die transiente Elastographie und die bioelektrische Impedanzanalyse sind nichtinvasive und patientenfreundliche Methoden mit geringen Kosten, die jederzeit wiederholbar sind, durch Funktionspersonal durchgeführt werden und dadurch unabhängig vom ärztlichen Personal sind. Die Untersuchungsdauer der Leber betrug in dieser Studie durchschnittlich 2,7 min, und das Verfahren kann zu Recht als *EKG des Hepatologen* bezeichnet werden [4]. Die Teilnehmer wurden über auffällige Befunde informiert und auf die Notwendigkeit einer weiteren Abklärung hingewiesen. Bei Teilnehmern, die im Anschluss in

der Hochschulambulanz zur Ernährungsberatung vorstellig wurden, wurde der Therapieverlauf mittels transienter Elastographie und bioelektrischer Impedanzanalyse überwacht.

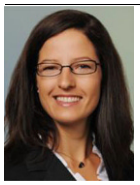
Die ermittelte Prävalenz für das metabolische Syndrom wird in unserer Kohorte aufgrund der fehlenden HDL-Werte bei einem Großteil der Teilnehmer unterschätzt. Ursächlich hierfür ist das Anforderungsverhalten der Arbeitsmediziner. Im Rahmen der betrieblichen Gesundheitsförderung konnte auch nicht gewährleistet werden, dass alle Teilnehmer mindestens 8 h nüchtern waren, sodass auch nicht durchgehend Nüchtern-Glukosewerte zur Verfügung standen.

Die Deutsche Leitlinie zu Fettlebererkrankung empfiehlt hinsichtlich der Therapie aus hepatologischer und kardiologischer Sicht eine Reduktion des Übergewichts und eine Steigerung der körperlichen Aktivität [23]. Der Arbeitsmediziner bzw. Betriebsarzt sollte darüber informieren sowie Beratungsgespräche über gesunde Ernährung und Bewegungsprogramme anbieten. Die Lotsenfunktion des Arbeitsmediziners bei aktuell 43 Mio. Beschäftigen hinsichtlich der Prävention von Lebererkrankungen wird durch die transiente Elastographie erleichtert und kann weiteren Beratungs- und Handlungsbedarf an der Schnittstelle zum Haus- und Facharzt anzeigen [16].

Fazit für die Praxis

- **Die Fettleberkrankheit ist initial meist symptomlos und geht nicht immer mit erhöhten Leberwerten einher, sodass neue Diagnoseverfahren benötigt werden.**
- **Die transiente Elastographie stellt ein nichtinvasives, risikofreies und schnelles Verfahren zur Beurteilung von Leberverfettung und Leberfibrose im Rahmen der betrieblichen Gesundheitsförderung dar.**
- **Die prozentuale Körperfettmasse und der Taillenumfang sind die stärksten Prädiktoren für die Leberverfettung.**
- **Die Befunde der transienten Elastographie und bioelektrischen Impedanzanalyse sind für den Mitarbeiter leicht zu verstehen und können zur Änderung des Lebensstils motivieren.**

Korrespondenzadresse



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Einhaltung ethischer Richtlinien

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Die beschriebenen Untersuchungen am Menschen wurden mit Zustimmung der zuständigen Ethik-Kommission, im Einklang mit nationalem Recht und gemäß der Deklaration von Helsinki von 1975 (in der aktuellen, überarbeiteten Fassung) durchgeführt. Von allen beteiligten Patienten liegt eine Einverständniserklärung vor.

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Appendix 4. Arslanow et al. Zeitschrift für Gastroenterologie 2016

Leserbrief „Zur Diagnose der Fettleberkrankheit“

A. Arslanow, F. Lammert

Die neue S2k-Leitlinie „Nicht-alkoholische Fettlebererkrankung“ der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten (DGVS) fasst den aktuellen Kenntnisstand zu Epidemiologie, Risikofaktoren, Diagnostik und Therapie zusammen [1]. Die Leitlinie definiert: „Die Steatosis hepatis (Fettleber) zeichnet sich durch eine Einlagerung von Fett in den Hepatozyten aus.“ Sie spricht explizit von „Patienten mit nicht-alkoholischer Fettleber (NAFL)“, die im Verlauf eine Steatohepatitis (NASH) und eine Zirrhose entwickeln können (S. 671). Diese Definitionen legen eine bimodale Verteilung, die die Bevölkerung in Patienten mit und Gesunde ohne Fetteber dichotomisiert, nahe. Der Krankheitsbegriff dient aber nicht nur zur Beschreibung, sondern auch dazu, ärztliche Handlungen und Aktionen anzumahnen [2, 3]. Nur als Randbemerkung sei eingefügt, dass im Englischen zudem streng „Disease“ (wie in NAFLD) von „Illness“ unterschieden wird: Der Mensch kann eine „Krankheit“ haben, ohne „erkrankt“ zu sein, so dass man streng genommen von „Nicht-alkoholischer Fettleberkrankheit“ sprechen müsste.

Die Diagnose der NAFLD wird heute mittels Serummarkern und daraus abgeleiteter Scoring-Systeme, bildgebender Verfahren (Ultraschall, Computertomografie, Magnetresonanzverfahren) und Leberbiopsie vorgenommen. Vor über 10 Jahren fand die Ultraschall-basierte Elastografie (z. B. Fibroscan®) Einzug in die Hepatologie. Diese nicht-invasive Methode wurde kürzlich durch den simultan ermittelten *Controlled Attenuation Parameter* (CAP) erweitert [4, 5]. Dieses Verfahren nutzt die dem Ultraschaller gut vertraute Schallabschwächung bei zunehmender Leberverfettung aus, die häufig zur semiquantitativen Beurteilung des Steatose-Grades herangezogen wird. Beim CAP-Verfahren wird die Schallabschwächung quantifiziert und als CAP-Wert in [dB/m] angegeben. Auch wenn dieses Verfahren keinen „Goldstandard“ darstellt, erlaubt es doch umfangreiche und longitudinale Verlaufsbeurteilungen der Fettleberkrankheit in bisher nicht dagewesenem Umfang. Am Universitätsklinikum des Saarlandes wurden im Zeitraum von Mai 2012 bis Dezember 2015 insgesamt 6814 Messungen mit dem Fibroscan® bei ambulanten Patienten der Inneren Medizin II (Schwerpunkte: Gastroenterologie, Hepatologie, Endokrinologie, Diabetologie und Ernährungsmedizin), bei stationären Patienten des gesamten Universitätsklinikums sowie im Rahmen von Studien an Patienten und Gesunden durchgeführt. Nach Abzug von 132 Test- und Trainingsmessungen berichten wir hier von 6682 Messungen. Bei einer medianen Untersuchungsdauer von 95 sec (Interquartilsabstand (IQR) 69 – 158 sec) wurde für die Kohorte ein medianer CAP-Wert von 245 dB/m (IQR 202 – 295 dB/m) dokumentiert; die Lebersteifigkeit als Maß für die Leberfibrose lag bei 7,3 kPa (IQR 5,2 – 12,2 kPa; **Abb. 1A, B**). Lange Zeit war die Erfassung des CAP-Werts auf den Einsatz der „M-Sonde“ des Fibroscan beschränkt; seit Kurzem kann dieser Parameter auch durch die „XL-Sonde“ erfasst werden, sodass insgesamt für > 90 % der Messungen ein CAP-Wert dokumentiert werden konnte. Technisch bedingt werden CAP-Werte zwischen 100 und 400 dB/m ausgedrückt, so dass Patienten mit niedrigen oder hohen Werten in die Randklassen fallen.

Aus ärztlicher Sicht wesentlich ist, dass die CAP-Werte keine bimodale Verteilung haben: Es gibt also nicht Menschen mit oder ohne Fettleber, sondern nur Individuen mit mehr oder weniger viel Fett in der Leber. Leberfett lässt sich – wie der Blutdruck – metrisch und nicht nur ordinal messen. Der mediane CAP-Wert von 245 dB/m zeigt nach den publizierten Voruntersuchungen an, dass viele Teilnehmer an der Untersuchung bereits eine „Fettleber“ haben [6]. Letztlich ist es eine Frage der Krankheitsdefinition und langfristiger Studien mit harten Endpunkten (Überleben, Zirrhosekomplikationen), welchen Schwellenwert wir der Diagnose der Fettleber zugrunde legen. Interessant ist auch, dass nur wenige Patienten sehr niedrige oder sehr hohe CAP-Werte und demnach eine Leber mit sehr geringer oder sehr starker Fetteinlagerung haben. Die Verteilung insgesamt kann mit der einer Allgemeinbevölkerung verglichen werden, da die Kohorte in ihrer Ätiologie und Krankheitsschwere sehr heterogen aufgebaut ist.

Die S2k-Leitlinie [1] empfiehlt das Screening auf NAFLD nur bei Personen mit Risikofaktoren (Adipositas, Dyslipidämie, Diabetes mellitus, Medikamente). Hinsichtlich der genetischen Prädisposition weist sie darauf hin, dass Patienten mit einer homozygoten Variante im *PNPLA3*-Gen (p. I148 M) eine höhere Prävalenz für die Entstehung einer NAFLD aufweisen. Patienten mit NAFLD sind aber häufig asymptomatisch mit normwertigen Transaminasen. Die CAP-Methode erlaubt nicht nur ein Screening auf NAFLD in der Allgemeinbevölkerung, sondern auch kurzfristige Verlaufskontrollen unter der in der Leitlinie als einzige Therapie bei Fettleber empfohlenen Lebensstilmodifikation [1]. Weitere Interventionsstudien mit Monitoring des CAP-Werts werden benötigt, um Behandlungsziele

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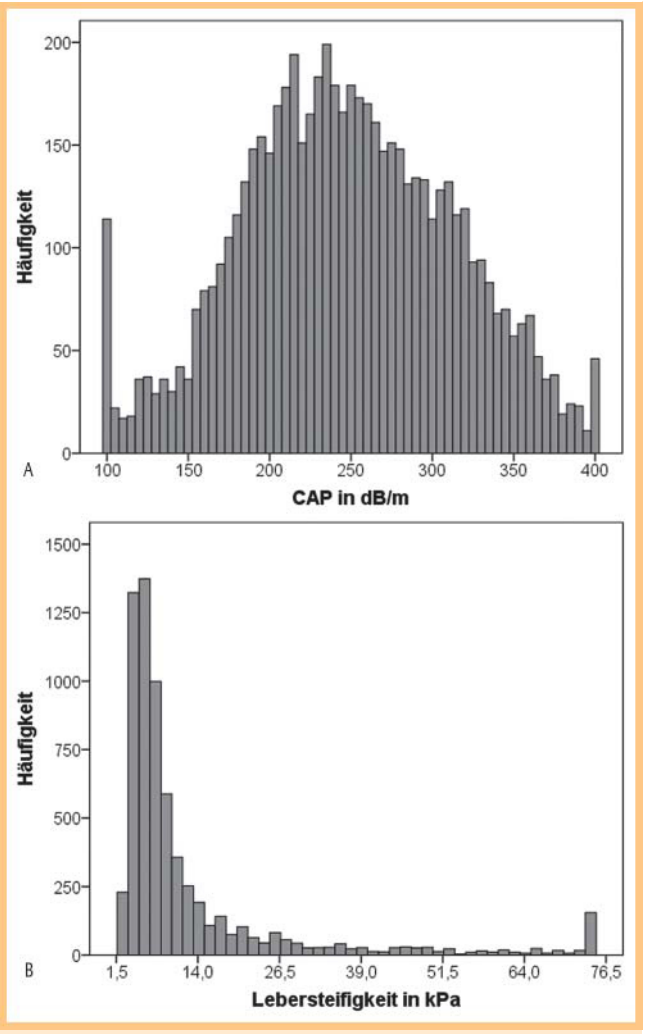

Abb. 1 Häufigkeitsverteilung der **A** CAP-Werte (N = 6099) und **B** Lebersteifigkeitswerte (N = 6682) mittels Ultraschall-basierter Elastographie (Fibroscan®) am Universitätsklinikum des Saarlandes.

zu definieren und letztlich ein „Treat-to-Target"-Konzept zu implementieren.

Die CAP-Messung zeichnet sich dadurch aus, dass sie patientenfreundlich und nicht invasiv ist. Die Durchführung durch Funktionspersonal, die kurze Messdauer und die geringen variablen Kosten zeichnen die Methode aus – sie kann daher mit Recht als „EKG des Hepatologen" bezeichnet werden.

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6 *Arslanow A, Stokes CS, Weber SN et al.* The common PNPLA3 variant p. I148M is associated with liver fat contents as quantified by controlled attenuation parameter (CAP). Liver Int 2016; 36: 418 – 426

Related Scientific Publications

Research prize "Clinical Gastroenterology" 2016

Awarded for „Short-Term Hypocaloric High-Fiber and High-Protein Diet Improves Hepatic Steatosis Assessed by Controlled Attenuation Parameter" (Anita Arslanow et al. *Clinical and Translational* Gastroenterology 2016 Jun 16;7(6):e176) by Gastroenterologische Arbeitsgemeinschaft Rheinland-Pfalz/ Saarland in Bad Kreuznach, Germany, October 2016

Publications independent of doctoral thesis

Anita Arslanow, Kaffee und die Leber – Ein Erfahrungsbericht vom 51. *International Liver Congress Der Gastroenterologe* 11:426-427

Małgorzata Jamka, Anita Arslanow, Annika Bohner, Marcin Krawczyk, Susanne N. Weber, Frank Grünhage, Frank Lammert, Caroline S. Stokes. Effects of Gene Variants Controlling Vitamin D Metabolism and Serum Levels on Hepatic Steatosis. *Digestion*. 2018 Mar 7;97(4):298-308 [Epub ahead of print]

Contribution at scientific conference by abstract submission

01/ 2014	**Poster** at German Association of the Study of the Liver, Tübingen, Germany
03/ 2014	**Poster prize** and **travel bursary** at Saarländisch-Pfälzische Internistengesellschaft, Neustadt (Weinstraße), Germany
04/ 2014	**Poster** at International Liver Congress, London, United Kingdom
04/ 2014	**Poster** and **travel bursary** at Deutschen Gesellschaft für Innere Medizin, Wiesbaden, Germany
05/ 2014	**Presentation** at European Society for Clinical Investigation, Utrecht, Netherlands
09/ 2014	**Short presentation** at Deutsche Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten, Leipzig, Germany
11/ 2014	**Presentation** at Gastroenterologische Arbeitsgemeinschaft Rheinland-Pfalz/ Saarland , Bad Kreuznach, Germany
01/ 2015	**Poster** at German Association of the Study of the Liver, Munich, Germany
02/ 2015	**Poster** and **travel bursary** at Monothematic Conference of the European Association for the Study of the Liver, Innsbruck, Austria
03/ 2015	**Presentation** at Deutsche Gesellschaft für Ernährung, Halle, Germany
04/ 2015	**Poster prize** and **travel bursary** at International Liver Congress, Vienna, Austria

05/ 2015	**Short presentation** and **travel bursary** at Deutsche Diabetes Gesellschaft, Berlin, Germany
09/ 2015	**Short presentation** at Deutsche Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten, Leipzig, Germany
11/ 2015	**Poster** at American Association for the Study of Liver Diseases, San Francisco, USA
11/ 2015	**Presentation** and **poster** at Gastroenterologische Arbeitsgemeinschaft Rheinland-Pfalz/ Saarland, Bad Kreuznach, Germany
10/ 2015	**Poster prize** and **travel bursary** at Falk Symposium 200, Freiburg, Germany
01/ 2016	**Poster** at German Association of the Study of the Liver, Düsseldorf, Germany
03/ 2014	**Poster** at Saarländisch-Pfälzische Internistengesellschaft, Neustadt (Weinstraße), Germany
04/ 2016	**Poster** and **travel bursary** at Deutsche Gesellschaft für Innere Medizin, Mannheim, Germany
04/ 2016	**Poster** at International Liver Congress, Barcelona, Spain
06/ 2016	**Poster** and **travel bursary** at Monothematic Conference of the European Association for the Study of the Liver, Porto, Portugal
09/ 2016	**Short presentation** at Deutsche Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten, Leipzig, Germany
10/ 2016	**Poster** at Cytokines, San Francisco, USA
01/ 2017	**Poster** at German Association of the Study of the Liver, Essen, Germany
04/ 2017	**Poster** and **registration bursary** at International Liver Congress, Amsterdam, Netherlands
09/ 2017	**Short presentation** at Deutsche Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten , Dresden, Germany
11/ 2017	**Poster** at Gastroenterologische Arbeitsgemeinschaft Rheinland-Pfalz/ Saarland, Bad Kreuznach, Germany

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