Hepatic Steatosis Detected by Ultrasonography as a Predictor of Insulin Resistance in Obese Patients.

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Running title: Liver steatosis and insulin resistance risk

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Abstract

Background and aims: The metabolic syndrome (MetS) is a worldwide health issue. Components of MetS are obesity, hypertension, insulin resistance/type 2 diabetes and dyslipidaemia. Non-alcoholic fatty liver disease (NAFLD) is closely related to the MetS, and particularly to insulin resistance. A significant feature of NAFLD is the presence of hepatic steatosis, which can be assessed by ultrasonography, by measuring the hepatorenal index (HRI), a comparison of liver and kidney acoustic echo densities. This study aims to investigate if HRI can predict insulin resistance.

Patients and methods: Ninety participants from the obesity clinic and the Sixth Tromsø Study were included. Homeostasis Model Assessment of Insulin Resistance (HOMA1-IR), body mass index (BMI) and HRI were measured. Steatosis was classified as mild (HRI 1.05-1.24), moderate (HRI 1.25-1.64) or severe (HRI ≥1.65). ROC analyses were performed, detecting insulin resistance at HOMA1-IR cut-off values 2.3 and 2.5. Groups of participants with BMI ≥30 (n = 46) and BMI ≥35 (n = 27) were analysed specifically.

Results: HRI at level 1.11 had sensitivity = 0.92 and specificity = 0.56 for predicting insulin resistance in all participants, whereas HRI at level 1.42 had sensitivity = 0.29 and specificity = 0.94. For BMI ≥30, HRI at level 1.11 had sensitivity = 0.94 and specificity = 0.54, and HRI = 1.42 had sensitivity = 0.33 and specificity = 0.96. For BMI ≥35, HRI = 1.17 had sensitivity = 0.93 and specificity = 0.77, whereas HRI = 1.42 had a sensitivity = 0.29 and a specificity = 0.92. (All test property values calculated for HOMA1-IR ≥2.3).

Conclusion: Mild hepatic steatosis diagnosed by ultrasound is a good predictor for diagnosing insulin resistance, especially in obese patients.

Introduction

Obesity is a major worldwide health issue due to its association with the metabolic syndrome (MetS). In 2008, more than 1.4 billion adults were overweight. Of these, about 500 million were obese (1). Components of the MetS are: hypertension, insulin resistance/type 2 diabetes and dyslipidaemia (hypertriglyceridemia and low levels of high density lipoproteins (HDL)) (2, 3).

Non-alcoholic fatty liver disease (NAFLD) is closely associated with the MetS, and is in some definitions considered part of the MetS. NAFLD is the most common type of liver disease in the developed world today, with a prevalence of approximately 30% (4, 5).

The hepatic lipid metabolism is vulnerable to metabolic dysfunction, resulting in the accumulation of lipid droplets in the hepatocyte. The 'two-hit theory' is a well-known model by Day et al(6), for describing the pathogenesis of NAFLD. The 'first hit' is a hepatocellular lipid accumulation due to an imbalance of lipid uptake and combustion. The 'second hit' is defined as a hepatocellular inflammation (NASH), due to imbalance between pro- and anti-inflammatory factors (4, 6).

One hypothesis on the cause of the first hit is glycolysis overload by a high dietary fructose intake, causing an increase in de novo lipogenesis and the accumulation of lipid droplets. The same mechanism is used to explain hepatic insulin resistance and ultimately the inflammation (7, 8).

The most common explanation of the pathophysiologic mechanism is, however, one or more of the following mechanisms: an increased inflow of free fatty acids due to insulin resistance in adipose tissue, altered processing of dietary lipids in the hepatocyte, impaired export of lipids and increased de novo lipogenesis (4, 5). It has also been suggested that the adipocyte's volume and production of adipokines are closely related, with the presence of and progression of NAFLD through increased inflammation. (5, 9)

Insulin resistance (IR) is considered to be the most important part of the MetS, being part of its pathophysiologic mechanism (10,11). IR is characterized by impaired lowering of blood glucose through a reduced uptake in muscles and lack of insulin's effect on reducing endogenous glucose production in liver. IR is also characterized by impaired insulin effect on lipid and protein metabolism, and also impaired effect on a number of other organs (10, 12).

A common way of assessing insulin resistance is by the Homeostasis model assessment of insulin resistance (HOMA1-IR) (13, 14)

Previous studies have shown that the optimal cut-off value of HOMA1-IR is 2.3 for detecting insulin resistance and MetS (sensitivity 77 % and specificity 67 %). The updated HOMA2-IR is more accurate, correcting for feedback relationships between insulin resistances in different organs. The optimal cut-off value for HOMA2-IR is 1.4, (sensitivity 79 % and specificity 62 %). However, HOMA2-IR has a limited range of reliable values, and calculating the index is much more complicated (15).

A second method for assessing insulin resistance is by Whole Body Insulin Sensitivity Index (WBISI), calculated from the fasting and mean postprandial values of blood glucose and serum insulin during an Oral Glucose Tolerance Test (OGTT). The formula used for calculating insulin sensitivity is Matsuda's ISI (16).

The gold standard for diagnosing hepatic steatosis is by liver biopsy (17), which also is the only way of diagnosing the presence of steatohepatitis. Liver biopsy, however, remains an invasive procedure with a risk of complications, and the need for biopsy in the diagnosis of NAFLD is much debated (18).

Increased levels of transaminases and γ-GT are used as an indicator of NAFLD when biopsy is contraindicated or not available. They are often combined with radiological methods like CT, MRI and ultrasound, where ultrasound is the cheapest method with the lowest risk of complications and no radiation dose, and therefore often preferred. Subjective assessment of steatosis in ultrasound does have a relatively large inter- and intraobserver variability. A way of

reducing this variability is by measuring the liver and kidneys' echogenicity on a grayscale from 0- 255, and using the mean value for computing a hepatorenal index (HRI)(19). HRI is a tool of quantifying the steatosis that is more reliable than subjective assessment alone.

Normally the liver and kidney cortex has the same echogenicity. Normal HRI is therefore in the range from 1.00 to 1.04. Hepatic steatosis is classified in mild (HRI 1.05-1.24), moderate (1.25-1.64) or severe steatosis (≥1.65) (19).

Insulin resistance in obese individuals is crucial for the risk of further development of the Metabolic Syndrome, and the presence of hepatic steatosis and insulin resistance is closely linked. Although the HOMA1-IR is relatively simple to calculate both in general practice and in specialist care, it tends not to be in mind of the clinician, unless there are other factors suggesting an underlying insulin resistance. Ultrasonography, being a risk-free procedure that is simple to perform, would be the natural choice for a screening procedure, and therefore, it is necessary to know its test properties.

Aim of study: To examine whether hepatic steatosis, quantified by HRI, is usable as a test for detecting insulin resistance, and to examine its test properties.

Materials and Methods

Participants were included both from a study performed at the obesity clinic at the University hospital of North Norway, and from a follow-up study on slightly elevated transaminases in the Sixth Tromsø Study (Figure 1). The Sixth Tromsø Study is previously described by Eggen et al. (20). All participants signed a written consent, which included permission to use their data for follow-up studies. The study performed at the obesity clinic was approved by The Regional Committee of Medical Ethics of North Norway, including approval of a bio bank.

Access to data and participants for the follow-up study for the Sixth Tromsø Study was approved by the Tromsø Study organisation. The follow-up study was covered by the main ethical approval given for the Sixth Tromsø Study by the Regional Committee of Medical Ethics of North Norway.

Patients at the obesity clinic with a BMI of 30 or more were included in the study. Exclusion criteria were medical treatment of diabetes mellitus, severe heart disease or severe kidney failure. These participants underwent an Oral Glucose Tolerance Test (OGTT), where the participants drank 75 g of glucose solved in water. Fasting and postprandial blood glucose and serum insulin levels were measured at 30-minute intervals, and HOMA1-IR and Matsuda's ISI was calculated:

$$HOMA1\text{-}IR = \frac{[fasting\ glucose] \times [fasting\ insulin]}{22.5}$$

$$WBISI = \frac{10000}{\sqrt{FBG \times FBI \times [mean\ glucose\ during\ OGTT] \times [mean\ insulin\ during\ OGTT]}}$$

Height, weight and blood pressure were recorded, blood samples for measurement of liver transaminases and γ-glutamyl transferase were collected, and abdominal ultrasound was performed as described below.

The participants included from the Tromsø Study follow-up were divided into three groups: participants with either transaminases or γ-glutamyl transferase of 2x Upper Limit of Normal

(ULN), participants with values between ULN and 2x ULN, and a selection of participants with normal values, matched for sex and age of the first two groups.

The group with liver enzyme levels of 2x ULN or more was followed up during the first few months after the Tromsø Study visits in 2008. The two other groups were followed up during 2013/2014. The same variables were recorded for all three groups: height, weight, transaminase and γ-glutamyl transferase levels, and fasting blood glucose, serum insulin, and triglyceride levels. Abdominal ultrasound was performed with measurement of HRI. We also collected data for blood pressure from the Tromsø study visits.

Abdominal ultrasound was performed using a Hitachi EUB-6500 HW with a 5 MHz convex EUP-C524 transducer (Hitachi Medical Corporation, Tokyo, Japan). Hepatic and renal parenchymal echogenic density on a grayscale (0-255) was recorded with the built-in histogram function. Values below 1.0 were corrected to 1.0. An average of three repeated measurements was used to calculate hepatorenal index (HRI) by the formula:

$$HRI = \frac{mean\ liver\ echogenicity}{mean\ kidney\ echogenicity}$$

All statistical analyses were carried out using IBM SPSS Statistics, version 21. ROC analyses were performed, detecting insulin resistance at HOMA1-IR values 2.3 and 2.5. Groups of participants with BMI ≥ 30 (n = 46) and BMI ≥ 35 (n = 27) were analysed specifically, in addition to the study population as a whole.

Results

We included 90 participants in the whole study (20 men and 70 women), of which 22 patients were included from the obesity clinic and 68 participants from the sixth Tromsø Study as shown in Figure 1. Median age for the study population as a whole was 64 years (range 21- 82), and median BMI was 30 (range 19.3- 51.2). Baseline characteristics are shown in Table 1.

<insert figure 1>

<insert table 1>

For the participants included from the obesity clinic, we calculated both HOMA1-IR and WBISI, in order to verify the reliability of HOMA1-IR in our dataset. Correlation between HOMA1-IR and WBISI is shown in Figure 2.

<insert figure 2>

Sensitivity and specificity values for different HRI levels for detecting a pathological HOMA1-IR are shown in Table 2. Summarised, the test has a high sensitivity and a relatively low specificity for HRI values corresponding to mild hepatic steatosis (HRI = 1.11), and a low sensitivity with a high specificity for HRI values corresponding to moderate hepatic steatosis (HRI = 1.42). Corresponding ROC curves are shown in Figures 3a – d.

<insert table 2>

<insert figure 3 a-d>

Discussion

In light of the obvious relationship between NAFLD and the MetS, and also in light of the many and severe complications of metabolic dysfunction, having good screening methods for detecting early signs of the MetS is of importance.

We chose the HOMA1-IR for this study because of its better applicability compared to the HOMA2-IR. Previous studies have shown an optimal HOMA1-IR cut-off value of 2.3. Our results show that using HRI as a screening test for detecting insulin resistance (IR) is possible, but it should only be used in groups where the prevalence of IR is high, that is, in people with a BMI of 30 or more. In this group, mild steatosis (HRI $\geq$ 1.11) diagnosed by ultrasound will detect 94 % of patients with a HOMA-IR of 2.0 or more. However, the specificity of the test is low (54 %). Therefore, this test will identify patients with high risk of having IR. We also calculated test properties for HOMA-IR levels of 2.0 and 2.5, because the clinical significance of the HOMA-IR limit may vary, depending on your goals. A limit of 2.0 detects all patients with a genuine insulin resistance, but renders too many patients with a false positive result. A cut-off limit of 2.0 is also not clinically relevant because of a large degree of overlap with normal HOMA-IR values in the population. The results of this analysis are therefore not described in this paper. A limit of 2.5 gives a more clinically applicable HOMA-IR value, but with a higher number of false negative results.

In a previous study of the test properties of HRI, show that a HRI cut-off value of 1.49 has a sensitivity of 100 % and a specificity of 91 % for detecting a 5 % steatosis, diagnosed by liver biopsy (19). Our results show that a HRI cut-off at 1.42 will have a specificity of 91 % in the group with BMI of 30 or more. A HRI result of 1.49 will therefore be diagnostic, both for having an actual hepatic steatosis, and also an actual insulin resistance.

Although a HRI cut-off level of 1.11 will give many false positive results, this is acceptable in a screening test, since the verification of IR is relatively simple through calculating HOMA-IR and/or WBISI.

One of the strengths of this study is that the study population is similar to the patient group that will be relevant for this screening, both because of overweight/obesity and because of pathological liver blood tests. An extrapolation to the general population is however not possible.

We did not perform liver biopsy to confirm the results of the ultrasound examination. Therefore, one cannot say for certain that the participants with HRI <1.49 have hepatic steatosis. The correlation between the actual steatosis and insulin resistance is beside the scope of this article, since this correlation is generally accepted.

One of the weaknesses of the HRI as a means of assessing steatosis is its variability. There is a certain degree of both inter- and intraobserver variability, but the results are also dependent on the type of ultrasound equipment used. HRI values in different studies may not be directly comparable as a result of this. Also, in later ultrasonography models, one of the features available is the option to highlight the liver tissue over other tissues, which will influence on the results. One needs to be aware of this as a source of bias when choosing this method.

The results of our study need confirmation by further studies with more patients/participants included, since the obese group, and particularly the group with morbid obesity had few cases with insulin resistance.

Conclusion: Mild hepatic steatosis (HRI ≥1.11) diagnosed by ultrasound is a good test for detecting insulin resistance, especially in obese patients. The specificity of the test is however low. Patients with a hepatorenal index of 1.42 or more are likely to have a true positive result for insulin resistance, i.e. having a HOMA-IR value of 2.3 or more.

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Legends to figures

Figure 1: Flowchart of included participants from the Sixt Tromsø Study and the Obesity clinic, UNN

Figure 2: Correlation between WBSI and HOMA-IR for 18 overweight or obese participants.

Figure 3a: ROC curve of HOMA-IR >2.3 by hepatorenal index in participants with BMI ≥30

Figure 3b: ROC curve of HOMA-IR >2.3 by hepatorenal index in participants with BMI≥35

Figure 3c: ROC curve of HOMA-IR >2.5 by hepatorenal index in participants with BMI ≥30

Figure 3d: ROC curve of HOMA-IR >2.5 by hepatorenal index in participants with BMI ≥ 35

Figure 1:

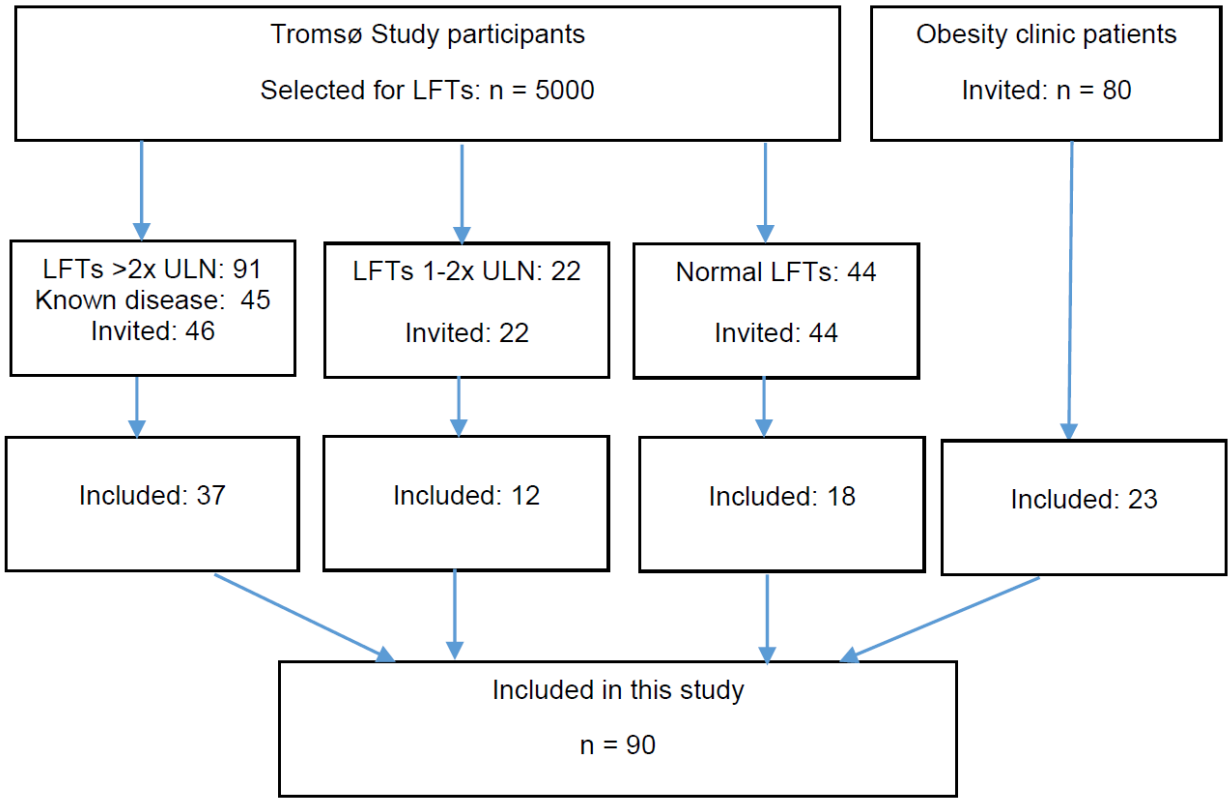
Tromsø Study participants
Selected for LFTs: n = 5000
Obesity clinic patients
Invited: n = 80
LFTs >2x ULN: 91
Known disease: 45
Invited: 46
LFTs 1-2x ULN: 22
Invited: 22
Normal LFTs: 44
Invited: 44
Included: 37
Included: 12
Included: 18
Included: 23
Included in this study
n = 90

Figure 2:

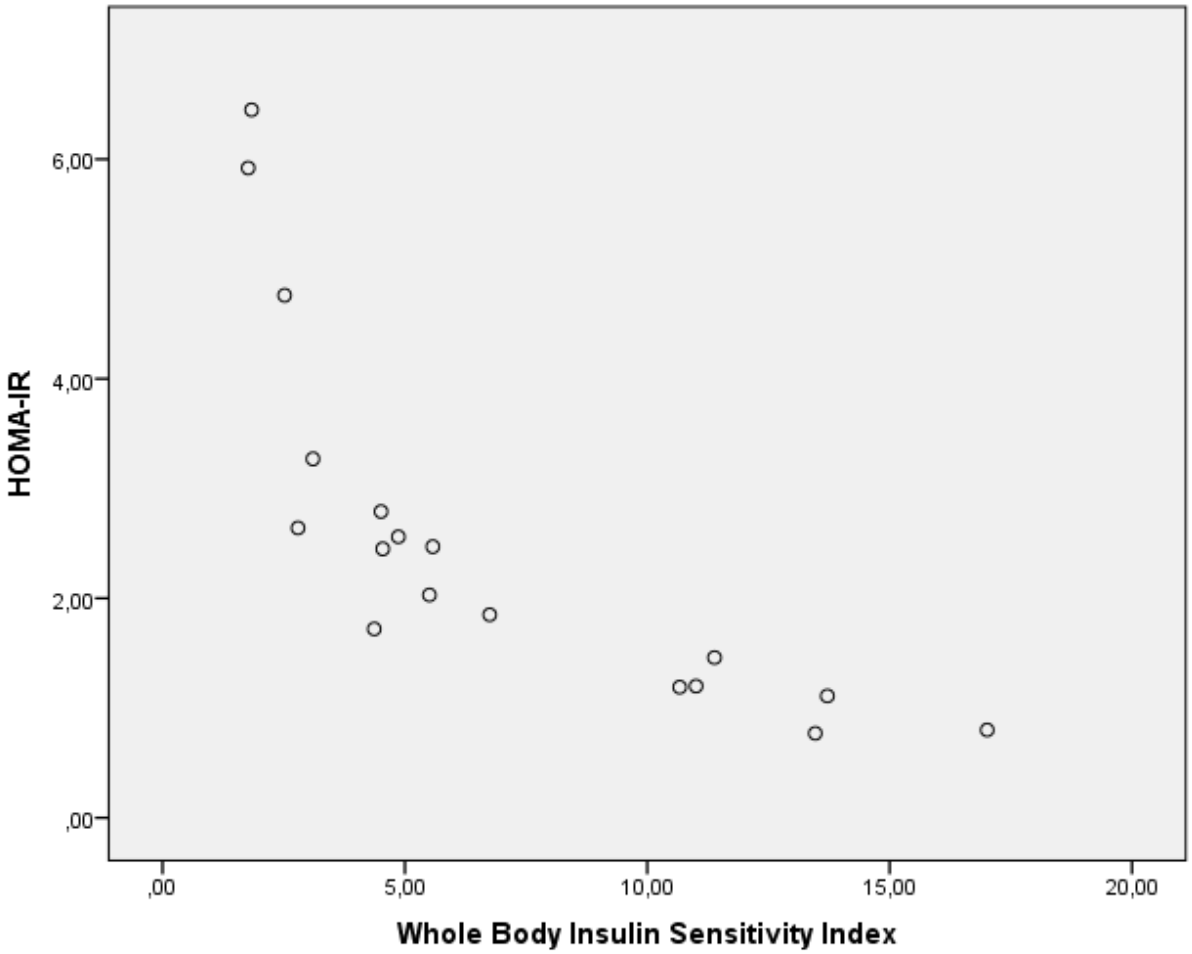
HOMA-IR
6,00
4,00
2,00
,00
,00
5,00
10,00
15,00
20,00
Whole Body Insulin Sensitivity Index

Figure 3a:

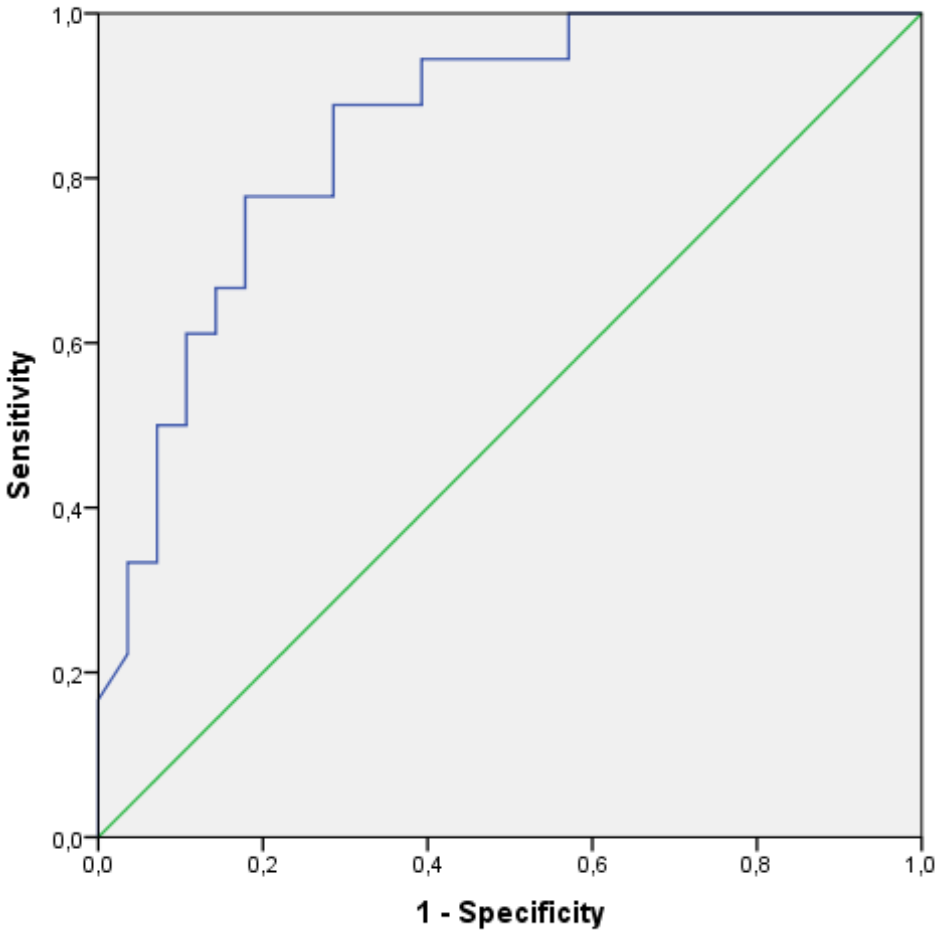

Fig 3b:

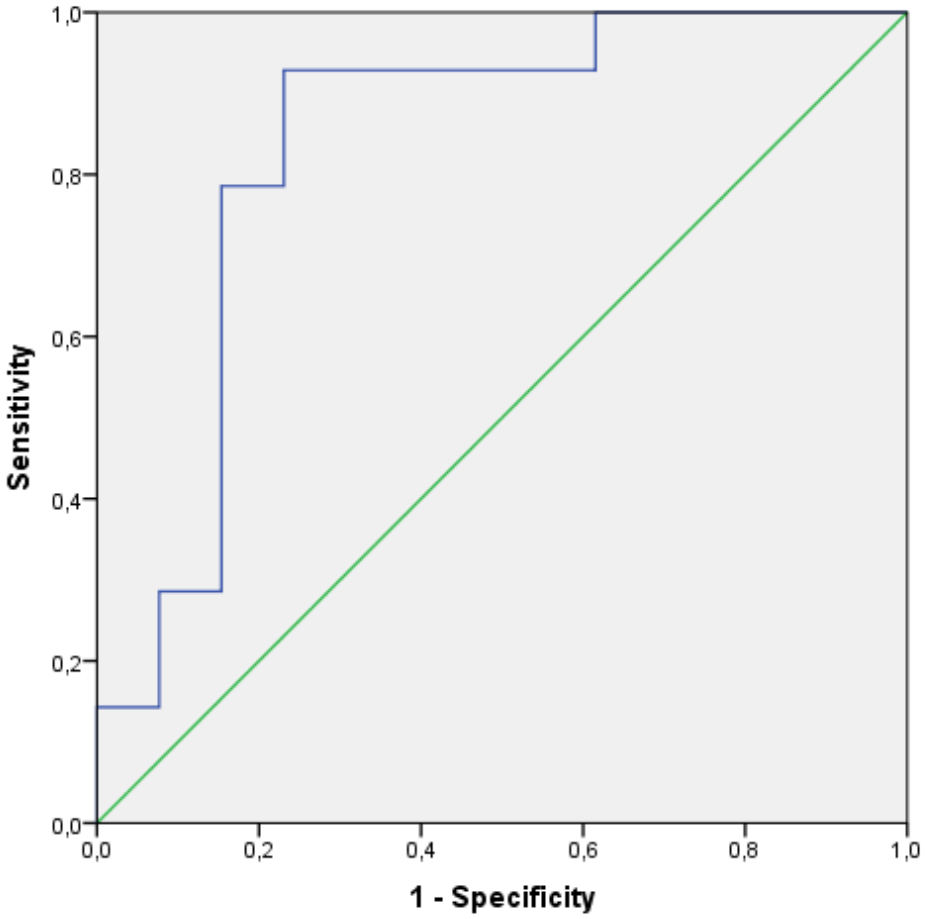

Fig 3c:

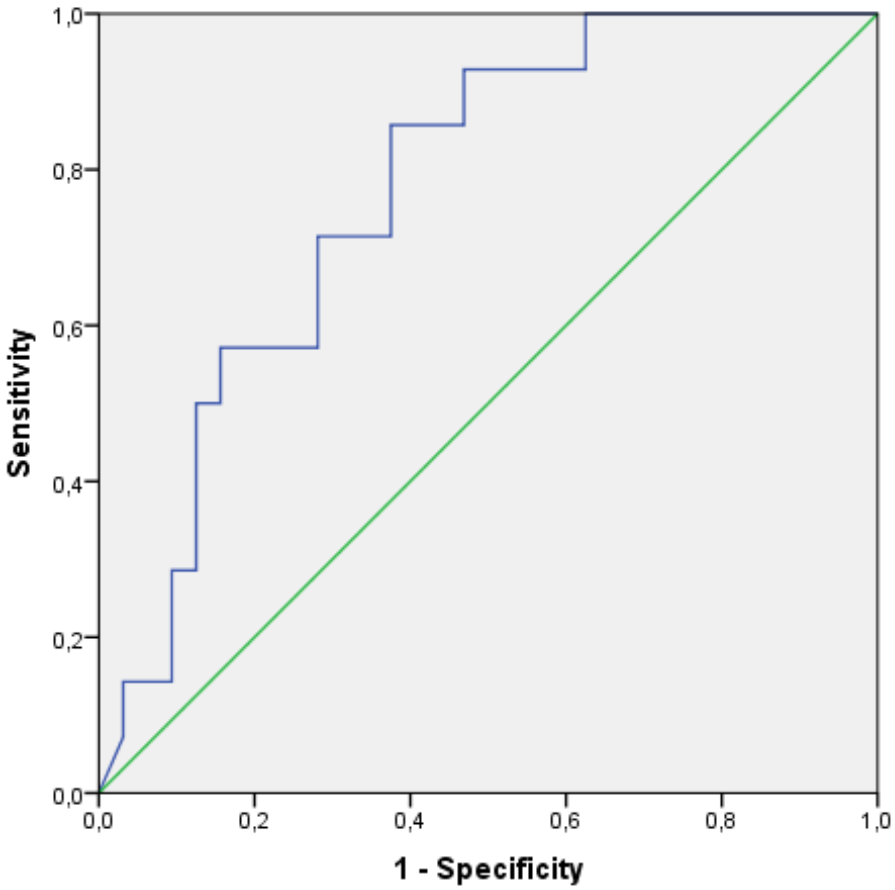

Fig 3d:

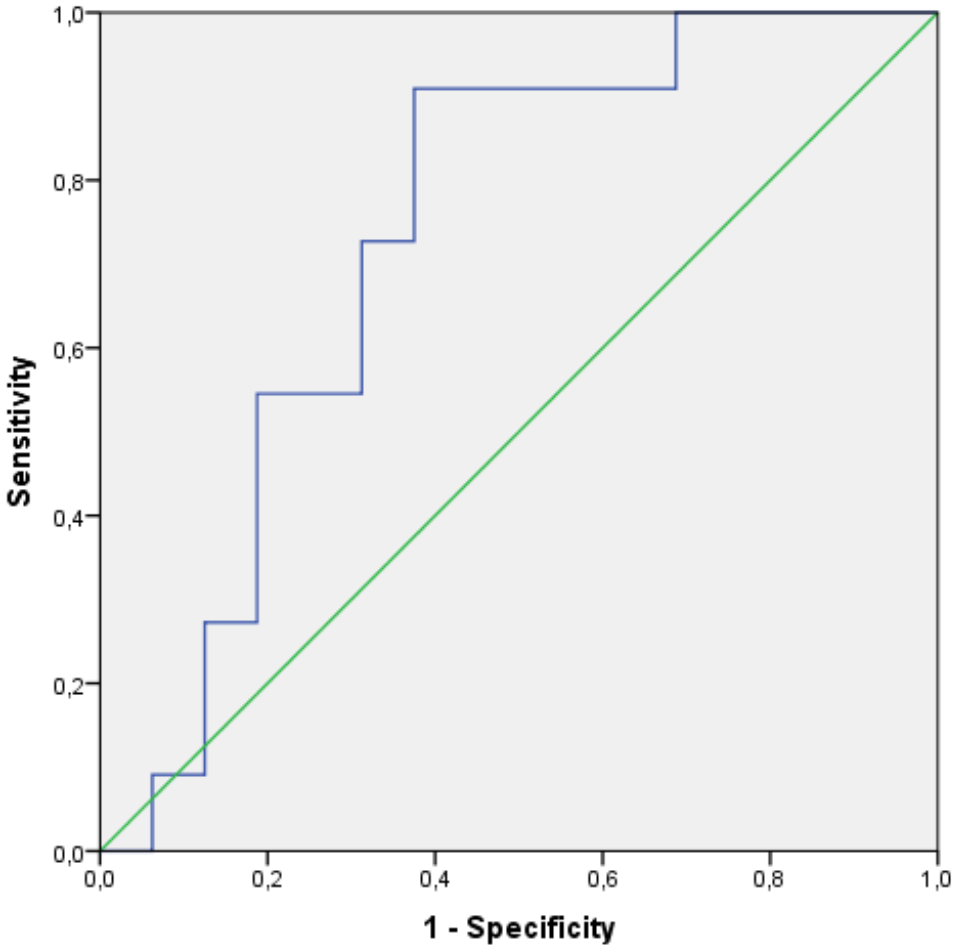

Table 1: Baseline characteristics of 90 participants in the study

Variables	Inclusion groups					
	Obesity clinic			Sixth Tromsø Study		
	N	Median(SD)	Range	N	Median (SD)	Range
Age, years	22	43,0 (12,76)	21-69	68	66,0 (10,84)	32-82
Height, cm	21	168,0 (6,79)	156-179	68	166,0 (9,08)	141-189
Weight, kg	22	113,0 (16,13)	83,5-148,0	68	81,3 (15,51)	50,6-123,5
Systolig BP, mmHg	22	126,0 (11,36)	112-159	68	137,0 (22,07)	96-213
Diastolic BP, mmHg	22	74,0 (8,16)	62-94	68	78,0 (8,42)	50-102
BMI, kg/m^2	21	41,4 (5,21)	31,8-51,2	68	28,0 (5,35)	19,3-45,6
ASAT, U/L	22	18,0 (9,32)	12,0-53,0	66	29,5 (1,23)	14,0-71,0
ALAT, U/L	4	38,5 (6,70)	29,0-45,0	66	39,0 (20,36)	14,0-102,0
γ-GT, U/L	21	29,0 (45,72)	13,0-198,0	65	82,0 (82,74)	14,0-398,0
ALP, U/L	4	88,0 (9,22)	80,0-99,0	66	81,0 (38,96)	36,0-322,0

Table 2: Sensitivity and specificity for different levels of HRI for the prediction of pathological HOMA1-IR

HOMA-IR ≥2.3

	All participants (n = 24)		BMI ≥30 (n = 18)		BMI ≥35 (n = 14)	
HRI cut-off level	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
1.11	0.92	0.56	0,94	0.54		
1.17			0.89	0.71	0.93	0.77
1.42	0.29	0.94	0.33	0.96	0.29	0.92

HOMA-IR ≥2.5

	All participants(n = 20)		BMI ≥30 (n =14)		BMI> 35 (n = 11)	
HRI cut-off level	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
1.11	0.90	0.53				
1.17			0.86	0.62	0.91	0.62
1.42	0.25	0.92	0.29	0.91	0.27	0.88