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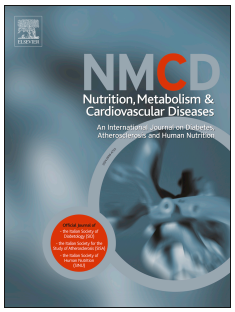

Insulin resistance, but not insulin response, during oral glucose tolerance test (OGTT) is associated to worse histological outcome in obese NAFLD

Federica Coccia, Moira Testa, Gloria Guarisco, Claudio Di Cristofano, Gianfranco Silecchia, Frida Leonetti, Amalia Gastaldelli, Danila Capoccia

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POSITIVE
ENERGY BALANCE

Buffering of excess energy in subcutaneous adipose tissue

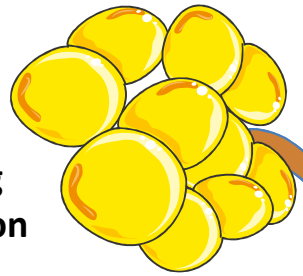
Muscle IR

Toxic effects of post load high glucose concentrations promotes liver steatosis

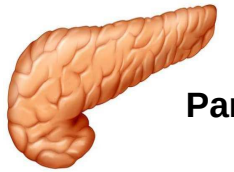
Oral glucose clearance during OGTT (OGIS) significantly reduces with increasing of histological liver damage (p<0,05)

Lipid spillover

Tissue remodeling and expansion



Pancreas



Increased insulin release due to decreased insulin sensitivity

Insulinogenic index was strongly associated to steatosis (p<0,0001)

↑Insulin
↑FFA

Increased insulin is associated to NASH

Higher insulin concentrations during OGTT in NAFLD/NASH than NO-NAFLD (p 0,04)

Insulin resistance, but not insulin response, during oral glucose tolerance test (OGTT) is associated to worse histological outcome in obese NAFLD

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Running Head
Altered glucose metabolism in obese with NAFL/NASH

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Abbreviations:

OGIS oral glucose insulin sensitivity index, **NAFLD** Nonalcoholic fatty liver disease, **IR** insulin resistance, **IS** insulin sensitivity, **NASH** Non- alcoholic steatohepatitis, **SS** simple steatosis, **HOMA-IR** homeostasis model assessment of insulin resistance, **MS** metabolic syndrome, **T2D** type 2 diabetes mellitus, **OGTT** oral glucose tolerance test, **BS** Bariatric Surgery, **LSG** laparoscopic sleeve gastrectomy, **HDL** high-density lipoprotein cholesterol, **ALT** alanine aminotransferase , **AST** aspartate aminotransferase, **γ-GT**γ-glutamyl-transpeptidase,

Abstract

Background Obese subjects are at high risk of nonalcoholic fatty liver disease (NAFLD) and diabetes (T2D) due to insulin resistance (IR). Since high glucose levels are as toxic as lipids for hepatic metabolism, we hypothesize that altered response to oral glucose tolerance test (OGTT) is associated to more severe NAFLD with significant/advanced liver damage.

Methods and Results We studied 90 subjects with morbid obesity (73F/17M, BMI=43.2±5,9Kg/m^2) undergoing bariatric surgery and intraoperative liver biopsy, and measured HbA1c, HOMA-IR (fasting Glucose x Insulin/22.5), OGTT glucose and insulin profile, and calculated OGIS (muscle insulin sensitivity), hepatic-IR (glucose[$AUC_{0\text{-}30}$] x insulin[$AUC_{0\text{-}30}$]) during OGTT, insulin response as (insulin[$dAUC_{0\text{-}120}$] /glucose[$dAUC_{0\text{-}120}$] or Insulinogenic Index (IGI= $(I_{30}\text{-}I_0)/(G_{30}\text{-}G_0)$).

Patients were divided in 3 groups according to liver biopsy: A (no-NAFLD, 23%), B (simple steatosis (SS), 53%) and C (NASH, 24%) with similar age, gender and BMI. Diabetes was 0% in no-NAFLD, 13% in SS, 35% in NASH.

During OGTT, OGIS decreased from A to C (422 vs 360 vs 338, $p<0.01$). Increased insulin concentrations, HbA1c, HOMA-IR and OGIS, not Hep-IR, were strongly associated to hepatic steatosis (p0.03, p0.0001 and p0.01 respectively) Hepatic fibrosis stage was mild as most of the patients had fibrosis grade-1 (69% vs. 8% no fibrosis) and associated to fasting insulin, HbA1c and HOMA-IR. Total insulin response was similar in the 3 groups, while IGI was strongly associated to steatosis ($r=0.48$, $p<0.0001$), but not to fibrosis.

Conclusions: in morbid obesity OGTT-indexes of IR, and not of insulin response, are markers of histological severity of liver disease.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease whose prevalence has linked to the global obesity epidemic [1]. Ongoing persistence of obesity with increasing rate of diabetes will further increase the risk of NAFLD/NASH, that is becoming the leading indication for liver transplantation in the United States [2, 3].

Obesity has been linked not only to initial stages of the disease but also to the progression of steatosis to nonalcoholic steatohepatitis (NASH), NASH-related cirrhosis and hepatocellular carcinoma (HCC) leading to an increased morbidity and mortality when combined with NAFLD [4], owing to cardiovascular and liver-specific mortality.

Moreover, NAFLD is also a risk factor for type 2 diabetes (T2D) [3, 5]. The worldwide prevalence of NAFLD in the general population has been estimated to range 6.3–33%, with a median of 25% [1] and is increasing with the rise in the incidence of obesity and T2D. Though, prevalence is over 85% in morbid obese [4] and 75.6% in patients with T2D independent of obesity. The prevalence of nonalcoholic steatohepatitis (NASH) has been estimated to range 3–5% [1], but it can be more than 10 times higher (30-90%) in subjects with morbid obesity undergoing bariatric surgery [4, 6].

Only few studies have investigated glucose metabolism and insulin sensitivity during OGTT in biopsy proven NAFLD and (nonalcoholic steatohepatitis) and none, to our knowledge, in subjects with morbid obesity. Rosso et al have recently shown that decreased insulin sensitivity during OGTT is associated to increased liver fibrosis but not steatosis in lean to mild obese patients with NASH [7].

Several features of Metabolic syndrome (MS), particularly T2D, dyslipidemia, hypertension and abdominal obesity, are risk factors for the presence of NASH [2, 8, 9] probably due to insulin resistance (IR), one of the main pathogenic mechanisms for the onset and progression of NAFLD [9-12].

In the lean or moderate obese NAFLD, the indexes of peripheral IR (HOMA-IR and OGIS) have been associated to presence of NAFLD and more severe liver damage [13-15]. Hepatic IR is also increased in NAFLD [11, 12]. OGIS was associated to increased liver fibrosis but not steatosis in lean to mild obese patients with NASH [7]. However, if this is true also in subjects with morbid obesity has not been investigated. Subjects with morbid obesity often have a lower degree of fibrosis [6] and the relationship among glucose tolerance, indexes of insulin sensitivity and severity of fibrosis and NAFLD might be different than in subjects with lower BMI. In morbid obesity, adipose tissue function is still preserved and subcutaneous adipose tissue can expand becoming a buffer for excess circulating lipids, thus limiting the accumulation as ectopic fat [16]. However,

adipocyte hypertrophy has been linked with a worse metabolic profile in several studies [16]; ongoing persistence of obesity with increasing rate of diabetes will further increase the risk of NAFLD/NASH. Bedossa et al shown that morbid obese subjects with presumably normal liver, have lower prevalence of diabetes, hypertension and hyperlipidemia than those with NAFLD/NASH and adipocyte size were increased in females while decreased in males. But he did not evaluate the impact of IR on severity of NAFLD [6].

IR is considered one main driver for NAFLD since high insulin concentrations stimulates adipogenesis and lipotoxicity [11]. Although fasting measures of IR like HOMA-IR are often associated to NAFLD [17], only OGTT derived IR indices were associated with liver damage and the OGIS index was the best predictor of significant fibrosis in a cohort of overweight or moderate obese subjects (≥F2) [14]. Moreover, the analysis of markers of NAFLD/NASH and imaging tests is difficult in this population. Ooi et al have proposed new thresholds for identfying fibrosis in morbid obesity [18] Other authors reported that, in morbid obesity, low adiponectin levels are associated with more severe liver histology [19] and that FibroTest, ActiTest, SteatoTest, and NashTest are useful in quantifying liver damage [20] But most of these exams are more expensive exams than those of our study

Thus, the aim of our study was to evaluate if altered glucose profile and reduced glucose clearance during OGTT were associated to more severe NAFLD (i.e: NASH and/or significant/advanced liver damage at liver biopsy in a well-characterized cohort of morbid obese subjects undergoing bariatric surgery (BS).

Materials and methods

PATIENTS

The study reports data on 90 subjects with morbid obesity undergoing BS at the Department of Medico-Surgical Sciences and Biotechnologies Division of General Surgery and Bariatric Center of Excellence, Sapienza University, Latina, Italy for which liver biopsy and metabolic measurements were available. The local ethics committee approved the present clinical investigations and all subjects gave written informed consent.

Bariatric surgery (BS) was indicated following to international guidelines [21-23]. All the contraindications to BS represented exclusion criteria for our study. Exclusion criteria included autoimmune, inflammatory or infectious diseases, viral hepatitis, cancer, known alcohol

consumption (>20 g/day) or kidney diseases. All patients underwent laparoscopic sleeve gastrectomy (LSG) and surgical liver biopsy during LSG. Subjects were grouped according to presence of absence of NAFLD according to EASL-EASD-EASO guidelines [2] as:

- **Group A**, with presence of fat accumulation in less than 5% of hepatocytes, defined "NO NAFLD"
- **Group B** with steatosis alone or steatosis with lobular or portal inflammation, without ballooning or steatosis with ballooning but without inflammation, defined "simple steatosis (SS)"
- **Group C** characterized by joint presence of steatosis, ballooning and lobular inflammation and so defined "NASH".

CLINICAL AND LABORATORY ASSESSMENT

Patients underwent a complete medical history, physical examination and fasting blood tests preoperatively. Type 2 diabetes (T2D) was defined in agreement with American Diabetic Association criteria or the use of antidiabetic drugs [24]. Hypertension, dyslipidemia and MS were identified according to International Diabetes Federation [25].

Glucose tolerance was evaluated before surgery by OGTT in subjects without known diabetes (n=77), at 0, 30, 60, 90 and 120 min of OGTT to assess glucose and insulin. Area under the curve (AUC) of glucose and insulin during OGTT was calculated.

MEASURES OF FASTING and OGTT INSULIN RESISTANCE

HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) was calculated as (fasting_insulin (mU/L) x fasting_glucose (mmol/L))/22.5 [13, 14].

OGIS (Oral Glucose Insulin Sensitivity index) is a dynamic index insulin sensitivity that represents glucose clearance and reflects whole body insulin sensitivity during OGTT [14, 26].

Hepatic IR index: was calculated as the product of area under curve (AUC) for glucose and insulin during the first 30 min of the OGTT (glucose0-30[AUC] x insulin0-30[AUC]) [27].

MEASURES OF OGTT INSULIN RESPONSE

We evaluated insulin response by calculating

Insulino-Genic Index (IGI) obtained by the ratio of $(I_{30}-I_0)/(G_{30}-G_0)$

Insulin Response obtained by ratio of the incremental areas under the curve of insulin to glucose, calculated from 0 to 120 min dAUC-I/dAUC-G

LIVER BIOPSY

Surgical liver biopsies of the left lobe were systemically performed during LSG using the same standardized procedure with a scalpel blade and each liver fragment measured at least 1 500µl di NH_4OH 5M cm^2 on cut section. Tissue samples were formalin-fixed and paraffin-embedded. Serial sections were stained with H&E picrosirius red and Perls' iron stain. A single liver pathologist reviewed all biopsies. Histological features of NAFLD, i.e., steatosis, inflammation, hepatocyte ballooning, and fibrosis, were scored according to Kleiner et al [28].

The diagnosis of NASH was defined by the local pathologist according to the joint presence of steatosis, hepatocyte ballooning, and lobular inflammation with or without fibrosis on liver biopsy[2].

In regard to fibrosis, stage 1 referred to perisinusoidal fibrosis in zone 3 (perivenular area: delicate [1A] and dense [1B]), and detection of portal fibrosis without perisinusoidal fibrosis was defined as 1C. Stage 2 was characterized by perisinusoidal and portal/periportal fibrosis. Stage 3 was defined as bridging fibrosis and stage 4 as cirrhosis [28].

STATISTICS

Data are reported as mean ± standard deviation, 95% CI, for continuous normally distributed variables and number and frequency (%) for categorical variables. Comparisons between groups were performed using the two tailed Student t test or analysis of variance (ANOVA) for normal continuous variables and the Wilcoxon or $\chi 2$ test for categorical variables. All IR/IS indexes were calculated using previous published formulas [12,13, 22, 23]. To evaluate the association between several surrogate indexes of IR/IS and fibrosis, steatosis or liver inflammation, Pearson correlation analysis was used. Univariate and multivariable logistic regression analyses were used to assess IR/IS indexes associated with the severity of liver disease. Values of $P < 0.05$ were considered statistically significant. All calculations were performed with SPSS version 22.0.

Results

Clinical and histological characteristics of study subjects

Baseline characteristics of the 90 morbid obese patients are shown in **Table 1**. Most patients were females (81%). Mean BMI was 43.2 kg/m^2. T2D was detected in 16%, hypertension in 28%, dyslipidemia in 49% and MS in 43% of the cohort.

In the entire cohort prevalence of NAFLD was 77%. We have distinguished 3 different groups according to liver biopsy results (**Table 1**): group A without NAFLD, composed by 21 subjects

(23%) mainly females (19/2 F/M); group B with SS, composed by 47 patients (53%), 35 females and 12 males; group C with NASH, composed by 22 patients (19%), 19 females and 3 males.

The degree of steatosis, lobular inflammation and ballooning was significantly different among the 3 groups, as expected, whereas fibrosis stage was different only between NO-NAFLD (group A) and NAFLD (group B and C). Most of the subjects had SS or early NASH with fibrosis F1; 10 SS and 2 NASH subject had fibrosis F2, one NASH had F3 but none had cirrhosis. Fibrosis stage was higher in IGT and T2D (69% F1; 31% ≥F2, respectively) than NGT (8% F0, 80% F1, 8%≥ F2)

Group A, B e C were compared for demographic, anthropometrical, clinical and metabolic characteristics (**Table 1**). The three groups showed similar age, gender and anthropometric characteristics. As regard to serum liver enzymes, ALT and γ-GT increased significantly with the severity of liver disease ($p<0.01$). In particular, these values were significantly higher in patients with SS or NASH (Group B and C) than in no-NAFLD patients (Group A) although no significant differences were observed between patients with SS (Group B) or NASH (Group C). Patients with NASH showed lower HDL concentrations compared to patients without NASH ($p=0.04$). No significant difference was observed for total and LDL cholesterol. Triglycerides and uric acid were significantly higher in NAFLD patients (group B and C) than NO-NAFLD (**Table 1**).

Prevalence of metabolic and cardiovascular complications, T2D hypertension, dyslipidemia and MS, is also reported in **Table 1**. In this cohort the great majority of the subjects had normal glucose tolerance, (NGT were 61%), impaired glucose tolerance (IGT) was present in 23% while diabetes was present in 13% (**Table 1**), i.e., 13 T2D patients of which 6 were newly diagnosed, 4 patients had a history of T2D lower than 5 years and 3 patients had a history of T2D of 5-10 years. Patients with SS or NASH showed increased prevalence of complications although the differences were significant only for T2D and MS. It is important to note that none of the subjects in group A (no NAFLD) had T2D.

Glucose and insulin changes during fasting and OGTT

Fasting glucose was higher in patients with NAFLD (Group B and C) compared to patients with no NAFLD, but no significant difference was observed between Group B and C (**Table 1)**. HbA1c was significantly higher in NAFLD compared to subjects without NAFLD (**Table 1**). Fasting insulin concentrations were elevated compared to healthy non-obese subjects, but similar among the three groups. HOMA-IR was elevated compared to normal values [13] but it was similar in the 3 groups.

Glucose tolerance was assessed during a standard oral glucose tolerance test (75g) performed before surgery. Glucose and insulin concentrations during OGTT are shown in **Figure 1;** OGTT insulin concentrations and insulin AUC increased significantly from group A to C indicating reduced insulin sensitivity (**Figure 1**).

The insulin sensitivity index during OGTT (i.e., OGIS index, that is a surrogate measure of insulin sensitivity and estimates mean glucose clearance during OGTT) was reduced significantly with the degree of liver disease, reaching lowest values in patients with NASH whereas hepatic IR index increased from group A to C (**Table 2**). On the other hand, the insulin response during OGTT, measured as early insulin response (IGI, from 0-30min) or as dAUC-I/dAUC-G (from 0-120min), was not different among the 3 groups (**Table 2**).

Associations between metabolic parameters and liver histology

In the entire cohort HbA1c was significantly associated to worse steatosis grade at liver biopsy ($r=0.482$, $p<0.0001$), liver inflammation ($r=0.325$, $p<0.006$) and fibrosis ($r=0.34$, $p<0.004$). Also fasting glucose was associated with steatosis grade at liver biopsy ($r=0.33$, $p<0.005$), but not to hepatic inflammation ($r=0.11$, $p=0.35$) or fibrosis ($r=0.21$, $p=0.077$).

HOMA and OGIS, but not Hepatic-IR, were associated to steatosis ($r=0.31$ and $r=-0.34$, respectively, $p<0.01$). HOMA, but not OGIS, correlates also with fibrosis grade ($r=0.41$, $p<0.02$), while the associations with hepatic inflammation did not reach statistical significance.

Fasting insulin concentrations were associated to worse steatosis grade at liver biopsy ($r=0.280$, $p<0.025$) and fibrosis ($r=0.46$, $p<0.0002$) but not to liver inflammation. Insulinogenic index (IGI, i.e., early insulin response during OGTT), but not dAUC-I/dAUC-G (total insulin response to the glucose load), was associated to steatosis grade at liver biopsy ($r=0.482$, $p<0.0001$), but not to fibrosis or liver inflammation.

Discussion

In our study, 90 patients with morbid obesity were enrolled, studied for glucose tolerance and liver biopsies were systematically performed during LSG independently of any symptoms. Based on the results of liver biopsy, subjects were categorized as no NAFLD (group A), SS (group B) or NASH according to the European guidelines [2]. The three groups were similar for age, gender, BMI (**Table 1**), but serum liver enzymes and fasting glucose significantly increased in SS and NASH.,

while only a non-significant trend was observed for increased AUC-glucose during OGTT. Prevalence of T2D was 0 in subjects without NAFLD (group A), while it was 15% and 35% in SS and NASH respectively.

In our cohort of morbid obese subjects 23% were without NAFLD, 53% had SS and 24% had NASH. Although the prevalence of NAFLD and NASH in the general population has been now estimated to be 25% and 6% respectively [1], the prevalence in morbid obesity is much higher but still unknown, in part because of difficulties in performing imaging techniques and because liver biopsy, i.e., the gold standard for diagnosis of NASH, is usually performed only in subjects with suspect of severe liver disease. Moreover, the association between prevalence of NAFLD and glucose intolerance or T2D is also unknown. In morbid obese subjects liver biopsy is usually taken during BS. In the review of available studies on prevalence of NAFLD/NASH comprising a large cohort of severely obese patients (n=1620) Machado et al [4] reported 91% prevalence of NAFLD (85-98%) but wide variability in prevalence of NASH (24-98%, mean 37%). Other following studies reported a similar prevalence of NASH, i.e., Bedossa reported 35% [6] and Petrick 32%[29], while Lassailly a much lower prevalence (7.7%) [30]. The prevalence of NASH in our study was 24%, lower than Bedossa (35%) [6] or Petrick (32%) [29], but similar to Machado [4]. As expected, the degree of steatosis, lobular inflammation and ballooning was significantly different among the 3 groups observing the highest-grade cases in group with NASH.

NAFLD is strongly associated to IR and is a major risk factor for T2D [1]; for this reason the current European guidelines for the management of NAFLD [2] have suggested the screening for NAFLD and IR in all subjects at risk. Many studies have shown that subjects with morbid obesity have increased IR not only in the muscle but also in the liver and adipose tissue [10, 11, 14, 31, 32]. Fasting IR, estimated using HOMA-IR, is associated to a worse and harmful liver lipidome, enriched in saturated and monounsaturated triacylglycerols, free fatty acids and dihydroceramides [33]. We observed that HOMA-IR tended to increase from no-NAFLD to SS and NASH, but only OGIS that represents a measure of whole body insulin sensitivity, not fasting insulin nor HOMA-IR, were significantly lower in morbidly obese NASH compared to SS.

Although fasting IR was often found independently associated with severe steatosis and presence of NASH [4, 30, 34], in previous studies there was no report of glucose tolerance or IR state in response to glucose challenge. Souto et al analyze the degree of tissue damage in liver biopsies of 521 obese patients, concluding that higher hepatic fibrosis were observed in diabetic (56.4%)

compared with prediabetic (29.2%), and normoglycemic patients. But they focused only on fasting glucose tolerance [35].

Our results showed that subjects with morbid obesity have an impairment in both glucose tolerance and insulin sensitivity during OGTT worsening with the degree of liver damage/fibrosis. It appears that post-prandial high glucose concentrations might exert toxic effects in these patients. This may be explained by the fact that insulin is the main driver of de novo lipogenesis and lipid synthesis from glucose since it activates SREBP-1c [36]. Thus, the combination of high glucose and high insulin after glucose load stimulates the conversion of glucose into triglycerides and promotes hepatic steatosis [36]. Since high glucose levels are toxic for the liver and post-prandial hyperglycemia often precedes fasting hyperglycemia, OGTT could represent a simple and useful exam to investigate liver damage in a noninvasive way in patients with morbid obesity.

Fibrosis is the most important prognostic factor in NAFLD and is correlated with liver-related outcomes and mortality [37]. A higher stage of fibrosis is accociated to a worse glucose tolerance as confirmed by Strey et al that reported that T2D is as an independent risk factor for severe fibrosis [38]. Previously, Rosso et al [14] performed OGTT in NAFLD non-diabetic patients and found that subjects with higher degree of liver fibrosis (F3-F4) also had higher glucose excursions; moreover, peripheral glucose clearance (measured using OGIS) was significantly reduced in proportion to degree of liver fibrosis. The association with fibrosis appears to be mediated by post-load glucose metabolism, independently of BMI and the degree of steatosis. Similar association between OGIS and liver fibrosis was previously observed by Svegliati et al [15] in non-obese or moderate obese subjects with NAFLD or HCV. Also in this study reduced glucose clearance during OGTT expressed by OGIS reached lowest values in patients with with NASH but it's not significantly associated to fibrosis degree, as reported by Rosso [14] and Svegliati et al [15] probably because, unlike these studies, most of our patients had early fibrosis (<F2). On the other hand, HbA1c was associated to increased hepatic steatosis, inflammation and fibrosis. These results suggest that, in subjects with morbid obesity, liver damage severity and fibrosis are associated more with metabolic impairment than BMI and confirmed the importance of screening for alterations in glucose metabolism, as well as for NAFLD.

The strong association between liver steatosis and obesity poses subjects with severe obesity at higher risk not only of NAFLD but also of related comorbidities. Obesity has increased morbidity and mortality when combined with NAFLD, owing to cardiovascular and liver-specific mortality. As expected, prevalence of diabetes and other comorbidities associated to IR and metabolic

syndrome, as hypertension and dyslipidemia, was higher in patients with SS and NASH. These subjects, despite increased insulin concentrations during OGTT have reduced insulin sensitivity and glucose clearance (measured by the OGIS index).

It is important to notice that patients without NAFLD and patients with SS or NASH had similar BMI, gender and age. This suggests that in patients with morbid obesity the severity of liver damage depends not so much on the degree of obesity and BMI, as suggested by Bedossa et al[6], but rather on the degree of metabolic impairment and presence of comorbidities. Indeed, previous studies have not evaluated the association among severity of NAFLD, and so many OGTT derived IR-indices in morbid obesity. Our findings are in agreement with the recent meta-analysis by Lu et al that, although for NAFLD patients, obesity could predict a worse long-term prognosis, it may not be an independent factor for the development of NASH or advanced fibrosis [39]. Also the study by Rosso et al found that glucose tolerance worsen in subjects with liver fibrosis independent of obesity, while steatosis does not [14]. Also Machado [3] reported that NASH was not related with age or BMI, as in our study, but he found an association between male sex and NASH/hepatic fibrosis. In our study, most patients were females so a possible role of the gender on NAFLD differences in morbid obesity could not be evaluated.

In our cohort serum liver enzymes, ALT and γ-GT, although within normal ranges, increased significantly with the severity of liver disease, similar to Bedossa et al [6]. Elevated γ-GT and ALT, although used often as markers of fatty liver disease, can be in the normal ranges also in NASH subjects [40, 41]. Increased ALT and γ-GT, but within normal ranges are associated to increased hepatic IR [42]. The Fatty Liver Index (FLI),that uses γ-GT, was found associated to peripheral IR measured by the clamp in the RISC study [43]. In the KORA study and the DESIR study increased γ-GT concentrations and FLI were significantly associated with prediabetes and diabetes and were also associated with incident hypertension in the DESIR and RISC studies [43-45].

NAFLD is asymptomatic but associated to an increased cardiovascular risk [1]. So, due its high prevalence in obesity, to identify serum biomarkers able to predict the severity of liver tissue in this population is a challenge. Noninvasive scores of steatosis or fibrosis described in literature [46] show often a poor accuracy in severe obesity, as described by Blond et al, that applied the recent EASL–EASD–EASO clinical practice guidelines for the management of NAFLD in a retrospective cohort of 385 subjects with morbid obesity, using the NAFLD Fibrosis Score (NFS) and its combination with Transient Elastography (TE) to screen for advanced fibrosis, and found an excessive number of individuals to address to specialist [47].

A strength of this study is that morbid obesity associated NAFLD was characterized simultaneously for histological, clinical and metabolic characteristics including not only fasting glucose tolerance but also several OGTT derived indices of IR. In particular, we found that some OGTT derived indices of IR, as decreased peripheral insulin sensitivity measured by OGIS, were more meaningful than fasting IR indices and could be a marker of severe liver histology in NAFLD morbid obese subjects. A limitation of the present study might be in the relatively low number of subjects, mostly females, and the fact that the 3 different groups are not homogeneous as sample size and this could explain why some results, although encouraging, do not reach statistical significance.

Conclusion:

In morbid obese subjects with NAFLD, a reduced glucose clearance during OGTT (OGIS), leading to increased insulin secretion due to IR, is associated to a worse liver tissue damage and could be a possible mechanism of onset and progression of NAFLD. This finding could be useful for clinicians suggesting that in clinical practice OGTT should be performed not only to identify glucose intolerance but also NAFLD severity.

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'Authorship Statement'**:**

• *Guarantors of article:* F.L. and A.G: are the guarantors for this manuscript.

• *Specific author contributions:* F.L. and D.C. designed the research study; F.C., G.S. and A.G. contributed to the design of the study; F.C., M.T., G.G, D.D.C., G.S., performed the research and collected the data; F.C., F.L., A.G., D.C. analyzed the data and wrote the paper.

• *ALL authors approved the final version of the article, including the authorship list.*

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Conflicts of Interest

The authors declare no conflict of interest for this paper

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Table 1 Comparative data between patients without NAFLD (Group A), Simple Steatosis (Group B) and NASH (Group C). Comparisons were performed between subjects without NAFLD (A) vs patients with NAFLD (B+C) and between subjects with simple steatosis (B) vs subjects with NASH (C)

	Entire cohort (n=90)	**A NO NAFLD (n=21)**	**B Simple steatosis (n=47)**	**C NASH (n=22)**	P A vs B/C	P B vs C
Demografic						
Age (years)	40.29±11.65	41.11±12.52	39.07±11.28	42.47±11.96	ns	ns
Gender (F/M)	73/17	19/2	35/12	19/3	**ns**	ns
Clinical						
NGT/IGT/T2D (%)	61/23/16	78/22/0	59/26/15	47/18/35	**0.05**	ns
Diabetes Treatment (Only diet/metformin/insulin) (%)	23/69/8	--	14/72/14	17/83/0	**ns**	ns
Hypertension (%)	28	19	30	32	**ns**	ns
Dyislipidemia (%)	49	43	51	50	**ns**	ns
Metabolic Syndrome (%)	43	24	51	45	**0,05**	ns
Liver Biopsy						
Steatosis S0, S1/S2, S3/S4 (n)	21/41/28	21/0/0	0/32/15	0/9/13	**<0,0001**	**0,009**
Inflammation (Y/N) (n)	52/38	9/12	21/26	22/0	**0,008**	**<0,0001**
Balloning (Y/N) (n)	30/60	0/21	8/39	22/0	**0,001**	**<0,0001**

Fibrosis F0, F1, F2/F3,F4 (n)	8/69/13/0	4/17/0/0	4/33/10/0	0/19/3/0	**0,05**	ns
Anthropometric						
Weight (Kg)	118.14±17.75	113.66±15.75	121.36±16.57	114.75±21.31	ns	ns
Waist (cm)	126.79±13.31	119.88±14.07	128.18±12.92	120.4±9.88	ns	ns
BMI (Kg/m^2)	43,18±5.92	41.82±5.30	44.02±5.94	42,43± 6,36	ns	ns
Liver tests						
ALT (U/L)	29.61±16.15	18.06±5.03	31.48±16.34	36.05±17.64	**<0.001**	**ns**
AST (U/L)	23.77±10.07	20.30±4.91	24.57±11.39	25.05±10	**0.02**	**ns**
GGT (U/L)	27.15±21.92	14.27±5.64	32.02±26.86	31.73±7.83	**0.03**	**ns**
Fibrinogen (g/L)	362.75±55.69	377.4±24.51	375±51.76	286.25±43.86	ns	**0.02**
Platelets (x10^9/L)	281.33±59.97	279.87±53.10	274.39±60.59	300.5±63.85	ns	ns
Laboratory Parameters						
Total Cholesterol totale (mg/dl)	192.04±30.95	192.5±33.41	193.30±32.74	188.52±24.76	ns	ns
HDL (mg/dl)	49.36±11.54	50.72±11.74	51.17±12.24	43.68±7.56	ns	**0.02**
LDL (mg/dl)	114.72±27.75	119.94±31.26	112.89±29.01	114.21±21.10	ns	ns
Triglycerides (mg/dl)	137.45±55.99	108±49.65	142.05±59.40	153.10±44.11	**0.01**	ns
Uric Acid (mg/dl)	5.51±1.42	5.07±0.83	5.48±1.58	6.04±1.36	**0.05**	ns
Fasting Glucose (mg/dl)	101.38±31.14	89±6.62	105.5±38.82	103.35±18.38	**0.02**	ns
Fasting Insulin (mg/dl)	17.82±12.67	13.69±11.57	18.96±13.21	19.75±11.92	ns	ns
HbA1c (%)	5,6 ± 1,0	5,2 ± 0,2	5,7 ± 1,3	5,6 ± 0,6	**0,04**	ns

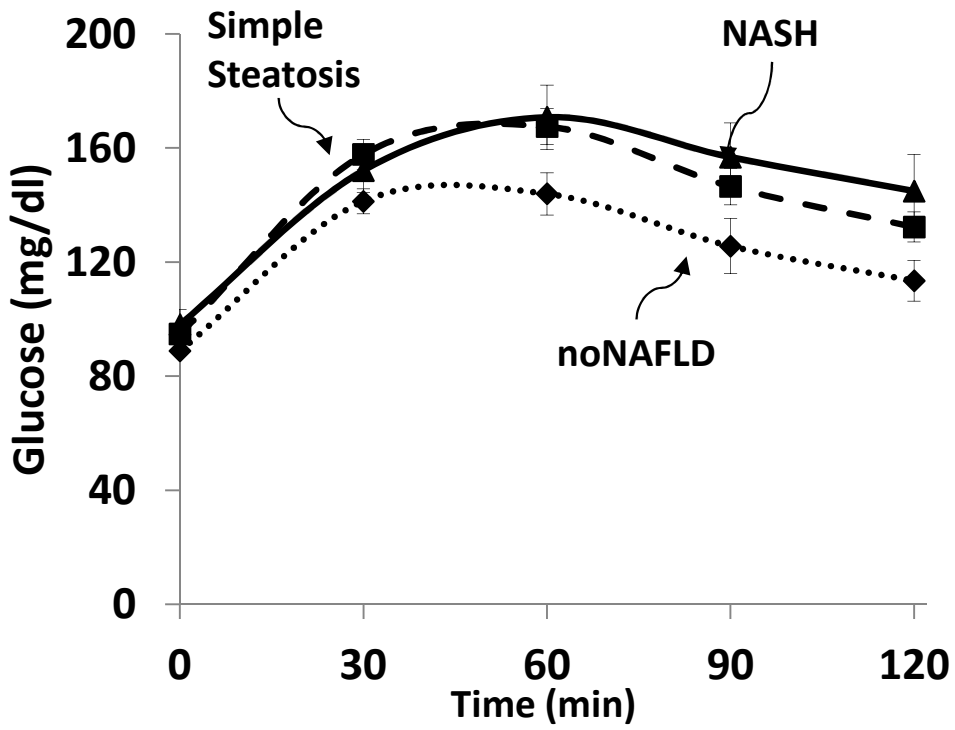
Table 2 Comparison of OGTT characteristics of patients of 3 different groups without known diabetes (n=77), without NAFLD (Group A), Simple Steatosis (Group B) and NASH (Group C). Comparisons were performed between subjects without NAFLD (A) vs patients with NAFLD (B+C) and between subjects with simple steatosis (B) vs subjects with NASH (C)

	A NO NAFLD (n=21)	**B Simple Steatosis (n=40)**	**C NASH (n=16)**	P A vs B/C	P B vs C
NGT/IGT	17/4	28/12	13/3	**0,05**	ns
AUC glucose OGTT (g/dl x 120min)	15384±2971	17124±4219	16690±5458	ns	ns
AUC insulin OGTT (mU/l x 120min)	6034±3202	9835±5908	11530.05±6642	**0.003**	ns
OGIS (ml/min m2)	422.09 ± 44,3	360,6 ± 63.3	337,9±62	**0.001**	**0,05**
HOMA IR	3.03 ± 2.64	4.5±3.3	4.8±2.8	**0.05**	**ns**
Insulinogenic Index (IGI)	0.86 ± 0.55	1.30±1.38	0.75±0.43	**ns**	**ns**
dAUC-I/dAUC-G	1.16 ± 0.68	1.61±1.28	1.66±1.12	**ns**	**ns**
Hepatic IR index (mg/ml x mU/ml)	21±15	46±44	48± 27	**ns**	**ns**

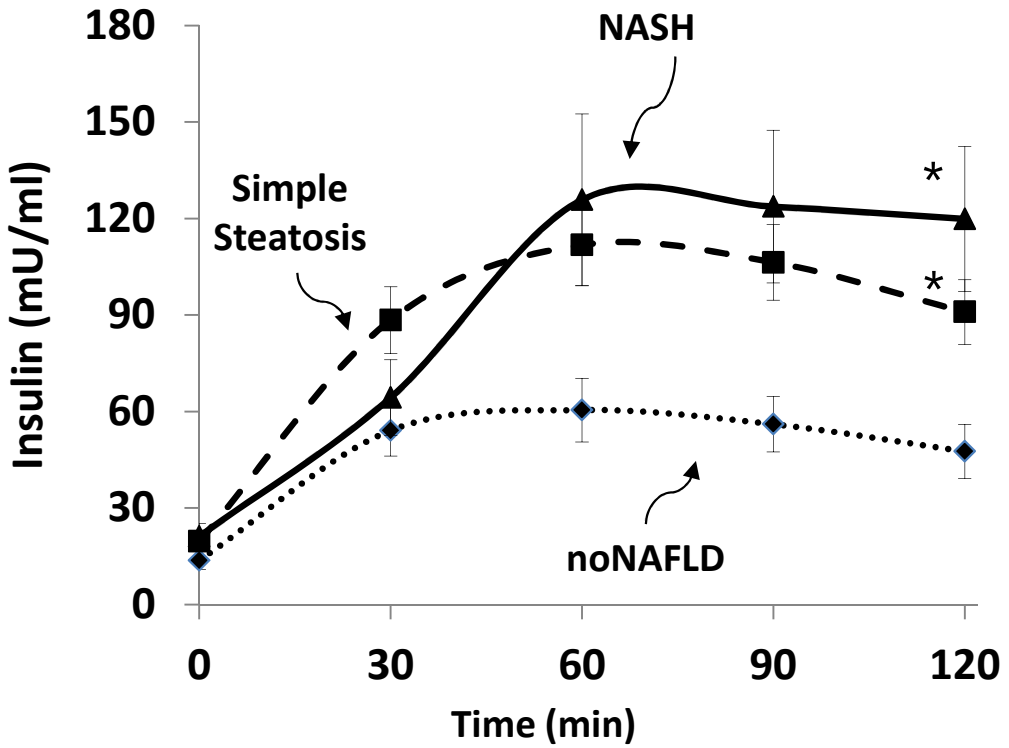
Figure 1 OGTT in subjects without known diabetes (n=77) in 3 different Groups. (Panel A) Glucose serum levels during OGTT with standard errors in Group A (no-NAFLD), B (Simple Steatosis) and C (NASH). (Panel B) Insulin serum levels with standard errors during OGTT in Group A (no-NAFLD), B (Simple Steatosis) and C (NASH). Dotted line represents Group A, dashed line Group B and continuous line Group C. Levels of significance: *p 0,04 Group B vs A; p 0,05 Group C vs A; p ns Group B vs C

FIGURE 1

A


Simple
Steatosis
NASH
noNAFLD
Glucose (mg/dl)
200
160
120
80
40
0
0
30
60
90
120
Time (min)

B


NASH
Simple
Steatosis
noNAFLD
*
*
Insulin (mU/ml)
180
150
120
90
60
30
0
0
30
60
90
120
Time (min)

HIGHLIGHTS

1. In our cohort of morbid obese patients, prevalence of NAFLD was 77% and NASH 24%
2. OGIS was associated to steatosis and decreased with liver histology severity
3. HOMA IR was associated to hepatic steatosis and fibrosis stage
4. AST, GGT and prevalence of comorbidities increased with liver injury