Diploma thesis

Effect of thoracic diameter and food intake on FibroScan® results: Comparison of the S- and M-probes

submitted by

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Graz, 23.11.2020

Affidavit

I hereby declare that the following diploma thesis has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of this thesis other than those indicated in the thesis itself.

Graz, 23.11.2020 Iohanes-Lorean Negrean eh.

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Abbreviations

A

ALT *Alanine aminotransferase*

AP *Alkaline phosphatase*

APRI *Aspartate aminotransferase to platelet ratio index*

ARFI *Acoustic radiation force impulse imaging*

AST *Aspartate aminotransferase*

B

BMI *Body-Mass-Index*

C

CAP *Continuous attenuation parameter*

CT *Computed tomography*

E

ELF *Enhanced liver fibrosis (test)*

F

FS *FibroScan®*

G

GGT *Gamma glutamyl transferase*

H

HA *Hyaluronic acid*

HBV *Hepatitis-B virus*

HCV *Hepatitis-C virus*

HIV *Human immunodeficiency virus*

I

INR *International normalized ratio*

IQR *Interquartile range*

L

LS *Liver stiffness*

M

MRE *Magnetic resonance elastography*

MRI *Magnetic resonance imaging*

N

NAFLD *Non-alcoholic fatty liver disease*

P

PBC *Primary biliary cholangitis*

PIIINP *Procollagen III aminoterminal peptide*

PP *Peak-to-peak*

PSC *Primary sclerosing cholangitis*

PT *Prothrombin time*

R

RTE *Real-time tissue elastography*

S

SCD *Skin-capsule-distance*

T

TC *Thoracic circumference*

TE *Transient elastography*

TIMP-1 *Tissue inhibitor of metalloproteinases 1*

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Zusammenfassung

Einleitung: Der FibroScan® stellt ein Verfahren zur nicht-invasiven und schmerzfreien Erfassung der Lebersteifigkeit (LS) und damit des Grades an Fibrose/Zirrhose dar. Laut Empfehlung des Herstellers soll bei Patienten/innen mit einem Thoraxumfang (TC) zwischen 45 cm und 75 cm (TC<75 Gruppe) eine S-Sonde, bei Patienten/innen mit einem Thoraxumfang über 75 cm (TC>75 Gruppe) eine M-Sonde verwendet werden. Bei der Untersuchung sollten die Patienten/innen zudem stets nüchtern sein. Im Rahmen dieser Studie wurde der Einfluss des Thoraxumfangs und der Einfluss standardisierter Flüssignahrung auf die Lebersteifigkeit in einer Gruppe gesunder Erwachsener untersucht und die Messwerte zwischen der S- und M-Sonde verglichen.

Methoden: Gesunde Erwachsene wurden in nüchternem Zustand sowie 30, 60 und 120min, nach Einnahme einer standardisierten Flüssignahrung (200 ml, 300kcal) mit der S- und M-Sonde vermessen. Lebersteifigkeit und Controlled Attenuation Parameter (CAP) wurden erfasst.

Ergebnisse: 50 gesunde Erwachsene (26 weiblich, 24±3 Jahre), 22 mit einem TC<75 cm und 28 mit einem TC>75 cm (Bereich 58-99 cm), wurden inkludiert. Die Lebersteifigkeit war in der TC>75 Gruppe signifikant höher. Der Vergleich der Messwerte der S- und M-Sonde zeigte eine exzellente Übereinstimmung mit minimalem Unterschied der verschiedenen Tests (Spearman-Korrelation $r=0.754$, $p<0.001$, Bland-Altman Bias 0.6 +/- 0.9 kPa, lineare Regression $r^2=0.557$, $p<0.001$). Nach Einnahme der Flüssignahrung wurden keine Veränderungen zwischen den Sonden im Vergleich zum Nüchtern-Wert festgestellt. Bei den Erwachsenen der TC>75-Gruppe, gab es keine Veränderungen des CAP-Werts nach Nahrungsaufnahme, in der TC<75 Gruppe sank er nach 120min jedoch leicht.

Diskussion: Die S- und M-Sonde zeigen, sowohl bei der TC<75- als auch bei der TC>75-Gruppe, eine gute Übereinstimmung was schlanke, gesunde Erwachsene angeht. Die standardisierte Flüssignahrung hatte keinen relevanten Einfluss auf die Lebersteifigkeit. Zusammenfassend lässt sich sagen, dass sowohl die S- als auch die M-Sonde bei schlanken, gesunden Erwachsenen unabhängig vom Thoraxumfang eingesetzt werden kann.

Abstract

Background & Aims: Fibroscan® (Echosens, France) to assess liver stiffness (LS) non-invasively and painlessly is commonly used to determine the degree of fibrosis/cirrhosis. The manufacturer proposes to use the S-probe in patients with a thoracic circumference (TC) between 45 cm and 75 cm (TC<75 group) and the M-probe for patients above 75 cm (TC>75 group), always in fasting condition. We compared the influence of TC as well as the impact of a standardized liquid meal on the LS of the S- and M-probe in healthy volunteers.

Methods: Healthy volunteers were assessed with the S- and the M-probe in fasted state and 30, 60 and 120 minutes after a standardized meal (200 ml Fresubin Energy Drink, Fresenius-Kabi, Germany). Liver stiffness and controlled attenuation parameter (CAP) were assessed.

Results: 50 heathy volunteers (26 female, 24+/-3 years) 22 with a TC <75 and 28 with a TC >75 (range 58-99cm) were included. The TC<75 group was mainly female (n=19, $p<0.001$) with lower BMI ($p<0.001$). LS measurements were significantly higher in the TC>75 group. Comparison of the measurements obtained with the S-probe and the M-probe showed excellent agreement with minimal bias on various tests (Spearman correlation $r=0.754$, $p<0.001$, Bland-Altman bias 0.6 +/- 0.9 kPa, linear regression $r^2=0.557$, $p<0.001$). After a standardized meal, no changes in LS were observed compared to baseline in any group. In volunteers with TC>75 CAP remained unchanged after the meal, but decreased slightly in the TC<75 group after 120min.

Discussion: The S- and M-probe show good agreement in non-obese healthy adults both in adults below and above 75 cm TC and the intake of the standardized meal did not have a relevant influence on LS. In conclusion the probes may be used interchangeably in non-obese healthy adults.

1 Introduction

1.1 Incidence of chronic liver diseases and the importance of fibrosis

The incidence of chronic liver diseases is increasing, especially in first world countries. Chronic liver diseases and their complications are a major cause of death and currently ranked on the 4th place in terms of life years lost due to chronic diseases. The causes for such diseases are diverse and include mostly infectious, genetic or autoimmune matters. Typical risk factors are obesity, metabolic syndrome, excess of alcohol consumption and an unhealthy lifestyle. Hepatitis B, C, autoimmune hepatitis, alcohol-related liver disease as well as non-alcoholic fatty liver disease (NAFLD) belong to chronic liver diseases.(4)

Table 1 shows chronic liver diseases and their percentage in liver cirrhosis.

Chronic Liver Diseases

Chronic Liver Diseases	Percentage in liver cirrhosis	Reference
Alcohol-related liver disease	About 50-55%	(5, 6)
Viral Hepatitis (B, C)	30-40%	(5-7)
Non-alcoholic fatty liver disease	15-25%	(6, 8)
Autoimmune hepatitis, primary sclerotic cholangitis, etc.	<5%	(6, 9)

Table 1: Chronic liver diseases and their percentage in liver cirrhosis.

Chronic liver diseases lead to histological changes characterized by deposition of fibrous tissue in the liver, which is called fibrosis. The endpoint of fibrosis is liver cirrhosis, which is the progression of fibrosis over time and the loss of cell architecture. Cirrhosis is associated with high mortality and high risk of liver decompensation, liver failure and a reduced synthesis capacity of the liver.(10) Because of this, the synthesis of proteins, peptides and hormones is reduced. Increased vascular portocaval resistance in the liver leads to portal hypertension, ascites and varices. Complications like spontaneous bacterial peritonitis, variceal bleeding and/or hepatocellular carcinoma can develop, ultimately causing death.(4) The detection of early stage fibrosis is really important for discovering and treating chronic liver diseases. Therefore, new diagnostic methods and devices are constantly developed. The focus is on non-invasive and painless methods.

This thesis will discuss one of these non-invasive methods – Transient Elastography (TE). TE is the basis of the FibroScan® (FS) (Echosens, Paris, France), a device used for grading and evaluating the degree of liver fibrosis.(11)

1.2 Liver biopsy

Currently liver biopsy still is the gold standard procedure in determining the degree of fibrosis in a patient suffering from chronic liver diseases. It is used for diagnosing liver diseases and staging fibrosis.

1.2.1 Different methods of liver biopsy & examination process

The most widely used method is the transthoracic percutaneous liver biopsy, which is carried out using a suction needle under ultrasound guidance and with a skin anesthetic (usually lidocaine 1%) applied beforehand. Other methods include the transvenous biopsy and the laparoscopic biopsy. In the transthoracic percutaneous approach suction-needles (Menghini needles) are used. Core-aspiration needles (Jamshidi

needles) or sheathed cutting needles are used as alternatives. The suction in the Menghini needle is generated by a syringe and a flat needle tip is used to acquire a liver tissue specimen. A specimen of about 10-15 cm is acquired and later analyzed histologically by pathologists using the METAVIR-Score.(12, 13) In the transvenous biopsy approach the liver tissue specimen is acquired by accessing the liver via the jugular or femoral veins.(14) In the laparoscopic biopsy approach the liver tissue specimen is acquired surgically under direct vision of the liver.(13) Prior to a liver biopsy it is recommended by experts to carry out an imaging of the liver with cross-sectional imaging devices or ultrasound as well as fasting of the patient for at least the night prior to the biopsy. Furthermore, antiplatelet and anticoagulation medications have to be discontinued up to 10 days prior to the liver biopsy. Heparin and heparin-related products should be discontinued 12 to 24 hours prior to the liver biopsy. Oral antidiabetic medications can be continued; however, insulin may have to be adjusted during and after the biopsy. (13, 15) After the biopsy the patient is under close observation for 3 hours and vital parameters (blood pressure and heart rate) are obtained every 15 min. In the absence of complications the patient can be discharged. (13)

1.2.2 Contraindications and complications

Many contraindications for liver biopsy depend on the operator and the patient's cooperation. Uncooperative behavior of the patient may result in wrong positioning or wrong breath-holding during the biopsy. In these situations deep sedation or general anesthesia can be useful. Moderate and massive ascites can cause complications by interfering with the biopsy needle while acquiring the liver tissue specimen. (13, 16) The biopsy of liver vascular lesions should generally be avoided and malignant lesions should be biopsied only under imaging guidance and with care as tumor vessels have an increased risk of bleeding.(13, 17) Impaired hemostasis is a contraindication, which is assessed using different parameters like international normalized ratio (INR),

prothrombin time (PT), platelet count, whole-blood clotting time and platelet function.(13, 18, 19)

There is a wide spectrum of possible complications due to liver biopsies. The complications are influenced by a variety of different factors like patient cooperation, coagulation status, operator experience, use of imaging, method used, needle diameter and needle type.(20, 21) Complications vary from pain and bleeding over organ perforation, hemobilia, neuralgia, pneumo- or hematothorax, peritonitis to ventricular arrhythmia and can ultimately result in death. Complications occur in 0.14 to 0.29% of all patients undergoing a liver biopsy.(22)

Table 2 shows the most common complications of a liver biopsy and their prevalence.

Complications of liver biopsy

Complication	Percentage	Reference
Pain	84%	(21)
Bleeding	0.7%	(23)
Infection	0.6%	(24)
Death	9/100.000	(25)

Table 2: Most common complications in liver biopsy in percent.

1.2.3 Histological analysis

The liver tissue specimen acquired by the biopsy is analyzed by pathologists using the METAVIR-Score to grade the degree of fibrosis and the activity of inflammation thereby determining the severity of the chronic liver disease. Under the microscope the specimen is examined for hallmarks of liver fibrosis and damage. The most important hallmarks are focal lobular necrosis, portal inflammation, piecemeal necrosis and

bridging necrosis. The findings are evaluated and assigned to a two-letter and two-number code by the pathologist depending on how severe the specimen looks. The letters used are A (Activity) and F (Fibrosis) and the numbers range from 0 to 3 for activity and from 0 to 4 for fibrosis (F0/A0=no fibrosis/no activity, F1/A1= portal fibrosis without septa/mild activity, F2/A2= portal fibrosis with rare septa/moderate activity, F3/A3= numerous septa without cirrhosis/severe activity, F4= cirrhosis) *(Figure 1, 2, 3 & 4)*.* (26) The METAVIR-Score is mostly used in patients with hepatitis C, but lately it has also been used in hepatitis B, NAFLD and autoimmune hepatitis.(26, 27)

Table 3 shows an overview of the METAVIR-Score.

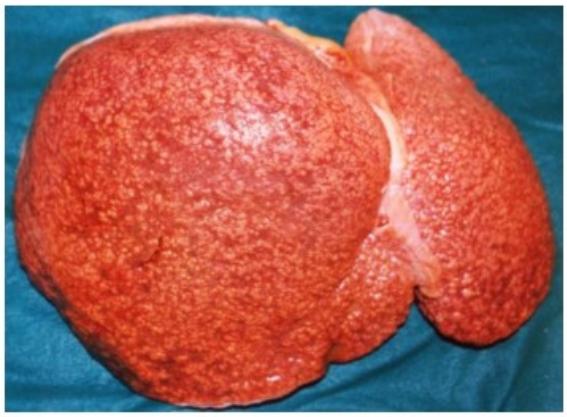


Figure 1: Macrosyopy of liver cirrhosis

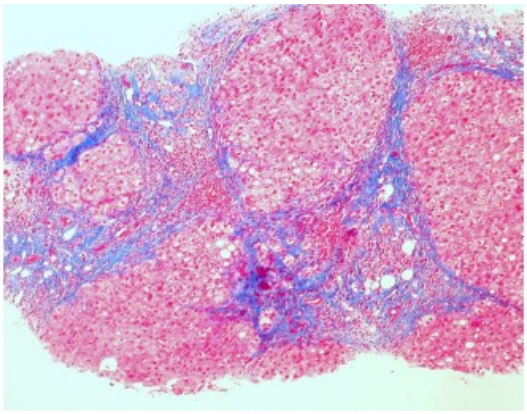


Figure 2: Histology of liver cirrhosis (METAVIR-Score F4)- parenchyma nodules in red and fibrous septa in blue

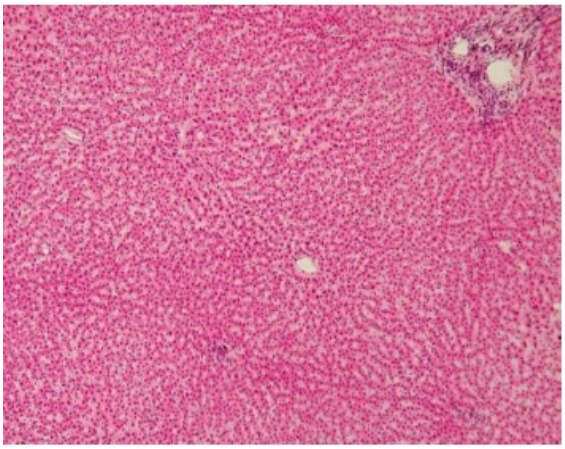


Figure 3: Normal liver tissue

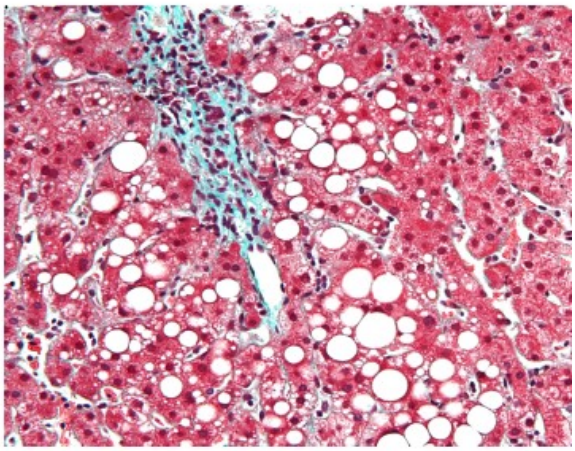


Figure 4: Histology of steatosis hepatis

**Figures 1, 2, 3 and 4 from commons.wikimedia.org*

METAVIR-Score

F - Fibrosis		A – Activity	
METAVIR - Score	**Histology**	**METAVIR - Score**	**Inflammation**
F0	No fibrosis	A0	No activity
F1	Portal fibrosis without septa	A1	Mild activity
F2	Portal fibrosis with rare septa	A2	Moderate activity
F3	Numerous septa without cirrhosis	A3	Severe activity
F4	cirrhosis	------	------

Table 3: METAVIR-Score code and corresponding meaning for Fibrosis and Activity.

1.3 Non-invasive procedures

Recently non-invasive procedures have been researched as an alternative for liver biopsy. Different methods and devices with an equally high accuracy were developed.(28) Notably, a method called transient elastography (TE) is nowadays widely used for diagnosing chronic liver diseases. Other non-invasive methods are tests and scores like the FibroTest and the Enhanced liver fibrosis (ELF) test or the FIB-4-Index (Fibrosis-4-Index).(29, 30) Conventional cross-sectional imaging methods like magnetic resonance imaging (MRI) or computed tomography (CT) are insensitive for diagnosing cirrhosis/fibrosis, because they are unable to detect typical cirrhosis-signs like nodules or the quality of the liver surface.(31)

1.3.1 Transient elastography, liver stiffness and continuous attenuation parameter

TE is an ultrasound-based, non-invasive method used to quantify liver fibrosis. The degree of liver fibrosis is quantified using a TE-utilizing device like the FibroScan® (FS) (Echosens, Paris, France), which measures the stiffness of the liver tissue (liver stiffness, LS).(11)

LS represents the resistance of the liver tissue during deformation and is expressed in kilopascal (kPa). Physically, stiffness is defined as a modulus of elasticity. Hooke's law of elasticity ($\sigma = E^*\varepsilon$, σ – applied stress, E – stiffness, ε – induced strain) states that the extension of a material is proportional to the applied stress on it. Following Hooke's law of elasticity, stiff materials react with low strain even if a lot of stress is applied and soft materials react with high strain even at low stress. Therefore, stiffer materials have less deformation and softer materials have more deformation if the same stress is applied. Thus, LS is higher in patients with fibrosis or cirrhosis, because fibrosis and cirrhosis make the liver tissue stiffer and therefore less deformable leading to higher LS.(32) LS depends on many factors. The main factor is the extracellular matrix of the liver, because it physically is a deformable structure which transfers external physical forces like ultrasound waves through the liver. Furthermore, the applied pressure on the liver surface by the operator holding the FS-probe also influences LS. The more pressure there is applied, the stiffer the liver will appear and the higher LS will be. Therefore, the FS displays the amount of pressure applied on its screen. Furthermore, the internal pressure of the liver due to blood flowing through the organ, causes LS to rise.(33) This is because increased blood flow induces an increase of resistance in the liver. LS also depends on the frequency of the shear waves passing the liver tissue. LS is higher at higher frequencies – responsible for this dependency is the viscous effect, which is influenced by frequency as well. LS is calculated through the velocity of reflected shear waves.(32)

Besides measuring LS, TE is also used for quantifying steatosis by measuring the continuous attenuation parameter (CAP). CAP is measured using the FibroScan ® and is expressed in dB/m (Decibel per meter).(34) CAP has high accuracy for non-alcoholic fatty liver disease and alcoholic liver disease and can assess steatosis better than body mass index (BMI) or waist circumference. (30, 31)(35) Steatosis (deposition of fat tissue in the cells of the liver) is physically shown to increase the attenuation of ultrasound waves and thereby decrease their intensity.(36)

CAP is derived from the formula of ultrasound intensity attenuation ($I_z = I_0 e^{-\alpha f z}$; I_z – resulting ultrasound intensity in W/m^2, I_0 – initial ultrasound intensity in W/m^2, α_f – ultrasound attenuation coefficient in dB/m, z – depth in meters).(36) α_f is mainly influenced by the frequency of the ultrasound wave and by the composition of the conducting organ (liver tissue). CAP is an ultrasound attenuation parameter (α_f) with a fixed frequency (usually 3.5 Megahertz, MHz) and is therefore only affected by the degree of steatosis.(36) Therefore, the higher the degree of steatosis, the higher the CAP-value will be with a physiological range from 100 dB/m to 400 dB/m.(36) Further advantages of CAP compared to invasive methods like liver biopsy, are that CAP can be obtained at the same time as LS and from the same region of interest since both parameters are measured using FS-probes.(36)

1.3.2 Measuring LS and CAP using FibroScan®

The FibroScan 502 Touch (FS) with software version C 3.2 (Echosens, Paris, France) is the most widely used device for assessing LS and CAP. The FS consists of a main body with a screen and different attachable probes. *(Figure 5)* Furthermore, a single channel ultrasound analog end for emitting and receiving reflected ultrasound signals and a shear wave generator are embedded in its main body.(32)

Figure 5: The FS 502 Touch with probes, as it was used for the study. (own picture)

The probes have different sizes and physical attributes. There is an S (small)-, M (medium)-, and XL (extra-large)-probe. *(Figure 6 & 7)* All probes can measure LS, only the M- and XL- probe can measure CAP. S-, M-, and XL-probes have a center frequency of 5 MHz, 5 MHz and 3.5 MHz, respectively. The diameter of the probe tip is 5 mm for the S-probe, 9 mm for the M-probe and 12 mm for the XL-probe.(37, 38)

Figure 6: From left to right – S-, M- and XL-probes. (own picture)

Figure 7: Probe tips – S-probe (top), M-probe (bottom right) and XL-probe (bottom left). (own picture)

LS is measured by holding the probe perpendicularly above the liver in an intercostal space on the right flank with the patient lying in supine position. The probes consist of an ultrasound transducer mounted on top of a vibrator axis emitting an ultrasound pulse in the form of a shear wave. A shear wave is a wave with sinusoid period and 50 Hertz (Hz) center frequency.(32) *(Figure 8)*

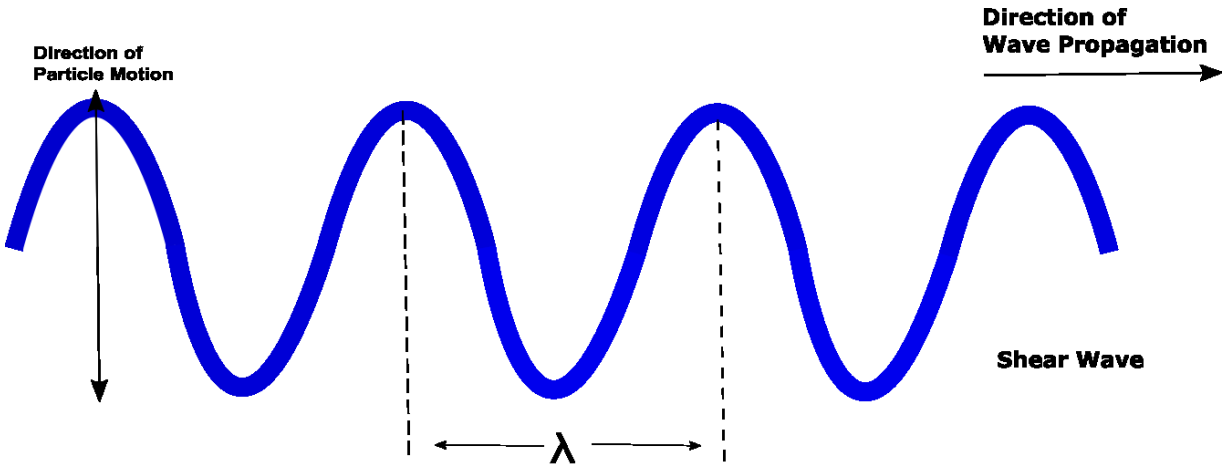


Figure 8: Illustration of a shear wave (center frequency of 50 Hz and a sinusoid period) that is typically generated by the FS. (own picture)

The amplitude varies depending on the probe and its peak-to-peak (PP). PP is the difference between the maximum and the minimum of a wave's positive and negative amplitude.(31) *(Figure 9)*

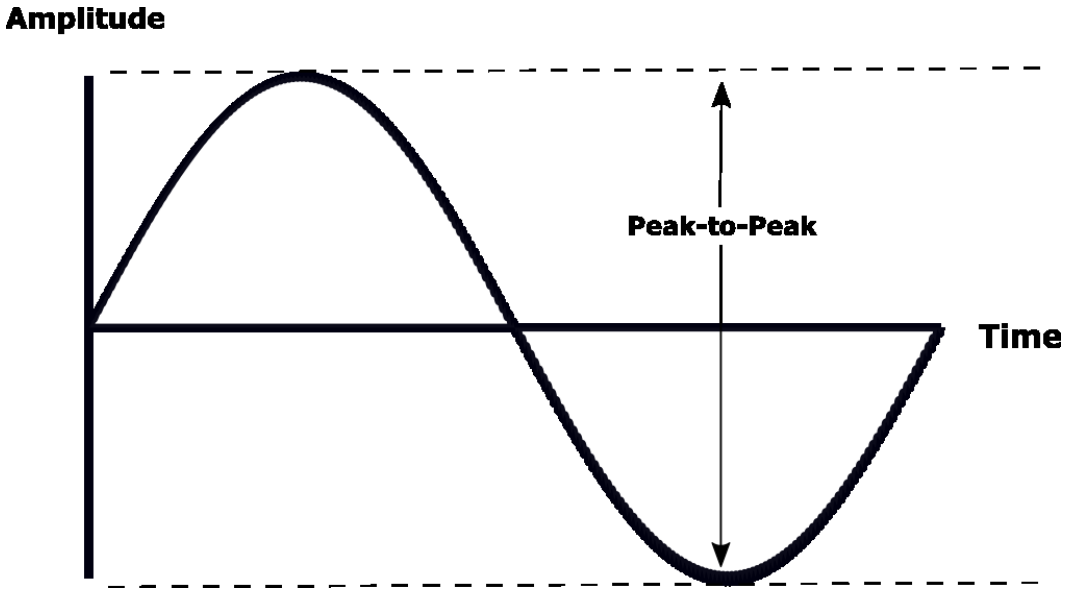


Figure 9: PP is the difference of the maximum and minimum of an amplitude over a certain time.

The PP is 1 mm in the S-probe, 2 mm in the M-probe and 3 mm in the XL-probe. Considering the relation of $\lambda = c/f$ (λ - wave length, c – wave velocity, f – frequency) this yields the shortest penetration depth for the S-probe with 20 mm to 50 mm and the largest for the XL-probe with 35 mm to 75 mm. The M-probe has a penetration depth of 25 mm to 65 mm. Penetration depth is the distance the ultrasound wave can travel in the body.(32, 37, 38)

All physical and technical characteristics of each probe are shown in Table 4.

Physical and technical characteristics of FS-probes

	S-Probe	M-Probe	XL-Probe	References
CAP measurable?	No	Yes	Yes	(39)
Center frequency (in MHz)	5	5	3.5	(37, 38)
Tip diameter (in mm)	5	9	12	(37, 38)
Recommended patient (in years)	<18	>18	>18	(39)
Recommended TC (in cm)	<75	>75	>75 and a Skin-capsule-distance >2.5 cm	(39)
Penetration depth (in mm)	20 to 50	25 to 65	35 to 75	(37, 38)

Table 4: Physical and technical characteristics of the FS- S-, M- and XL-probe.

The ultrasound shear wave is generated in the FS-probe and emitted into the liver over the probe tip. The LS is calculated using the velocity of the reflected wave and displayed on the screen. *(Figure 10)* The formula used is E = 3 ρV2 (E - stiffness, ρ - density, V - shear wave velocity).(32) The FS-screen consists of an ultrasound motion-mode and amplitude mode (M-mode and A-mode) imaging. The ultrasound imaging modes are used for liver localization and avoiding large vessels. Alternatively a brightness-mode (B-Mode) ultrasound device can be used beforehand to localize the liver. An elastogram is displayed as a time-depth function and is used to represent the induced strain in the liver tissue by shear wave deformation.*(Figure 10)* (32) LS is displayed as

the median, interquartile range (IQR) and interquartile range/median (IQR/Med in percent) in orange. *(Figure 10)* CAP is displayed as the median and IQR in blue. *(Figure 10)*

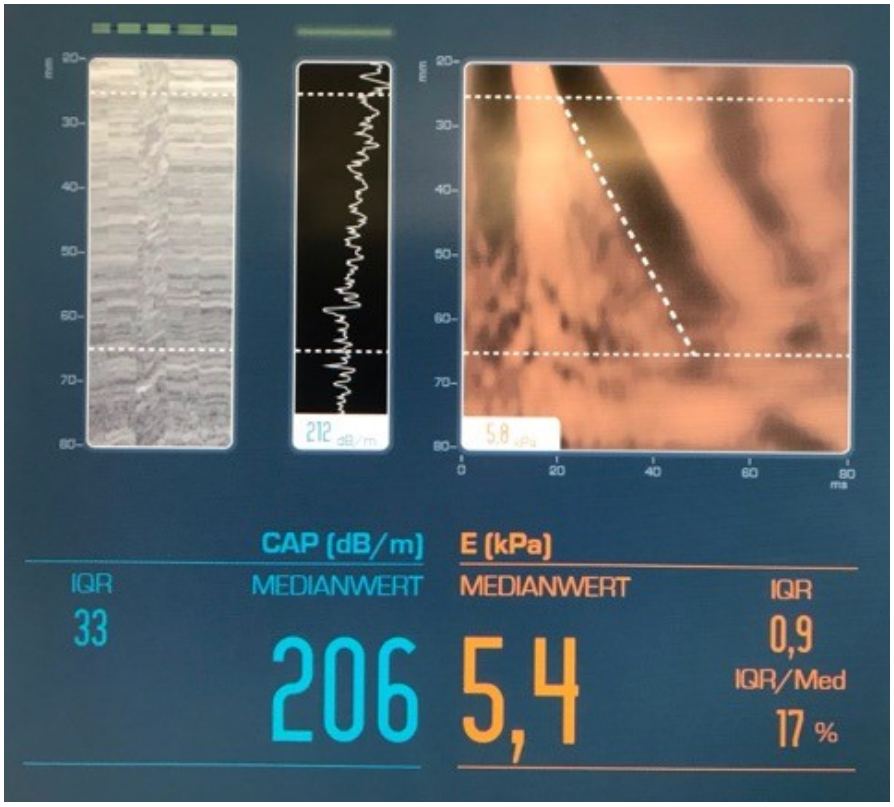


Figure 10: From top left to top right – motion-mode picture – amplitude-mode picture - elastogram. The CAP-value in blue and the LS in orange.

1.3.3 Cut-offs for patients and healthy subjects

LS cut-off values are different for patients with chronic liver diseases and for healthy adults. For patients with chronic liver diseases the right cut-off-value depends on the specific disease and its pathology and is displayed as the median liver stiffness measured in the respective group. As assessed by multiple studies, cut-offs for significant fibrosis (METAVIR-Score of F2) and cirrhosis (METAVIR-Score of F4) are around 7.9 kPa and 14 kPa respectively. Cut-offs for other common chronic liver

diseases are listed in Table 5. (11, 32, 56, 57) For healthy adults the normal LS cut-off is around 6.0 kPa as assessed by Colombo *et al.* (57)

LS cut-off values

	Cut-off value (kPa)		Reference
Healthy	**6.0 kPa**		**(40)**
Chronic liver disease	**Fibrosis (F2)**	**Cirrhosis (F4)**	**-----**
HBV	7.2 kPa	11.0 kPa	(44)
HCV	7.1 kPa	9.5 kPa	(41)
NAFLD	7.8 kPa	26.9 kPa	(42)
PBC & PSC	7.3 kPa	17.3 kPa	(45)

Table 5: LS cut-off values for the most common chronic liver diseases.

1.3.4 Other TE-based devices

TE is used in much more devices to assess stiffness and fibrosis.

The magnetic resonance elastography (MRE) uses TE in combination with magnetic resonance imaging to assess the stiffness of various organs. The accuracy of MRE is similar to FS; however, MRE has drawbacks like high-cost, high-effort and unfeasibility in patients with metal implants.(46)

Real-time elastography (RTE) is another method for assessing LS and fibrosis. It uses static elastography and B-mode-ultrasound imaging to measure and display LS. RTE is often part of conventional ultrasound probes and delivers a colour-coded image

(blue=hard tissue, red=soft tissue). In comparison to FS, RTE is slightly inferior in terms of fibrosis staging. *(Figure 11)* (3)

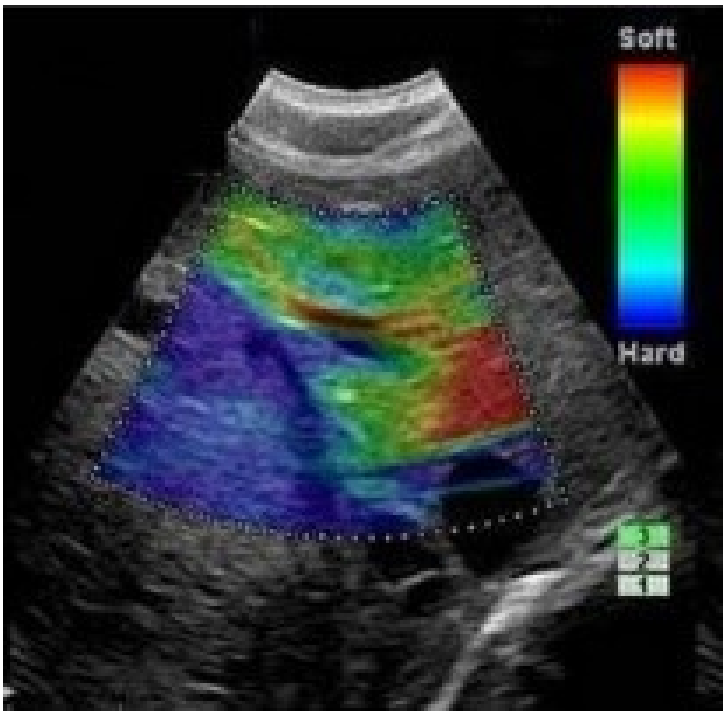


Figure 11: RTE of the liver. The colour-coded image shows the tissue elasticity distribution overlayed on the conventional B-mode ultrasound image. [from hindawi.com]

Acoustic radiation force impulse elastography (ARFI) is a new method for staging fibrosis. ARFI uses high-intensity ultrasound beams inducing tissue displacement of several micro-meters. This tissue displacement causes transverse waves traveling through-out the tissue with a certain velocity. The velocity of these transverse waves is measured via ultrasound and LS is calculated. The higher the velocity the stiffer the tissue. Studies show good correlation with FS, but the accuracy of FS is better especially in the fibrosis stages F2-4. (30, 47)

Table 6 shows an overview of the mentioned non-invasive devices.

Non-invasive devices for measuring LS

Name of the device	Method used for assessing fibrosis	Reference
Fibroscan 502 Touch® (FS)	Transient Elastography (TE)	(11)
Magnetic Resonance Elastography (MRE)	TE in addition to magnetic resonance of a MRI	(46)
Real-Time Elastography (RTE)	Static elastography implemented in conventional ultrasound probes	(3)
Acoustic Radiation Force Impulse Elastography (ARFI)	High-intensity US-beams causing tissue displacement which is measured via US	(47)

Table 6: Different TE-based devices/procedures and their operating principles to assess the degree of fibrosis/cirrhosis.

1.3.5 Fibrosis scores and tests

Other forms of fibrosis and cirrhosis assessment tools are scores and tests. Scores and tests are non-invasive methods using serum- and biomarkers to assess the degree of fibrosis.

FibroTest® (Biopredictive, Paris, France) is a score based on five different biomarkers and is used for detecting inflammatory activity and grading fibrosis. The biomarkers used are: Gamma- glutamyltransferase (GGT), total bilirubin, haptoglobin, alpha-2-macroglobulin and apolipoprotein A1. FibroTest® is often used in patients with HCV, HBV, and NAFLD and in patients with HIV-co-infection.(29, 48, 49)

The Enhanced Liver Fibrosis Score® (ELF) (Siemens Healthcare, Erlangen, Germany) is a score based on different biomarkers and is used for fibrosis staging. The three markers used are: Tissue inhibitor of metalloproteinases 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP) and hyaluronic acid (HA). ELF is especially accurate in stage F3 and F4 of fibrosis.(50, 51)

Fibrometer® (Echosens, Paris, France) is a score used in HCV patients for fibrosis grading. The biomarkers used are: Prothrombin index, aspartate aminotransferase (AST), alpha-2-macroglobulin, hyaluronate, urea as well as age and platelet count.(50)

Aspartate-aminotransferase to platelet ratio index (APRI) uses AST and the platelet count to determine different stages of fibrosis. It offers high accuracy and separation capacity (about 85%) and is often combined with other tests and scores like ELF or Fibrometer® to further its accuracy and reliability.(52)

The Fibrosis-4-Index (FIB-4) consists of age, aspartate- and alanine-aminotransferase levels and platelet count. It is used in the detection of liver fibrosis in HCV, NAFLD and HIV-co-infection patients, but can be used in almost all chronic liver diseases.(53)

NAFLD Fibrosis Score (NFS) is only used in NAFLD-patients and consists of age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio.(54)

An overview of the markers discussed is given in Table 7.

Fibrosis tests and scores

Test name	Markers/Parameters used	References
FibroTest®	Gamma-GT, total bilirubin, haptoglobin, alpha-2-macroglobulin and apolipoprotein A1	(29)
Enhanced Liver Fibrosis Score® (ELF)	Tissue inhibitor of metalloproteinases 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP) and hyaluronic acid (HA).	(30)
Fibrometer®	Prothrombin index, AST, a-2-macroglobulin, urea, age, platelet count	(55)
Aspartate aminotransferase to platelet ratio (APRI)	AST, platelet count, sex	(52)
Fibrosis-4-Index (FIB-4)	Aspartate- and alanine-aminotransferase levels, age, platelet count	(53)
NAFLD Fibrosis Score (NFS)	Age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio	(54)

Table 7: Tests and scores and their constituting markers.

1.4 Comparison of invasive and non-invasive methods

1.4.1 Advantages and disadvantages of liver biopsy

Advantages of liver biopsies are the amount of diagnostic information obtainable. A liver biopsy delivers information for staging of different chronic liver diseases and information for therapy planning that is very reliable and accurate.(13) However, there are many disadvantages. Liver biopsy is invasive; therefore has complications that range from pain to life-threatening bleeding and death. Furthermore, liver biopsies require preparation and training. The preparation includes patient examination,

considerations about his/her medications and diseases, an aseptic environment, assistance, sometimes general anesthesia and hours of observation after the biopsy. The accuracy of liver biopsies can be lowered by sampling errors if the acquired specimen are too few or too small in size.(13)

1.4.2 Advantages and disadvantages of TE and FibroScan®

Advantages of FS are the easy use, bed-side applicability and accuracy. FS is non-invasive and causes no complications or risks. It requires less training, preparation and precaution than liver biopsies and is easily repeatable. The accuracy of FS is comparable with liver biopsy regarding fibrosis staging, but lacks data to support its use with therapy decisions.(28, 32) The disadvantages of FS are the low reproducibility in early stages of fibrosis and in non-fasted patients regarding LS-values as well as a low sensitivity in mild steatosis regarding CAP-values. (34) (56) Furthermore, food intake influences FS-measurements in some chronic liver diseases. However, in this study no relevant influence of food intake on LS in non-obese healthy adults could be shown, especially if the recommended probe regarding thoracic circumference (TC) has been used. CAP did not change after meal intake in volunteers with a TC above 75 cm, but decreased after 2 hours in volunteers with a TC below 75 cm.(32, 57)

Overall, FS has very high accuracy, reliability and usefulness in most chronic liver diseases like hepatitis C (35), metabolic steatohepatitis (36), hepatitis B (37), alcoholic liver disease (38) and different biliary diseases. (39) FS rivals and sometimes tops the accuracy and precision of liver biopsy with the advantages of being non-invasive. (32)

Table 8 gives an overview of the comparison between liver biopsy and FS.

Comparison: Liver biopsy vs. FS

Comparison of liver biopsy to FS	Liver biopsy	FS	References
Invasive	Yes	No	(13, 32)
Complications (like pain, bleeding, death)	Yes	No	(22, 31)
Use for staging of liver diseases	Yes	Yes	(13, 32)
Use for therapy planning	Yes	No	(13, 32)
High accuracy and precision	Yes	Yes	(28, 58, 59)
Easy to repeat	No	Yes	(13, 32)
Lots of preparation and training needed	Yes	No	(13, 32)

Table 8: Direct comparison of Liver Biopsy and FS – major factors answered by yes/no-questions.

1.4.3 Advantages and disadvantages of scores and tests

Scores and tests are widely available and in comparison to TE or liver biopsy involve relatively small costs. However, not all commercially available scores and tests are cheap. Tests like the Fibrometer® and FibroTest® cost around 88€ and 111€ per sample.(60) Furthermore, an annually performed ELF-score has a higher total cost (determined via a Markov model) than liver biopsy, but it also offers a better incremental cost-effectiveness ratio per quality-adjusted life years than the liver biopsy.(61) However, TE-based devices like the FibroScan® are more expensive regarding its acquisition, but its maintenance cost is relatively low. They are well validated and have an applicability of over 95%. Scores and tests offer good reproducibility and can easily be applied on outpatients as well. The biomarkers are seldom liver specific, which may reduce the sensitivity of some tests.(50)

Limitations differ from test to test: The FibroTest® is influenced by hemolysis (increase of Haptoglobin) or Gilbert syndrome (increase of bilirubin) and often delivers false positive results. (39) The ELF Score® is very age- and sex- dependent, so it is necessary to define cut-off levels for each age-interval. The ELF Score® is really cheap compared to other methods and is available even in smaller centers. However, TE-methods are more reliable and accurate than the ELF Score®. (27, 39, 40) The Fibrometer® struggles with discriminating stages of fibrosis and offers an accuracy of about 69%, while the reliability of this test is higher if combined with other tests like APRI. (39, 41) The specificity and sensitivity of FIB-4 ranges from 52% to 90%. It is better at determining NAFLD in comparison to other biomarker-tests, but FIB-4 is not rivaling TE or liver biopsy regarding accuracy and reliability. (42)

1.5 Probe recommendation algorithm

The manufacturer Echosens proposes an algorithm for probe selection, because choosing the adequate probe is a necessity for obtaining valid LS-measurements (Figure 12). This recommendation algorithm is based on age, thoracic circumference (TC) and skin-capsule-distance (SCD) of the patient. However, the algorithm ignores adults with a TC below 75 cm and therefore causes a gap in the recommendation algorithm. (64)

This is because the algorithm does not check the TC of adults and only determines probe selection by assessing the SCD. In this study 22 adult volunteers were below 75 cm regarding TC. This shows the importance of closing the gap in the recommendation algorithm.

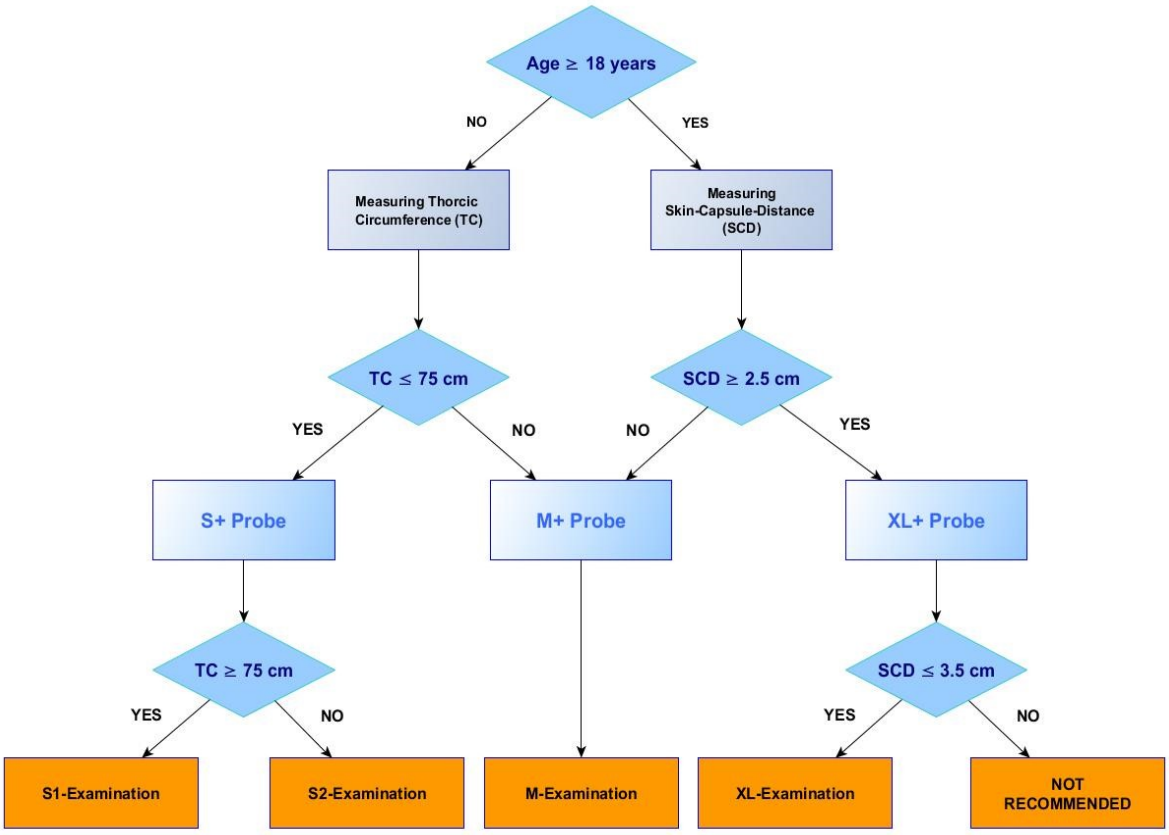


Figure 12: Probe selection algorithm as it is recommended by Echosens. (own image)

1.6 Aims and hypotheses

The aim of this study was to compare the FibroScan® Touch 502 S- and M-probes regarding differences in measuring quality and to assess if the probes can be used interchangeably. This is important, because the manufacturer does not suggest an algorithm for adults with a TC below 75 cm. The only recommendations exist for adults with a TC above 75 cm and for children below and above 75 cm. The manufacturer states that using the probes interchangeably (in this case using the M-probe on an adult with a TC below 75 cm) causes a lack of accuracy and a deviation of the measurements by more than 30%.(39)

Secondly, we investigated possible influences of food intake on the accuracy of measurements regarding LS in both probes and CAP in the M-probe. There is a lot of data describing the influence of food intake in patients with chronic liver diseases for the M-probe. However, for the S-probe there is little data available.(62)

We hypothesized that the S- and M-probes can be used interchangeably without a lack in quality or accuracy of LS- and CAP- measurements in healthy non-obese adults with a TC below 75 cm and that food intake does not affect the quality and accuracy of the probes in these adults either.

2 Methods and Materials

2.1 Patient recruitment and study design

In the period from May 2019 to July 2019 after the affirmation of the study protocol by the institutional review board of the Medical University of Graz (31-345 ex 18/19) and registration of the study at clinicaltrials.gov (NCT03947359), the recruitment of the in total 52 healthy non-obese adults began. The majority of the volunteers were students enrolled in the Medical University of Graz. The study took place from July 2019 until November 2019 at the University Hospital of Graz, Department of Gastroenterology and Hepatology. The study was carried out as an open-label cohort study. The selection of the participants was based on beforehand established inclusion and exclusion criteria. The study was performed in accordance with the declaration of Helsinki.

Table 9 shows all the inclusion and exclusion criteria of the trial.

Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Age > 18 years	Acute or chronic liver disease
TC > 45 cm and < 75 cm for one group; TC > 75 cm for the second group	Any other disease or circumstance that may impact on the result of the study
No history of liver disease	-----
Signed informed consent	-----

Table 9: Inclusion and exclusion criteria for the participant recruitment.

Each of the participants fasted for at least 6 hours prior to the first measurement and every participant read and signed the informed consent. A blood sample was drawn to assess their liver enzyme status and a case report form (CRF) was completed by the operator for each participant *(See point 2.2).* The participants were first measured with the S-probe and then with the M-probe - the duration of each individual measurement was 5 minutes. Within 5 minutes after the baseline measurement every participant ingested a 200 ml and 300 kcal standardized liquid meal (Fresubin Energy, Fresenius-Kabi, Germany). Measurements took place at baseline (after at least 6 hours of fasting), at 30 minutes, 60 min. and 120 min. after meal ingestion. *(Figure 13)*

For the statistical analysis the participants were split by their TC in the below 75 cm group (Below75cmTC) and the above 75 cm group (Above75cmTC) as well as analyzed as a whole cohort.

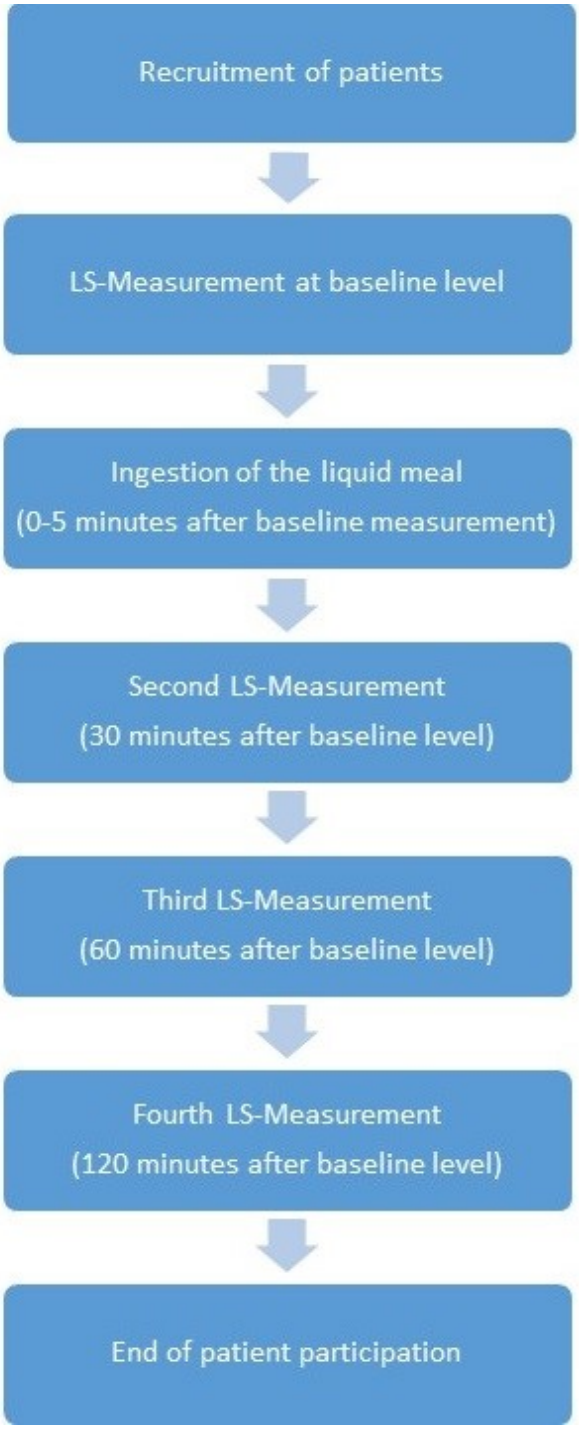


Figure 13: Study Flowchart – A total of eight individual measurements with both probes was applied on every subject (2 measurements per time point). (own image)

2.2 Case report form

A paper-based case report form (CRF) was completed for every participant. The questions are multiple-choice and free-text questions.
The topics of the questions are listed in Table 10.

CRF-Questions

Question	Content
Inclusion- and exclusion criteria	See *Table 2*
Demographic data	Sex, age, date of birth
Anthropometric data	Weight, height, thoracic circumference, BMI
Vital signs	Blood pressure, Heart rate
Lifestyle	Smoking habits, alcohol consumption, sports
Medical history	Diseases, start/stop-date
Medications	Trade name, indication, frequency, start/stop-date
Liver stiffness	LS and CAP values as well as IQR/Median%, CAP-IQR were noted here
Comments	-----
Adverse events	In case of adverse events during the trial

Table 10: Questionnaire filled out before the measurements.

The CRF included sections to tick-off if the informed consent was signed and to note the visit date and the end-of-visit-date.

2.3 FibroScan®

The FibroScan® Touch 502 (Echosens, Paris, France) was used to assess the participants' LS and CAP. All the measurements were carried out using the S- and M-probes of the device.

2.3.1 Liver stiffness

The FS S- and M-probes were placed on the right flank in an intercostal space on the level of the right liver lobe. The participants were laying down in supine position with the right arm elevated above the head and breathing normally. The probe was held with both hands for maximum stability and the probe's tip was held perpendicularly to the participant's skin. The spot of the baseline measurement was marked with a skin-friendly pen to ensure the same spot was used again for the next measurements. LS-IQR and the IQR/median% were measured the same way. A measurement was acknowledged as valid if the median of 10 individually valid measurements was recorded and the IQR/median% was <30%.

2.3.2 Continuous attenuation parameter

CAP has been measured over the same probes. Besides CAP, also the CAP-IQR was measured.

2.4 Blood sample and laboratory parameters

Liver parameters containing ALT, AST, GGT and alkaline phosphatase were determined using standard laboratory methods. Furthermore, renal parameters

(creatinine), inflammatory parameters (C-reactive protein), a complete blood count (erythrocytes, leukocytes, thrombocytes) and a differential blood count (neutrophilic-, eosinophilic-, basophilic granulocytes, lymphocytes, and monocytes) were determined. Additionally, bilirubin and albumin were determined. The venous blood sample (15.5 ml) was drawn from cubital veins and analyzed immediately. Participants with elevated liver parameters were afterwards excluded from the study.

2.5 Statistical analysis

Participants' data was collected using Microsoft Excel 2013.The statistical analysis was carried out using the Statistical Package for the Social Sciences version 26 (SPSS; IBM, USA). Normal distribution of the continuous values (LS and CAP) was assessed using Kolmogorov-Smirnov-test, Shapiro-Wilk-test and Q-Q-Plots. Since the data was mainly not normally distributed, a Mann-Whitney-U test was used to evaluate significant differences between the continuous LS- and CAP-values respectively. A Friedman-test with multiple comparisons and Bonferroni post-hoc test were carried out to assess differences of LS and CAP over time. Correlation and agreement between the values of the 2 probes have been assessed by Spearman-correlation, interclass-correlation and Bland-Altman-plots.

The analysis was carried out for the whole cohort and for the Below75cmTC and Above75cmTC groups separately. Results were considered statistically significant at $p<0.05$ and are reported as median plus confidence intervals (CI).

3 Results

3.1 Baseline characteristics and descriptive statistics

From July 2019 to November 2019, 52 subjects, 26 male and 26 female, underwent LS-measurements with the Fibroscan® 502 Touch using the S- and M-probes. A blood sample was drawn to assess liver parameters. 2 patients (both male) were excluded, because of elevated liver parameters. Ultimately, 50 participants were included in the analysis. The mean age of the participants was 24 ±3 years with a BMI of 22.2 ±2.7 kg/m^2 with an average TC of 77 ±8 cm. The whole cohort was split into 2 sub-groups (Below75cmTC group and Above75cmTC group) regarding TC.

All the anthropometric characteristics and the blood parameters are shown in Table 11 and Table 12. Descriptive statistics are shown below in Table 13.

Anthropometric characteristics of the cohort

Variable	N	Mean	Standard deviation	Median	95%-CI
Age	50	24	3	23	23,08-24,68
Height	50	174	9	174	171,11-176,07
Weight	50	67	12	65	63,94-70,62
BMI	50	22,2	2,7	22,0	21,39-22,92
TC	50	77	8	77	74,48-79,24

Table 11: Anthropometric Data of the participants for the whole cohort. Age in years, height and TC in cm, weight in kg and BMI in kg/m^2.

Blood parameters and liver enzymes

Variables	Mean	Standard deviation	Reference Values
ALT	18	8	Female: 10-35 U/l Male: 10-50 U/l
AST	24	7	Female: 10-35 U/l Male: 10-50 U/l
GGT	18	6	Female: 9-36 U/l Male: 12-64 U/l
AP	59	13	30-120 U/l
Albumin	4,9	0,3	3.7-5.3 g/dl
Creatinine	0,85	0,15	Female: 0.66-1.09 mg/dl Male: 0.81-1.25 mg/dl
Bilirubin (total)	0,67	0,33	≤1 mg/dl
C-reactive peptide	2,4	2,4	≤0.5 mg/dl

Table 12: Blood parameters and liver enzymes of the whole cohort. ALT, AST, GGT and AP in U/L. albumin in g/dL, creatinine and bilirubin in mg/dL and C-reactive peptide in mg/L.

3.2 Fibroscan assessment in the whole cohort

All participants were assessed with the S- and M- probes. The measurement results are shown as median plus upper/lower CI of the median. The cut-off value for LS in healthy subjects is 6.0 kPa.(32) At baseline the median LS with the S-probe was 5.1 kPa (CI of 4.5-5.3 kPa) and the median LS with the M-probe was 4.4 kPa (CI of 4.0-4.8 kPa).

Baseline measurements

Variables at baseline	Mean	Standard deviation	Median	95%-CI of median	Interquartile range
LS S-Probe	5,0	1,3	5,1	4,5-5,3	1,6
LS M-Probe	4,4	1,3	4,4	4,0-4,8	1,9
CAP	188	48	191	173-203	57

Table 13: Baseline measurements for the S- and M-Probe plus CAP for the M-Probe.

3.2.1 Below75cmTC Group

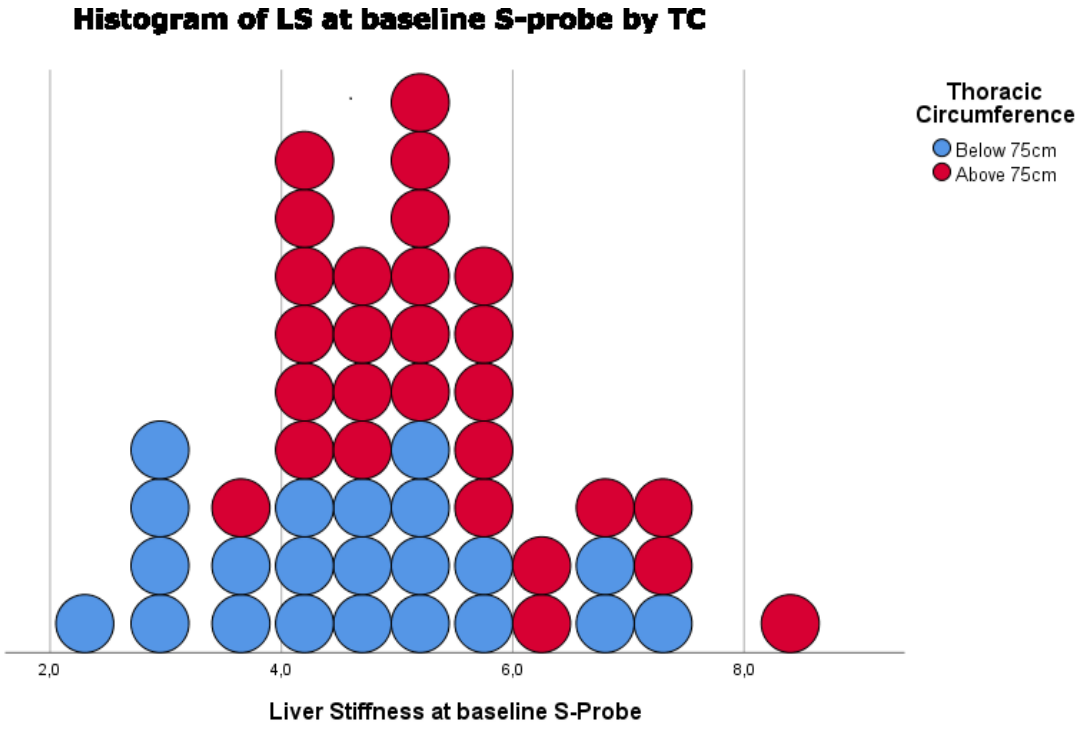
The Below75cmTC group was mainly female (19 female, 3 male; $p<0.001$) with a BMI of 20.2 ±1.7 kg/m^2 and a TC of 69 ±4 cm. The Below75cmTC group showed a median LS of 4.6 kPa with a CI of 3.7-5.2 kPa for the S-probe and a median LS of 3.8 kPa and a CI of 3.3-4.6 kPa for the M-probe with a median CAP of 185 dB/m.

3.2.2 Above75cmTC Group

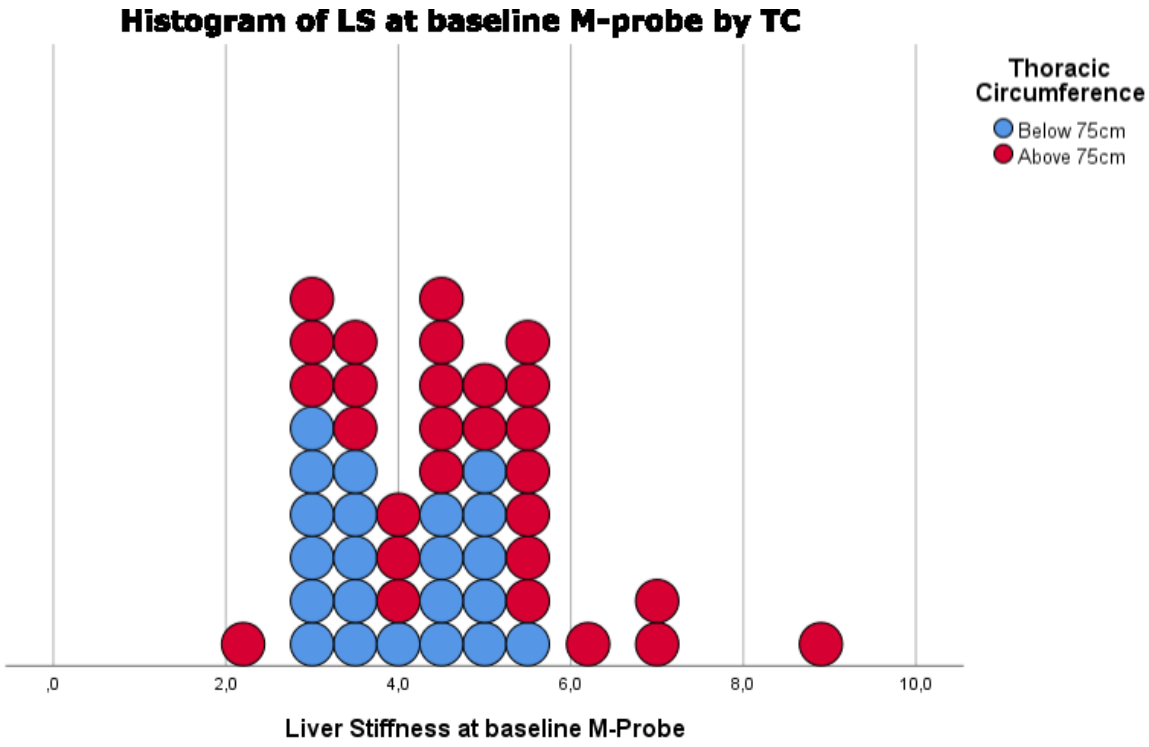
The Above75cmTC group was mainly male (21 male, 7 female; $p<0.001$) with a higher BMI of 23.7 ±2.4 kg/m^2 and a TC of 83 ±6 cm on average. The Above75cmTC group showed a median LS of 5.3 kPa with a CI of 4.6-5.6 kPa for the S-probe and a median LS of 4.6 kPa with a CI of 4.2-5.3 kPa for the M-Probe with a median CAP of 199 dB/m.

3.3 Comparison of differences between the Above75cmTC group and Below75cmTC group regarding LS

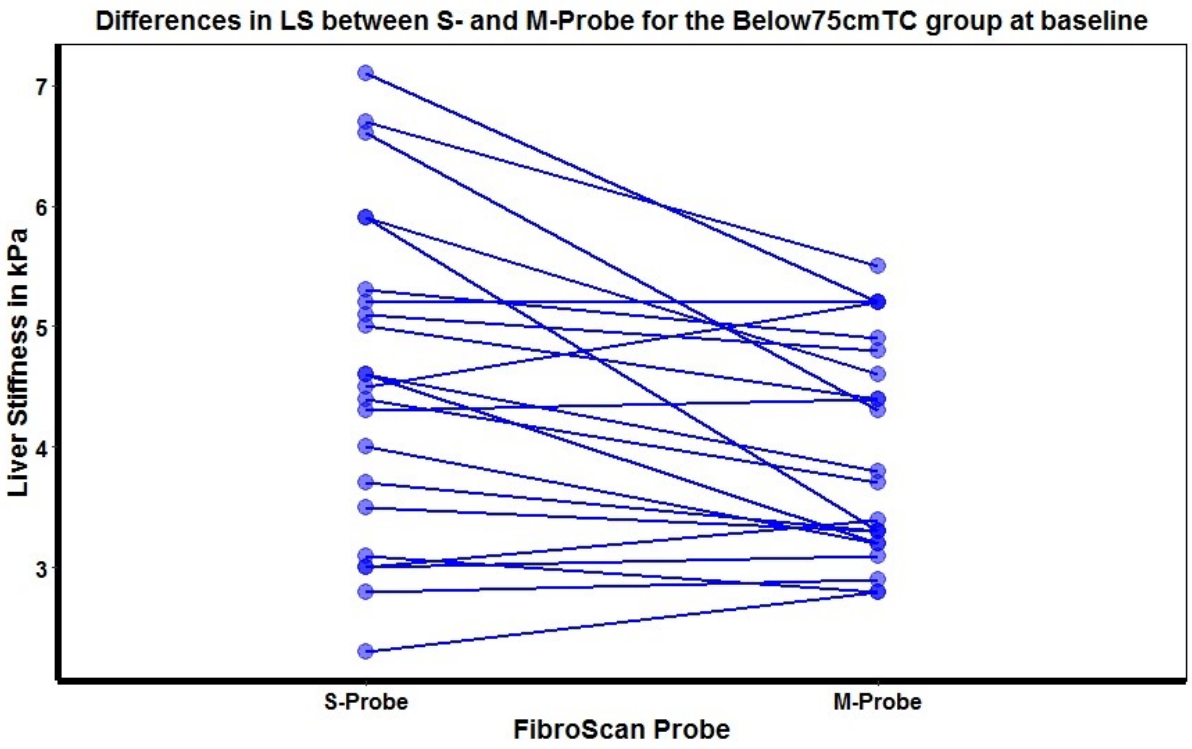
The S- and M-probe measurements differed significantly from each other (p=0.041 and p=0.028 respectively) and were higher with the S-probe for the Below75cmTC group and for the Above75cmTC group compared to the M-probe. *(Graph 1, 2, 3 & 4)*



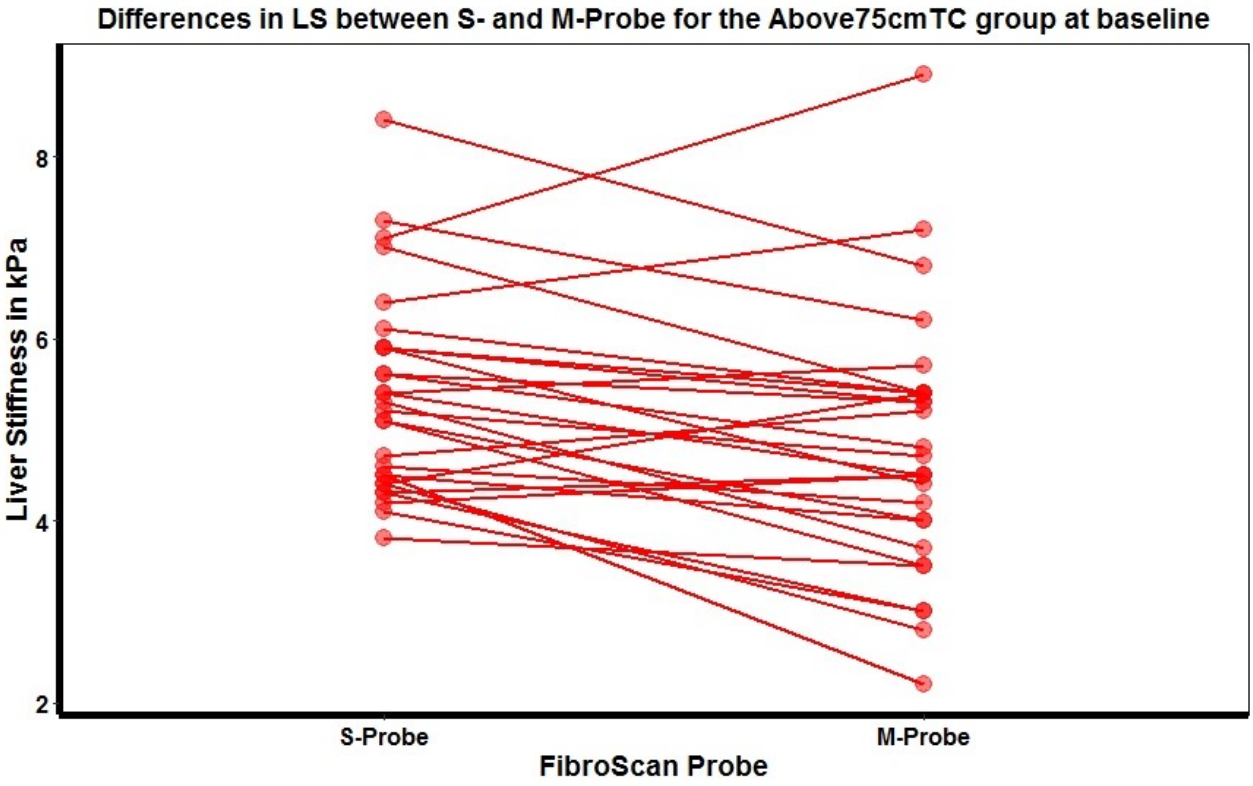
Graph 1: Histogram of LS with S-probe in TC below 75- and TC above 75 groups.



Graph 2: Histogram with M-probe in TC below 75- and TC above 75 groups.



Graph 3: Dot plot of each participant's LS-values in the Below75cmTC group with both probes – connecting lines between each participant's S- and M-probe baseline values.

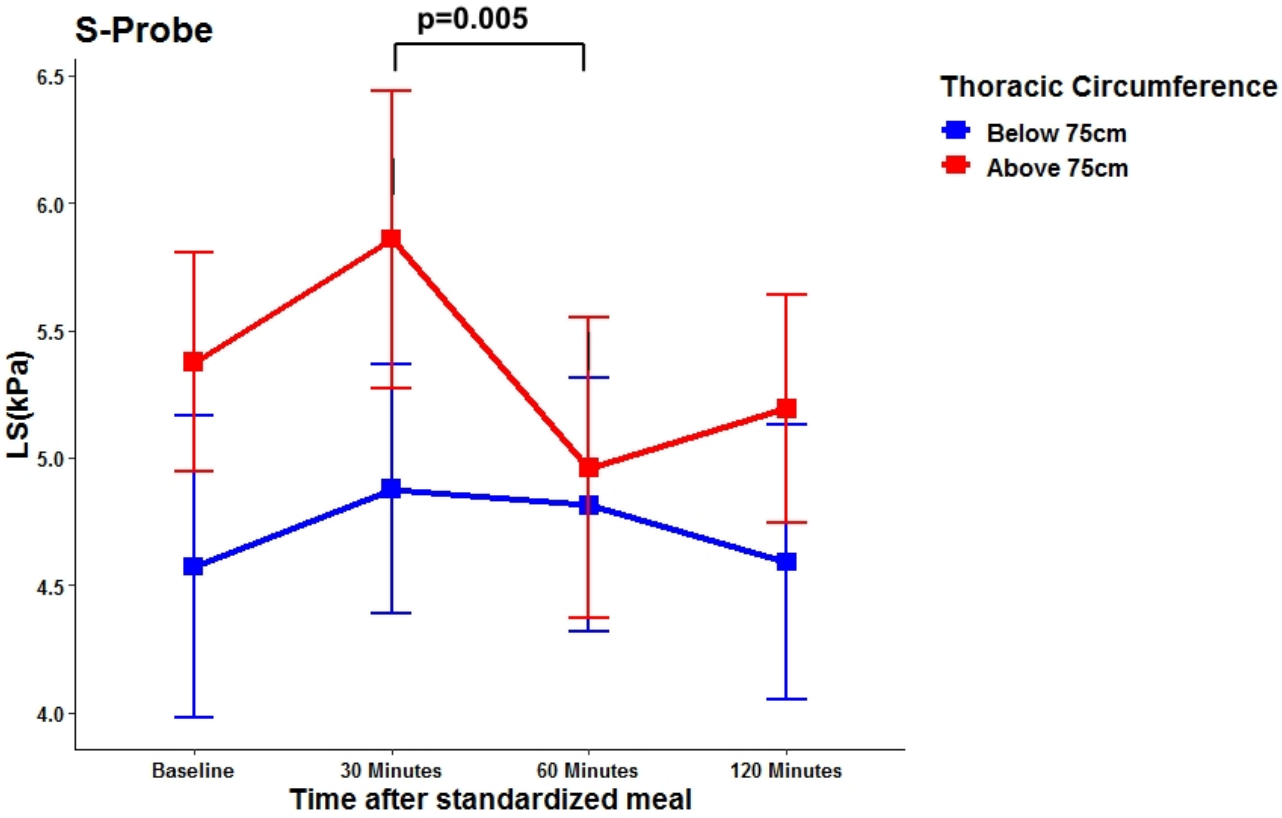


Graph 4: Dot plot of each participant's LS-values in the Above75cmTC group with both probes – connecting lines between each participant's S- and M-probe baseline values.

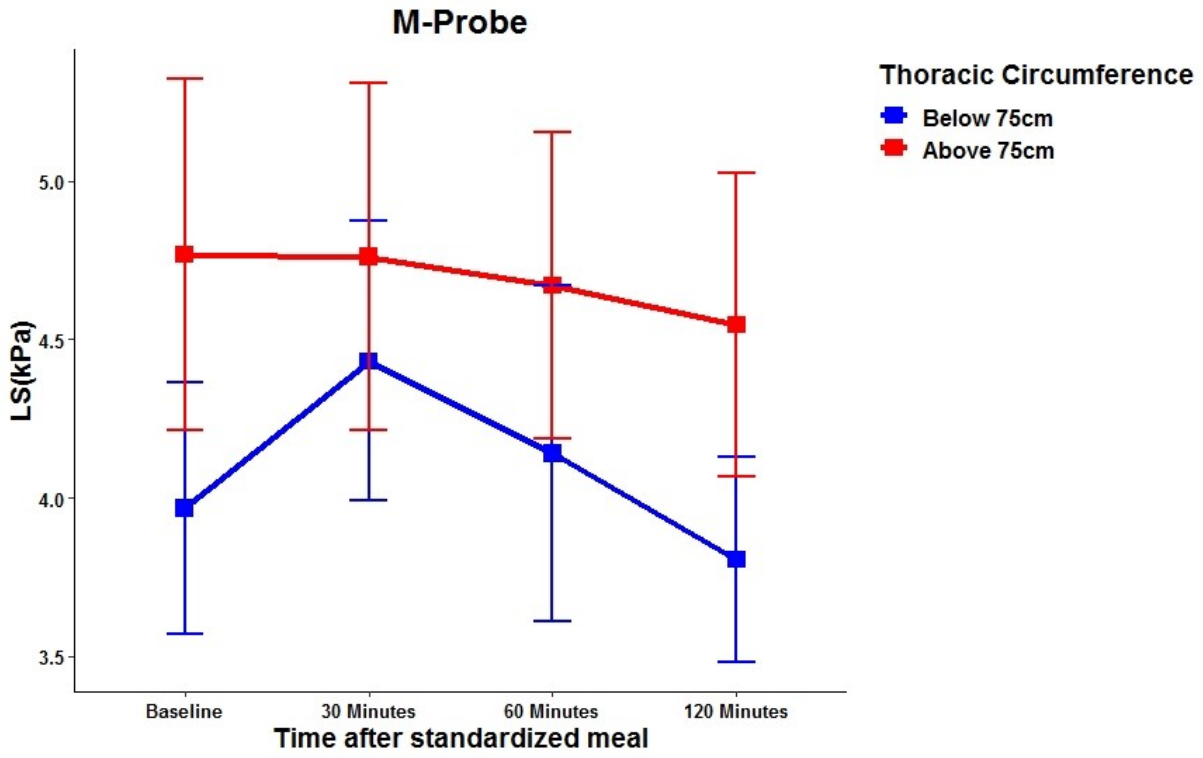
3.4 Differences over time between Below75cmTC and Above75cmTC groups regarding LS and CAP after meal ingestion

After the Bonferroni post-hoc test with multiple comparisons, the effect for the whole cohort using the S-probe was localized between time point 30 minutes and time point 60 minutes (Power=0.1; adjusted p=0.025). For the Above75cmTC group using S-probe the effect is localized between the same time points (Power=0.2; adj. p=0.005). With both probes the effect for the whole cohort is not significant after adjustment by the post-hoc test (Power=0.1; adj. p=0.057), with the same result regarding the Below75cmTC group and Above75cmTC group (Power=0.2; adj. p=0.052). *(Graph 5 & 6)* CAP does not change immediately after meal ingestion. However, CAP decreases

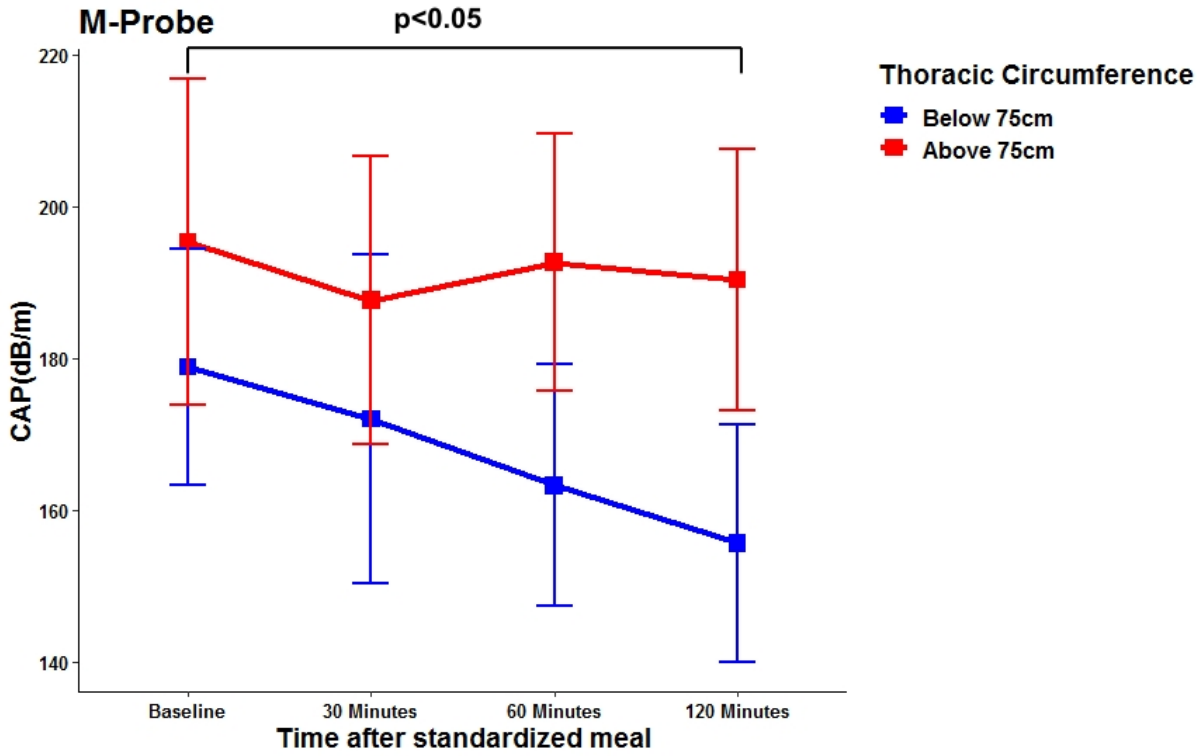
significantly 120 min. after meal ingestion in the Below75cmTC group (p=0.066). *(Graph 7)* CAP-IQR did not differ over time in both groups.



Graph 5: The graph shows the change of LS over time from baseline to 120min after food intake in the S-probe. The blue line represents the Below75cmTC-group, the red line represents the Above75cmTC-group. LS and error bars shown as mean plus CI of the mean.



Graph 6: The graph shows the change of LS over time from baseline to 120min after food intake in the M-probe. The blue line represents the Below75cmTC-group, the red line represents the Above75cmTC-group. LS and error bars shown as mean plus CI of the mean.



Graph 7: The graph shows the change of CAP over time from baseline to 120min after food intake in the M-probe. The blue line represents the Below75cmTC-group, the red line represents the Above75cmTC-group. CAP and error bars shown as mean plus CI of the mean.

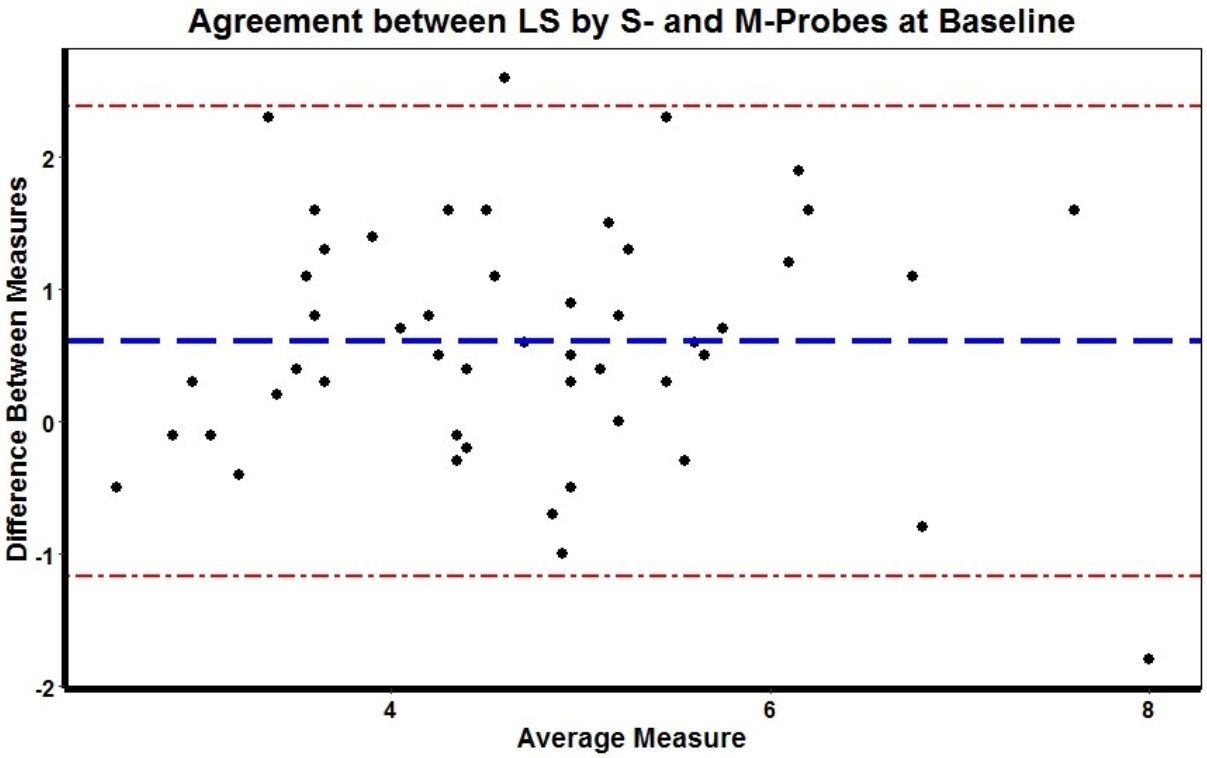
3.5 Agreement between the S- and M-probe in the whole cohort

Agreement was determined using Spearman-correlation, linear regression, interclass-correlation-coefficient and Bland-Altman-Plot.

Spearman-correlation showed excellent agreement ($r=0.755$; $p<0.001$) as well as linear regression ($r^2=0.557$; $p<0.001$) and Interclass-correlation-coefficient (ICC) for the whole cohort (Coefficient=0.805, CI=0.498-0.909; $p<0.001$).

The Bland-Altman plot shows very good agreement with a bias of 0.58±0.9 with the limits of agreement being between -1.2 and 2.3. On average the S-probe measures -1.2 units less or 2.3 units more than the M-probe and measures about 0.58±0.9 units more in total than the M-probe. Since the line of mean differences is between the

95%-limits-of-agreement, the bias is not significant and the agreement between the probes is considered very good.(63) *(Graph 8)*



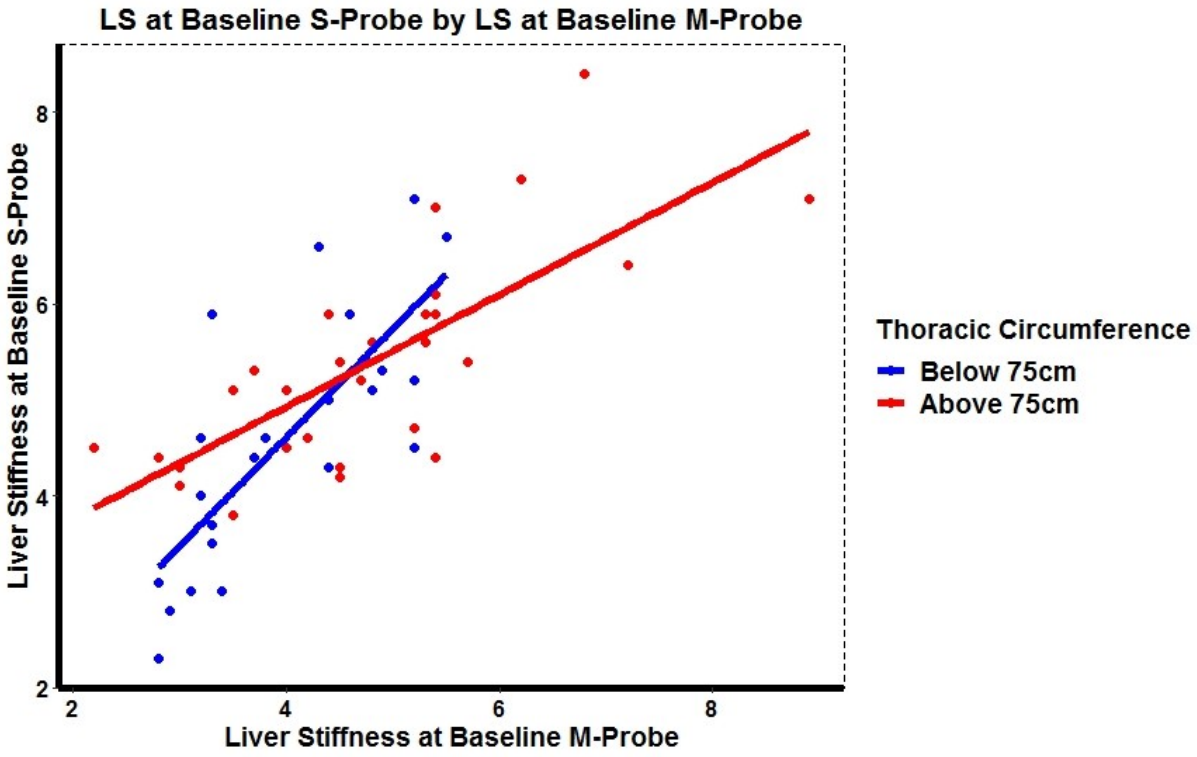
Graph 8: Bland-Altman-plot of S- and M- probes at baseline. The black dots represent the difference of measure between each measurement of each probe. The red lines are the upper and lower limits of agreement containing 95% of values. The blue line is the mean of the difference between measures.

3.5.1 Agreement between S- and M-probe considering the Below75cmTC group

In the Below75cmTC group Spearman-correlation (r= 0.762; p<0.001) and ICC (Coefficient=0.767, CI=0.323-0.911; p<0.001) show excellent agreement and excellent linear regression (r2 =0.575; p<0.001). *(Graph 9)*

3.5.2 Agreement between S- and M-probe considering the Above75cmTC group

Spearman-correlation (r=0.758; p<0.001) and ICC (Coefficient=0.796, CI=447-915; p<0.001) show excellent agreement in the Above75cmTC group and excellent linear regression (r2 =0.565; p<0.001). *(Graph 9)*



Graph 9: Linear regression – S-Probe at baseline (y-axis) plotted against M-Probe at baseline (x-axis). Whole Cohort. Below75cmTC group as blue line and dots. Above75cmTC group as red line and dots.

4 Discussion

The aim of the study was to compare the FibroScan® Touch 502 S- and M-probe regarding measuring quality and to assess if the S- and M-probe can be used interchangeably. Furthermore, we investigated the influence of food intake on measurement quality regarding LS in both probes and CAP in the M-probe. The study shows that the S- and M-probe of the FibroScan® can be used interchangeably in non-obese adults in fasting state or after a light meal in the Below75cmTC group.

Furthermore, it closes a gap in the recommendation algorithm of the manufacturer by showing that in adults with a TC below 75 cm the S-probe and the M-probe can be used interchangeably.

The manufacturer offers no recommendation for adults with a TC below 75 cm regarding probe selection. To confirm this claim, a cohort of 50 healthy non-obese adults was recruited and split into 2 groups according to their TC (Below75cmTC and Above75cmTC groups).(64) Each subject of each group was measured with both probes at several time points over a course of 2 hours to obtain data. In comparison to the M-probe, the S-probe yielded higher LS-values in the whole cohort, as well as in the Below75cmTC- and Above75cmTC groups. The difference regarding LS-values was about 15% in all groups. This is lower than the 30% difference in LS-values the manufacturer proposes when selecting the probe in disagreement with the probe recommendation algorithm. The correlation between the S- and M-probe was excellent in the whole cohort as well as in the Below75cmTC- and Above75cmTC groups. Variances were comparable between the Below75cmTC- and Above75cmTC groups. Only 4 participants exceeded the LS cut-off-value of 6.0 kPa. This could be because of lower feasibility of the FS in early stages of fibrosis or no fibrosis. Furthermore, a comparison between the S- and M-probe has not been done before in healthy adults. However, Pradhan *et al.* have compared the feasibility of the S- and M-probe in adults with a TC below 75 cm and suffering from chronic liver diseases.(65) Pradhan *et al.* demonstrated that the M-probe is superior in patients with a TC below 75 cm regarding LS-feasibility compared to the S-probe. In their cohort of 59 participants (41 patients

and 18 healthy controls) the median LS for the S- and M-probe was 4.5 kPa and 4.4 kPa respectively. This result is very similar to our result of a median LS of 4.6 kPa and 3.8 kPa for S- and M-probe respectively. However, Pradhan *et al.* had a cohort composed of healthy participants as well as patients suffering from chronic liver diseases, which makes the results of our studies less comparable. Another limitation of our study is that we did not assess SCE in our participants, which might have offered an answer to the difference in LS-values between the S- and M-probe. As Pradhan *et al.* have demonstrated, a skin-capsule-distance (SCD) over 15 mm leads to higher LS-values of about 10% with the S-probe compared to the M-probe in patients with a TC below 75 cm. This is because of more subcutaneous adipose tissue between the skin and the liver, which interferes with the S-probe's penetration depth of about 20 mm.(65) This is important because Pradhan *et al.* concluded that the S-probe might overestimate LS in patients with a high SCD. The about 15% higher median LS-values with the S-probe compared to the M-probe in our study could be because of a high SCD. However, since the FS is inaccurate in assessing LS at missing or low fibrosis (F0 and F1), one reason could be that our cohort was entirely composed of healthy adults. Furthermore, we did not see measuring the SCD fit due to our patients being healthy and having a BMI below 25 kg/m^2.

Considering FS probe comparisons, literature on comparisons between the S- and M-probe regarding LS in adults, is scarce. However, various authors have compared the FS XL- and M-probes with a similar study design. Ledinghen *et al.* and Şirli *et al.* have demonstrated that a difference between M- and XL-probes exists and is significant with the median LS of the XL-probe being significantly lower than the M-probe. The difference in LS-values between XL- and M-probes was about 20%. (43) This is similar to our results as well as to the results of Pradhan *et al.* where a higher SCD leads to higher LS-values due to interference with the probe's penetration depth. However, Ledinghen *et al.* pointed out that reliability of FS-measurements is associated with BMI and decreases in the M-probe the higher the BMI is. Additionally, Durango *et al.* also demonstrated that the XL-probe is less accurate than the M-probe, especially in patients with a high BMI (BMI>30 kg/m^2) and a high SCD (SCD>20mm).(66) This is important, because the aforementioned studies share a similar study design to our

study in terms of probe comparison. However, the number of included patients is higher (286 patients at Ledinghen *et al.* 371 patients at Durango *et al.* and 216 patients at Şirli *et al*.), which might lead to a higher reliability in these studies. Moreover, one limitation could be that the lower overall age and no inclusion of patients with liver diseases might lower generalizability in our study.(43, 67)

Also, there are several studies comparing the S- and M-probe in children. Kim *et al.* demonstrated that the S-probe tends to overestimate LS in children suffering from biliary atresia with a TC >45 cm.(38) The overestimation of the S-probe is in line with our results and the results of Ledinghen *et al.* However, Kim *et al.* pointed out that this could also be explained by an underestimation by the M-probe.(38, 43) Engelmann *et al.* demonstrated good feasibility of LS measured with M-probe in children aged 0-18 years. The median LS in a sub-group of children aged 12-18 years was 5.1 kPa which is very similar to the median LS in our Below75cmTC group.(68)

In summary, our study shows that there is no major difference between the S- and M-probe in our cohort of non-obese healthy adults. The results are comparable in both groups (Below75cmTC and Above75cmTC) and do not deviate to the extent the manufacturer states. However, variance was comparable for both probes in the Below75cmTC group, but was higher with the S-probe compared to the M-probe in the Above75cmTC group. LS-measurements were slightly higher in the S-probe regardless of TC, however, this overestimation of the S-probe is clinically not relevant in most cases. The difference between S- and M-probe of LS regarding TC, as stated by the manufacturer, could not be demonstrated. Therefore, S- and M-probe could be used interchangeably to measure LS at least in the Below75cmTC group. Our results clarify probe selection in healthy adults with a TC below 75 cm, thus saving time. This is important, when the M-probe is not available due to maintenance, because the S-probe could be used alternatively.

The second aim of this study was to analyze the influence of food intake on LS- and CAP-measurements. Participants ingested a 200 ml and 300 kcal containing standardized liquid meal (Fresubin Energy, Fresenius-Kabi, Germany) after the

baseline measurement. Further 3 measurements with each FS-probe were performed over a time window of 2 hours. After the standardized liquid meal, there was no significant change in LS regarding the Below75cmTC- and Above75cmTC group with both probes. Furthermore, variances did not differ over time in both probes. CAP decreased significantly in the Below75cmTC group 120 min. after meal ingestion compared to baseline.

Previous studies showed an influence of meal ingestion on LS- and CAP-measurements depending on the liver disease of the patient and calories of the meal.(62, 69-76) Alvarez *et al.,* demonstrated a significant increase in LS of approximately 30%, compared to baseline, 30min. after the ingestion of a standardized liquid meal (330ml, 495kcal) in patients with various liver diseases and different stages of fibrosis. Mederacke *et al.,* demonstrated a similar LS increase after the ingestion of a standardized continental breakfast (600kcal) for up to one hour after meal ingestion.(62, 72) The reason for LS-increase is explained by Mederacke *et al.* with an increase in blood flow in the hepatic artery and the portal vein.(72) This was first demonstrated by Szinnai *et al.* and other authors like Berzigotti *et al.* Arena *et al.* and Barone *et al. ,* who demonstrated the same increase in more recent studies.(33, 69, 70, 76) However, in comparison to our study the aforementioned studies all had a cohort composed of patients suffering from liver diseases. Furthermore, their administered meal contained almost double the amount of kcal compared to our study, which could influence generalizability. Our results are not in line with the aforementioned studies and suggest that LS-measurement is not significantly influenced by ingestion of a light meal in the S- and M- probes regarding healthy non-obese adults. Lemoine *et al.* demonstrated in a group of 76 healthy controls that LS is significantly higher 30 min. after meal ingestion. However, the meal contained 850 kcal which cannot be compared to the light meal of 300 kcal we administered.(71)

Furthermore, the influence of food intake on CAP is a controversial topic in currently available literature.(73-75) Vuppalanchi *et al.* demonstrated an increase in LS after meal ingestion, but demonstrated no influence on CAP with only a 3% change compared to baseline.(75) However, this change was not significant and the 3 hours

fasting period suggested by Vuppalanchi *et al.* is not in line with our results, where LS returned to baseline after 120 min. and CAP decreased significantly after that time.(75) Silva et al. like Vuppalanchi *et al.* demonstrated an increase in LS without an increase in CAP after the ingestion of a 600 kcal solid meal.(74) However, Silva et al. only measured LS and CAP at baseline and 30 min. after meal ingestion, which could reduce comparability to our results where a CAP was also observed after 60 min. and 120min. after meal ingestion.(74) A similar result was demonstrated by Ratchatasettakul *et al.* where LS and CAP peaked 60 min. after meal ingestion (600 kcal), but returned to baseline after about 120 min.(73) In our study however, there was no influence of a light standardized liquid meal (300kcal) on LS with S- and M-probe in healthy non-obese adults. CAP measurements decreased 2 hours after meal ingestion significantly only in the Below75cmTC group. Fasting periods prior to the examination varied in the aforementioned studies. Mederacke *et al.* had an eight hours fasting period prior to examination, while Alvarez *et al.* had an overnight fasting period. Other authors like Berzigotti *et al.* chose the same fasting period as we did in our study with a minimum of 6 hours prior to the examination.(62, 70, 72)

We have demonstrated that a standardized light meal does not influence LS and does not influence CAP immediately. LS did not change significantly over time in either probe. CAP decreased significantly after 120 min. only in the Below75cmTC group. These results should be considered when treating or diagnosing patients like pediatric patients or patients with eating disorders that cannot fast for a long period of time. However, further studies in these patient groups need to be conducted to verify this claim.

In conclusion, this study shows that the S- and M-probes can be used interchangeably in non-obese adults and that a light meal prior to the examination has no clinically relevant impact on LS-measurements. Furthermore, the results close the gap in the probe selection algorithm by showing that S- and M-probes are not different in adults with a TC below 75 cm. *(Figure 14)* These results save time, improving FS handling and clarifying further procedure of patients that do not fit in the proposed probe selection algorithm by the manufacturer. Additionally, since long fasting causes psychological stress, these results are potentially important for patients with eating disorders or pediatric patients. However, further studies need to be done in pediatric patients and patients with eating disorders to confirm this claim.

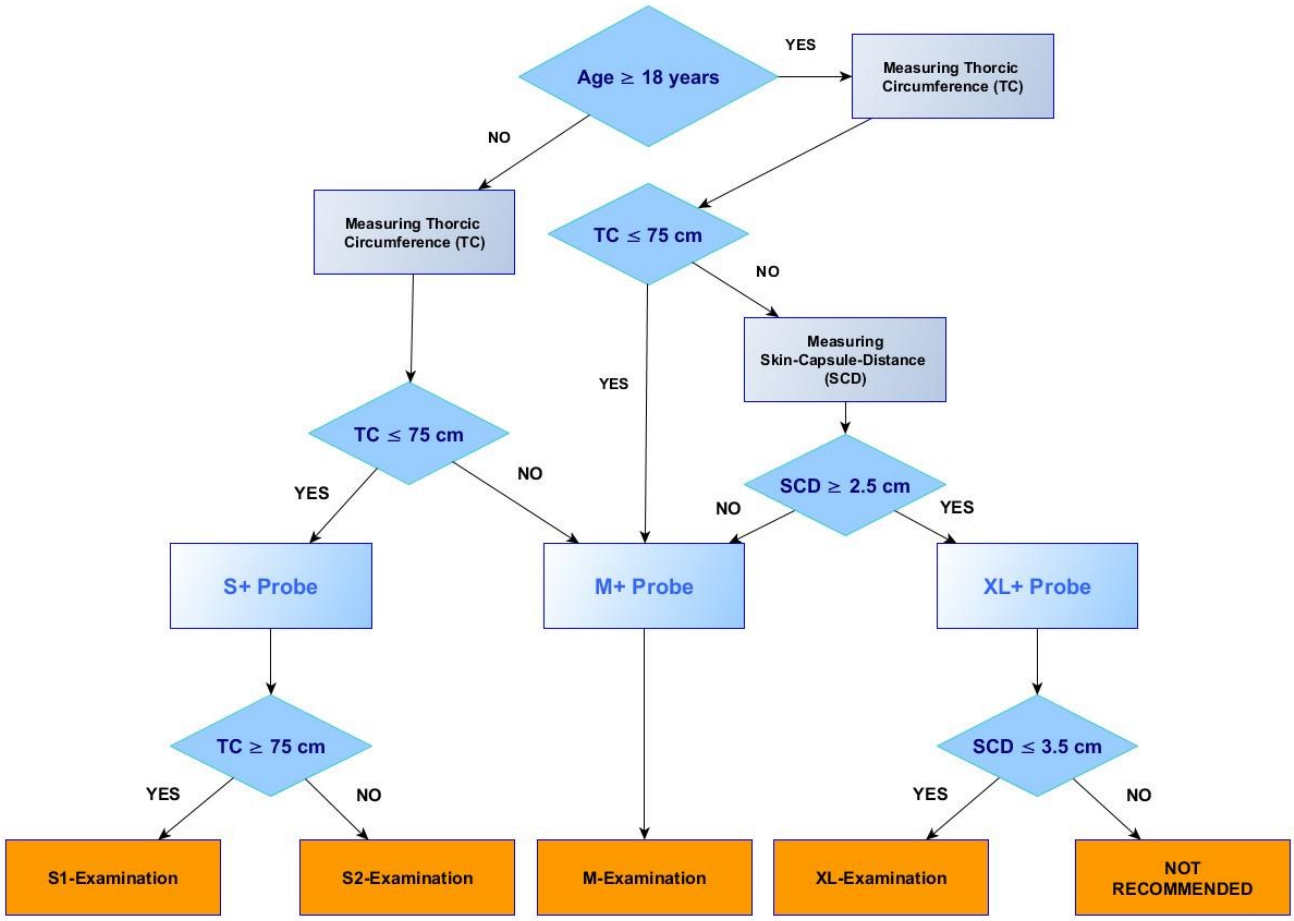


Figure 14: Probe selection algorithm expanded by our data on LS-measurements in adults with a TC below 75 cm- closing the recommendation gap for this group of patients.

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Appendix

Case Report Form

Version 1.0 05.04.2019

Effect of thoracic diameter and food intake on Fibroscan® results:

Comparison of the S - and M-probes

Principal Investigator:

Assoz. Prof. Dr. Vanessa Stadlbauer-Köllner

Visit date*	Done	Comments
_____/_____/_____	[_]	

*This study will only require one visit from the patient

Visit

Informed Consent		
1. Informed Consent signed prior to any study related procedure?	**[_] yes**	**[_] no**
Date informed consent obtained: _____/_____/_____ Physician Initials _____		

Inclusion Criteria (must be answered "yes")		
2.a. Age >18y if part of the adult population or age between 11y and 18y if part of the paediatric population	[_] yes	[_] no
2.b. No history of liver disease	[_] yes	[_] no
2.c. Informed consent signed by the patient and/or his legal representative	[_] yes	[_] no
2.d. Thoracic diameter of >75cm if part of the adult population or between 45cm and 75cm if part of the paediatric population	[_] yes	[_] no
Exclusion Criteria (must be answered "no")		
2.e. Acute or chronic liver disease	[_] yes	[_] no
2.f Any other condition or circumstance, which, in the opinion of the investigator, would affect the patient's ability to participate in the protocol	[_] yes	[_] no

1. Demographic Data	
1. Sex	[_] male [_] female
2. Date of Birth	_____/_____/_____
3. Age	_____ years

2. Anthropometric measurements	
Weight *(without shoes and overcoat)*	___ ___ ___ , ___ kg
Height	___ ___ ___ , ___ cm
BMI	___ ___ , ___ kg/m^2
Thoracic Diameter	___ ___ , ___ cm

3. Vital Signs	
Blood pressure *(after 5min resting)*	____/____mmHg

Heart Rate *(after 5min resting)*	_____ min^{-1}

4. Lifestyle

Smoking	[_] yes [_] no [_] ex smoker since _____ years of smoking _____ **Packyears _____**
Alcohol consumption	Units/week _____ Nature of alcohol consumed Beer/Lager [_] yes [_] no Wine [_] yes [_] no Spirits [_] yes [_] no Average number of days/week alcohol consumed _____ Current alcohol consumption [_] yes [_] no
Sports	Hours/week _____ h

5. Medical History (go to Annex 1)

6. Medication (go to Annex 1)

7. Liver Stiffness

LS at baseline level – S-probe	_____ kPa , IQR:_____, IQR%_____,
LS at baseline level – M-probe	_____ kPa, IQR:_____, IQR%_____ CAP:_____dB/m, CAPIQR_____

LS 30 minutes after food intake – S-probe	_____ kPa, IQR:_____, IQR%_____
LS 30 minutes after food intake – M-probe	_____ kPa, IQR:_____, IQR%_____ CAP:_____dB/m, CAPIQR_____
LS 60 minutes after food intake – S-probe	_____ kPa, IQR:_____, IQR%_____
LS 60 minutes after food intake – M-probe	_____ kPa, IQR:_____, IQR%_____ CAP:_____dB/m, CAPIQR_____
LS 120 minutes after food intake – S-probe	_____ kPa, IQR:_____, IQR%_____
LS 120 minutes after food intake – M-probe	_____ kPa, IQR:_____, IQR%_____ CAP:_____dB/m, CAPIQR_____

10. Comments

Please enter any relevant information

Time	Comment	Initials

11. End of Visit

Date:

_____/_____/_____

Signature Investigator:

_____

Annex 1

Medical History

Disease	Start date	Stop date

Medication

Trade name	Indication	Frequency	Start date	Stop date

Annex 2

Adverse Events	
4.1.1.1.1.1.1.1 4.1.1.1.1.1.1.2 Type of Adverse Event	o Adverse Event o Serious Adverse Event
Date when the adverse effect was detected:	____/___/____ DD MM YY
Ongoing? o YES o NO End of adverse effect: o death Date:	____/___/____ DD MM YY
Detailed Description:	
4.1.1.1.1.1.1.3	
Actions taken:	

Outcome:	☐ Recovered ☐ Improved ☐ Unchanged ☐ Worsened ☐ Death