HELLENIC REPUBLIC
National and Kapodistrian University of Athens

ATHENS UNIVERSITY OF APPLIED SCIENCES

Increased Risk of Non-Alcoholic Fatty Liver Disease in Women with Gestational Diabetes Mellitus:

A population-based cohort study, a systematic review and meta-analysis.

By

Aikaterini Lavrentaki, MD

(N**:** 20150212)

A thesis submitted to Athens Medical School, National and Kapodistrian University of Athens for the degree:

MSc "Research in Female Reproduction"

Examination Committee:

Supervisor: **A. A. Tahrani**
Members: **G. Mastorakos, K. Nirantharakumar**

ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
ΙΑΤΡΙΚΗ ΣΧΟΛΗ
&
ΤΕΧΝΟΛΟΓΙΚΟ ΕΚΠΑΙΔΕΥΤΙΚΟ ΙΔΡΥΜΑ ΑΘΗΝΑΣ
ΤΜΗΜΑ ΜΑΙΕΥΤΙΚΗΣ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ
«ΕΡΕΥΝΑ ΣΤΗ ΓΥΝΑΙΚΕΙΑ ΑΝΑΠΑΡΑΓΩΓΗ»

Αυξημένος κίνδυνος εμφάνισης λιπώδους διήθησης του ήπατος σε γυναίκες με ιστορικό διαβήτη κύησης:

μια πληθυσμιακή- αναδρομική μελέτη κοόρτης, μια συστηματική ανασκόπηση και μετα- ανάλυση.

ΛΑΥΡΕΝΤΑΚΗ ΑΙΚΑΤΕΡΙΝΗ

ΙΑΤΡΟΣ

Α.Μ.: 20150212

Τριμελής Εξεταστική Επιτροπή:

1. Α.Α. Tahrani - επιβλέπων
2. Γ. Μαστοράκος
3. K. Nirantharakumar

Αθήνα 2019

Acknowledgements

I would like to thank Professors Georgios Mastorakos and Georgios Valsamakis who gave me the opportunity to accomplish my dissertation abroad and awarded me a research scholarship covering the expenses for my transportation and accommodation in the UK, as well as for providing a project grant for accessing the database of the University of Birmingham.

Many thanks to Maria Riga for her valuable support, effort and hours of work for all the procedures necessary to visit the University of Birmingham, to cover the expenses and to fulfill my thesis. Her advice and the communication with her all these months were of great help to me in order to fulfil this study.

I am indebted to my supervisor Dr Abd A. Tahrani, NIHR Clinician Scientist at the University of Birmingham, for his endless support and advice on this project and for the time he has spent with me although he had a demanding schedule. I am grateful to Dr Tahrani for teaching me about diabetes mellitus, other areas of medicine like sleep medicine and obstructive sleep apnoea, as well as the difficult art of writing scientific, medical papers and the critically reviewing of research publications. I also thank him for giving me the opportunity to write under his supervision a review for obstructive sleep apnoea and its interplay with the endocrine system/ metabolism; and being by my side all the time I have spent in Birmingham.

I am grateful to Dr Krish Nirantharakumar, senior clinical lecturer at the Institute of Applied Health Research, for his guidance and support on this project especially in concern to design of this study and analysis of the data.

I do not forget to thank the IBR (Institute of Biomedical Research - University of Birmingham) department for technical support and all the colleagues on the 2nd floor who were always willing to help me and made me feel welcome.

Finally, I am also thankful to Anu Subramanian and Tom Thomas for their help and input in the statistical analysis and writing of the Methods of this study.

Funding. This study was funded by the Master's Program: ''Research in Female Reproduction'' of Athens Medical School, National and Kapodistrian University of Athens (Approval: KE 70/3/9359, Prot. Number: 14400/2018)

Table of Contents

Abbreviations

ADA	american diabetes association
BMI	body mass index
CI	confidence interval
GDM	gestational diabetes mellitus
IR	insulin resistance
IRR	incidence rate ration
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
OGTT	oral glucose tolerance test
OR	odds ratio
THIN	the health improvement network

Abstract

Background

Gestational diabetes mellitus (GDM) is associated with adverse perinatal outcomes, and increased risk of post-natal type 2 diabetes and cardiovascular disease. However, whether GDM increases the risk of developing incident Non-Alcoholic Fatty Liver Disease (NAFLD) is unclear and has not been well examined in previous studies. This is important considering the significant health burden of NAFLD and the opportunity to interfere in high risk population in order to reduce the risk of developing end-stage liver disease.

Objectives

To examine whether women with gestational diabetes mellitus (GDM) are at increased risk of developing Non-Alcoholic Fatty Liver Disease (NAFLD) compared to women without GDM.

Research Design and Methods

We conducted a population-based retrospective matched-controlled cohort study utilising The Health Improvement Network (THIN), a large primary care database representative of the United Kingdom population, between 01/01/1990 to 31/05/2016 followed by a systematic review of available literature. The study population included 9,640 women with GDM and 31,296 controls without GDM, matched for age, body mass index (BMI) and time of pregnancy. All study participants were free from NAFLD diagnosis at study entry.

Results

The median (range) follow-up duration was similar in women with and without GDM (2.95 (1.21-6.01) vs 2.85 (1.14-5.75) years respectively).Unadjusted incidence rate ratio (IRR) for NAFLD development in women with vs without GDM was 3.28 (95% CI 2.14 - 5.02), which remained significant after adjustment for a wide range of potential confounders (IRR 2.70; 95%CI 1.744 - 4.19). When women were censored when they developed type 2 diabetes during follow-up the risk of NAFLD in GDM remained high (IRR 2.46: 95% CI 1.51 - 4.00).

The meta-analysis of 3 studies (including the current study) showed increased NAFLD risk in women with vs without GDM (OR 2.60; 95% CI 1.90-3.57, I_2=0%).

Conclusions

Women with GDM are at increased risk of developing NAFLD in their later life compared to women without GDM regardless of the development of type 2 diabetes. Clinicians should have a low threshold to investigate women with history of GDM for the presence of NAFLD. Further studies to identify best screening strategies are needed

Περίληψη

Ο διαβήτης κύησης σχετίζεται με ανεπιθύμητα περι-γεννητικά αποτελέσματα, και αυξημένο κίνδυνο για εμφάνιση διαβήτη τύπου 2 και καρδιαγγειακή νόσο στην μετά τον τοκετό ζωή των γυναικών. Παρ' όλα αυτά, η συσχέτιση του διαβήτη κύησης με αυξημένο κίνδυνο για εμφάνιση μη αλκοολικής λιπώδους διήθησης του ήπατος παραμένει αδιευκρίνιστη και όχι καλά μελετημένη σε ως τώρα μελέτες. Η μελέτη της σχέσης αυτής είναι σημαντική λόγω των σημαντικών υγειονομικών επιβαρύνσεων της πάθησης (μη αλκοολική λιπώδης διήθηση), αλλά και της δυνατότητας παρέμβασης σε ομάδες υψηλού κινδύνου για την πρόληψη της ηπατικής νόσου.

Σκοπός

Να μελετηθεί εάν οι γυναίκες με διαβήτη κύησης έχουν μεγαλύτερο κίνδυνο εμφάνισης μη αλκοολικής λιπώδους διήθησης ήπατος σε σύγκριση με γυναίκες χωρίς ιστορικό διαβήτη κύησης.

Σχεδιασμός μελέτης και μεθοδολογία

Πραγματοποιήσαμε μια πληθυσμιακή, αναδρομική, μελέτη κοόρτης με αντιστοιχισμένη ομάδα μελέτης κάνοντας χρήση του Βρετανικού δικτύου βελτίωσης Υγείας (The Health Improvement Network - THIN). Πρόκειται για μια μεγάλη βάση δεδομένων που περιέχει δεδομένα πρωτοβάθμιας φροντίδας της υγείας στο Ηνωμένο Βασίλειο, αντιπροσωπευτική του πληθυσμού της χώρας. Η συλλογή των δεδομένων αφορά στην περίοδο μεταξύ 01/01/1990 και 31/05/2016, ενώ η παρούσα μελέτη συνοδεύτηκε και από μια συστηματική ανασκόπηση της διαθέσιμης σχετικής βιβλιογραφίας. Συνολικά ο πληθυσμός της μελέτης κοόρτης ήταν 9,640 γυναίκες με διαβήτη κύησης και 31,296 γυναίκες χωρίς ιστορικό διαβήτη κύησης (ομάδα ελέγχου), αντιστοιχισμένες για την ηλικία, τον δείκτη μάζας σώματος και τον χρόνο της κύησης. Όλες οι συμμετέχοντες στην μελέτη δεν έπασχαν από λιπώδη διήθηση του ήπατος κατά την εισαγωγή τους στην μελέτη.

Αποτελέσματα

Ο διάμεσος χρόνος (διάστημα) παρακολούθησης ήταν παρόμοιος και για τις δύο ομάδες γυναικών, με ή χωρίς ιστορικό διαβήτη κύησης [2.95 (1.21-6.01) και 2.85 (1.14-5.75) χρόνια αντίστοιχα]. Ο μη διορθωμένος λόγος της συχνότητας επίπτωσης [

Unadjusted incidence rate ratio (IRR)] για την εμφάνιση μη αλκοολικής λιπώδους διήθησης στις γυναίκες με διαβήτη κύησης έναντι των γυναικών χωρίς διαβήτη κύησης ήταν 3.28 [95% διάστημα εμπιστοσύνης (SD): 2.14 - 5.02], το οποίο και παρέμεινε σημαντικά υψηλό μετά και την διόρθωση για τους πιθανούς συγχυτικούς παράγοντες (IRR 2.70; 95% ΔΕ: 1.744 - 4.19). Ακόμη και όταν από τη μελέτη αφαιρέθηκαν οι γυναίκες που αργότερα ανέπτυξαν σακχαρώδη διαβήτη τύπου 2, ο κίνδυνος εμφάνισης μη αλκοολικής λιπώδους διήθησης στις γυναίκες με διαβήτη κύησης παρέμεινε υψηλός (IRR 2.46: 95% ΔΕ 1.51 - 4.00) ακόμη και επί απουσίας διαβήτη τύπου 2.

Η μετα-ανάλυση 3 μελετών (συμπεριλαμβανομένης και της παρούσας μελέτης-κοόρτης) ανέδειξε επίσης αυξημένο κίνδυνο για λιπώδη διήθηση στις γυναίκες με ιστορικό διαβήτη κύηση έναντι εκείνων χωρίς (OR 2.60; 95% ΔΕ 1.90-3.57, I_2=0%).

Συμπεράσματα

Οι γυναίκες με διαβήτη κύησης εμφάνισαν αυξημένο κίνδυνο για λιπώδη διήθηση του ήπατος στην μετά τον τοκετό ζωή τους σε σχέση με εκείνες τις γυναίκες που είχαν ελεύθερο ατομικό αναμνηστικό για διαβήτη κύησης; και μάλιστα ανεξάρτητα από την ανάπτυξη διαβήτη τύπου 2. Οι θεράποντες κλινικοί θα πρέπει να βρίσκονται σε εγρήγορση για τη διερεύνηση των γυναικών με θετικό ιστορικό διαβήτη κύησης όσον αφορά στην εμφάνιση λιπώδους διήθησης του ήπατος. Περαιτέρω μελέτες χρειάζονται για την ανάπτυξη στρατηγικών ελέγχου των ομάδων υψηλού κινδύνου.

GENERAL SECTION

Introduction

1. Gestational Diabetes Mellitus (GDM)

1.1 Definition

GDM is traditionally defined as any degree of glucose intolerance with onset or first recognition during pregnancy[1]. However, as the obesity and type 2 diabetes epidemic has resulted in more women of childbearing age with dysglyceamia and type 2 diabetes and an increased number of them undiagnosed[2], the above definition is imprecise without excluding the cases of women with type 2 diabetes prior to gestation. Hence, according to the recent guidelines of the American Diabetes Association GDM is: «diabetes that is first diagnosed in the second or third trimester of pregnancy that is not clearly either preexisting type 1 or type 2diabetes»[3] (**Table I**).

Whereas, women that are diagnosed with diabetes in the first trimester should be classified as having preexisting pre-gestational diabetes (type 2 diabetes, type 1 diabetes or monogenic diabetes). According to ADA recommendations it is reasonable to test women at their initial prenatal visit with standard diagnostic criteria, if they have risk factors for type 2 diabetes[3].

1.2 Epidemiology

Gestational Diabetes Mellitus (GDM) is common with a prevalence of 5.4% (3.8-7.8)[4] in Europe rising to 19.19% [15.5, 23.6] in other countries such as India[5] . The prevalence of GDM has increased over the last 20 years primarily fuelled by the obesity epidemic and the trend toward older maternal age [6, 7].

Although GDM normally disappears after delivery, women who have been previously diagnosed with GDM are at a greater risk of developing gestational

diabetes in subsequent pregnancies. Several studies show a recurrence rate of GDM between 35 and 48% , associated with weight gain between pregnancies, older age, multiparous, insulin use and ethnicity (Hispanics, Asian); and the magnitude of recurrence risk increases with the number of prior GDM episodes[8-14]. The interval between pregnancies provides an important window for diabetes prevention through lifestyle change[15].

Increasing maternal glycaemia is associated with negative pregnancy outcomes. There are data showing that for an increase in fasting plasma glucose of 1 SD (6.9mg/dl [0.4mmol/L]), or of 1SD in the 1-hour plasma glucose level (30.9 mg/dl [1.7mmol/L]) and of 1 SD in the 2-hour plasma glucose (23.5 mg/dl [1.3mmol/L]) odds ratio for birth weight above the 90th percentile were 1.38, 1.46 (1.39 to 1.53), and 1.38 respectively; for primary cesarean delivery 1.11, 1.10 and 1.08; and for neonatal hypoglycemia 1.08, 1.13, and 1.10 respectively[16]. There are also significant positive associations of maternal glycaemia with preeclampsia (OR for each 1-SD increase in each glucose measure 1.21- 1.28), with shoulder dystocia or birth injury OR were approximately 1.20[16]. The 1-hour and 2-hour plasma glucose levels are significantly related to premature delivery, intensive neonatal care, and hyperbilirubinemia[16].

On the other hand, good glyceamic control in women with GDM is considered beneficial, as it can significantly reduce the likelihood of serious neonatal morbidity, fetal macrosomia, cesarean sections and neonatal intensive care unit admissions[17]. In addition, GDM treatment can significantly reduce perinatal morbidity (death, shoulder dystocia, bone fracture, and nerve palsy) and improve maternal health-related quality of life[18].

1.3 Pathogenesis

GDM is caused by increased insulin resistance (IR) appearing in gestation, along with failure to compensate with β cells' increased insulin secretion[1]. Two main contributors to insulin resistance are increased maternal adiposity and the insulin desensitizing effects of hormones produced by the placenta[1].

Hormones contributing to IR are growth hormone, progesterone, placental lactogen and cortisol, whose increased levels in pregnancy lead to impaired glucose disposal[19]. Among them progesterone provides the major drive, increasing through gestation with a peak shortly before delivery; hence IR is greatest in the third trimester[1].

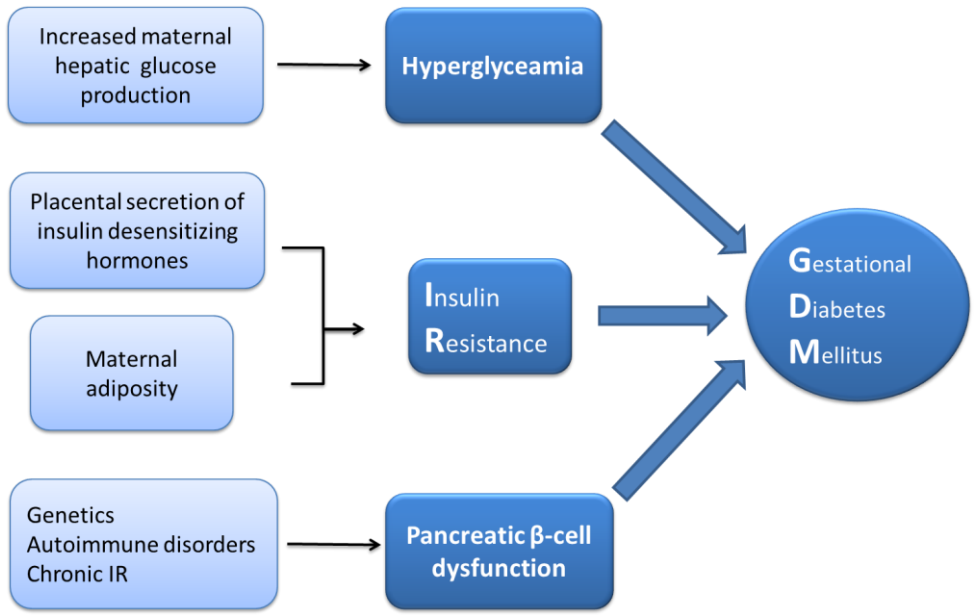
In pregnant women without diabetes, increased β-cell insulin secretory capacity/ response will compensate for reduced insulin sensitivity, and this is related to β-cell hypertrophy and hyperplasia (β-cell expansion in mid pregnancy by elevated prolactin and placental lactogen)[1]. However, women who have a deficit in this additional insulin secretory capacity will develop GDM.

Pancreatic β-cell defects can be revealed in a period of metabolic stress, such as pregnancy, and exacerbated by pregnancy-induced insulin resistance. In most cases impaired glucose tolerance is the result of insulin insufficiency due to pancreatic β-cells dysfunction on a background of chronic insulin resistance [17, 20]. Reduced glucose uptake and subsequent hyperglyceamia lead to β-cell overload for extra insulin secretion and finally result in β-cell failure and apoptosis- reduced β-cell number (glucotoxicity)[20]. Other less common causes of β-cell dysfunction are: autoimmunity and genetic abnormalities leading to impaired insulin secretion. Autoimmune β-cell dysfunction is due to the presence of antibodies (cytoplasmic islet cell antibodies, antibodies against GAD65, membrane tyrosine phosphatase and insulin) and

¨autoimmune GDM¨ subtype should be suspected in lean, Caucasian women with GDM [17]. Finally, there are highly penetrant genetic abnormalities that lead to impaired insulin secretion (maturity-onset diabetes of the young –MODY and mitochondrial diabetes) but in <5% of GDM cases. These patients are usually younger, with mild hyperglyceamia and no evidence of chronic IR and with relevant family history[17].

In addition, as maternal glucose levels are important for the fetus requirements, hyperglyceamia noticed in pregnant women is achieved not only due to IR in the liver, muscle and adipose tissue, but also due to increased maternal hepatic glucose production[1]. The pathogenic mechanisms are summarised in the **fig. I** below.

Fig. I. Gestational Diabetes Mellitus Pathogenesis



1.4 Risk factors

Increasing obesity rates and maternity age are major factors for GDM's higher incidence the last years, according to the National Institute for Health and Care Excellence (NICE) in the UK[21]. Other risk factors include maternal age >37 years, ethnicity with high prevalence of type 2 diabetes (South Asian, Afro-Caribbean, Middle Eastern), pregnancy weight >80kg or BMI>28kg/m^2, previous GDM, family history of diabetes, previous unexplained stillbirth, previous macrosomia/ polyhydramnios and polycystic ovarian syndrome[1, 19].

1.5 Strategies for diagnosis

It is important to perform a GDM risk assessment (see Risk Factors above) at the first prenatal visit and women with high risk should have an OGTT testing as soon as possible[3]. Lower risk women are screened for GDM at 24-28weeks with either of two strategies: 1. " One step" - 75g OGTT or 2. " Two-step" protocol with a 50g glucose load (non-fasting) followed by a 100g OGTT for positive results[1, 3]. See below **Table I** for Screening and diagnosis of gestational diabetes.

Table I. Screening and diagnosis of GDM

Blood Glucose testing at 24-28 weeks of gestation
One step strategy:
A 75g OGTT, plasma glucose measurement at 1 and 2 h after fasting (women not previously diagnosed with overt diabetes) Diagnosis of GDM when any of the following is met/ exceeded: • **Fasting: 92 mg/dL (5.1 mmol/L)** • **1 h: 180 mg/dL (10.0 mmol/L)** • **2 h: 153 mg/dL (8.5 mmol/L)**

Two-step strategy:			
Step 1: **A 50g GLT** (non- fasting), plasma measurement at 1h (women not previously diagnosed with overt diabetes) If plasma glucose level at 1 h after the load is ≥ 130mg/dL, 135mg/dL, or 140mg/dL (7.2mmol/L, 7.5mmol/L, or 7.8mmol/L), proceed to a 100g OGTT. **Step 2:** **A 100g OGTT (**when patient is fasting) GDM diagnosis is made if at least two* of the following plasma glucose levels are met/ exceeded:			
	Carpenter-Coustan criteria[22]	**or**	**NDDG criteria[23]**
Fasting:	95mg/dL (5.3 mmol/L)		105 mg/dL (5.8 mmol/L)
1h:	180 mg/dL (10.0 mmol/L)		190 mg/dL (10.6 mmol/L)
2h:	155mg/dL (8.6 mmol/L)		165 mg/dL (9.2 mmol/L)
3h:	140 mg/dL (7.8 mmol/L)		145 mg/dL (8.0 mmol/L)

NDDG: National Diabetes Data group

1.6 Treatment

The goal of therapy is prevention of fasting and postprandial hyperglycaemia with frequent follow-up visits every 1 to 2 weeks. Glycemic targets in pregnancy are stricter than in nonpregnant individuals and for optimal control glucose monitoring aims for the targets below:

- Fasting <95 mg/dL (5.3 mmol/L) and either

- One-hour postprandial <140 mg/dL (7.8 mmol/L) or

- Two-hour postprandial <120 mg/dL (6.7 mmol/L) [3].

After diagnosis, initial treatment includes dietary advice and moderate aerobic exercise[19]. Oral hypoglycaemic therapy is recommended when diet and exercise fail to maintain the target range of blood glucose levels, or when the fetus scans suggest

macrosomia, with metformin and glibenclamide commonly used[19]. Finally, insulin therapy is a choice to maintain glucose targets combined with diet, exercise and oral therapy[19].

1.7 Long-term considerations

While GDM, by definition, is limited to the time in pregnancy, its consequence might be life-long. It is well established that GDM is associated with negative impact on maternal and fetal outcomes (fetal macrosomia, small for gestational age, pre-eclampsia, eclampsia, cesarean delivery etc.) [16, 24] and increased risk of developing type 2 diabetes [25, 26].

More recently, there has been an increasing interest in exploring the long-term consequences of GDM other than type 2 diabetes and it was reported that women with GDM were at increased risk of incident hypertension and cardiovascular disease [27-29].

1.8 Post-partum follow-up

Women with GDM should be given lifestyle advice (weight control, exercise) and a fasting plasma glucose measurement 6 week post-natal and annually[19]. In addition, among ADA's recommendations is included to test women with GDM for persistent diabetes at 4–12 weeks postpartum, using OGTT and non-pregnancy diagnostic criteria[3]. In addition, it is recommended, that Women with a history of GDM should have lifelong screening for diabetes or prediabetes development at least every 3 years[3]. And those found to have prediabetes should receive intensive lifestyle interventions or metformin to prevent diabetes[3].

2. Non-alcoholic fatty liver disease (NAFLD)

2.1 Definition

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of disorders ranging from the simple fatty liver to non-alcoholic steatohepatitis, with increasing fibrosis leading eventually to cirrhosis[30]. NAFLD definition entails (1) excessive hepatic fat accumulation in the liver (detected by imaging techniques or histology) and (2) the absence of other secondary causes of hepatic fat; while patients with a history of significant ongoing or recent alcohol consumption have to be excluded (see **Table II** below) [31-36].

Table II. NAFLD diagnostic criteria & the threshold dose of alcohol consumption

	EASL	NICE	Asia-Pacific	AASLD
Criteria	Steatosis in > 5% of hepatocytes (imaging / histology) No other causes of Steatosis Insulin resistance	Excessive fat in the liver No other causes of Steatosis No significant alcohol consumption	Hepatic steatosis (imaging/ histology) No other causes of Steatosis No significant alcohol consumption	Evidence of hepatic steatosis (imaging/ histology) No other causes of Steatosis No significant alcohol consumption No coexisting chronic liver disease
Alcohol consumption threshold (men)	30 g/d	30 g/d	2 standard drinks per day 140 g/wk	21 standard drinks per week 294 g/wk
Alcohol consumption threshold (women)	20 g/d	20 g/d	1 standard drink per day 70 g/wk	14 standard drinks per week 196 g/wk

EASL: European Association for the Study of the Liver; NICE: National Institute for Health and Care Excellence; AASLD: American Association for the Study of Liver Diseases.

2.2 Epidemiology

Non-alcoholic fatty liver disease (NAFLD) is a serious public health challenge with rising prevalence globally fuelled by the global increase in the prevalence of obesity and type 2 diabetes [37-41]. Although there is a lack of large-scale epidemiological studies, there reports of NAFLD incidence between 18,5 to 31 cases per 1000 person years[38]. Global NAFLD prevalence is 24.24%, and positively correlated with economic status, while it is estimated to be 20 to 30% in Western countries and 5 to 18% in Asia[38]. In the USA economic and societal burden is also considered to be high, as over 30% of the population is affected by NAFLD and it has become a leading cause of chronic liver disease [41]. In addition, NAFLD is becoming the main cause of liver transplantation in the Western world [38, 42, 43].

2.3 Risk factors and Pathogenesis

Main risk factors for NAFLD development are obesity, type 2 diabetes and metabolic syndrome[44, 45]. Patients with dyslipidaemia (high triglyceride and low HDL cholesterol levels) develop NAFLD[44]. Furthermore, polycystic ovarian syndrome, sleep apnea and endocrine diseases such as hypothyroidism, hypogonadism and hypopituitarism, are related to NAFLD[44].

NAFLD is characterized by ectopic fat in hepatocytes. Possible pathogenetic mechanisms are increased hepatic synthesis of free fatty acids (FFAs), decreased B-mitochondrial oxidation of fats, deficient export of very-low-density lipoprotein (VLDL) and the increase of triglyceride deposits[44]. In patients with obesity, there is adipose tissue resistance to insulin leading to increased lipolysis and hence there is an increase in FFAs flow in the liver[44]. In addition, hyperinsulinemia and excess of carbohydrates are also related to de novo lipogenesis, while VLDL is not sufficient to compensate the triglyceride formation excess[44]. This excessive accumulation of fat in

the liver is responsible for the hepatocellular injury caused and progression to NAFLD[44]. Worth to note is that the abundant storage of adipocytes is associated with increased oxidative stress and the release of proinflammatory cytokines (tumor necrosis factor alpha TNF-a, interleukin 6 IL-6, resistin) participating in NAFLD genesis (inflammation, fibrosis, impairment of hepatic structure)[44] (**Fig. IIa,IIb**)

2.4 Screening

The European Association for the Study of the Liver (EASL), NICE and Asia-Pacific Guidelines recommend screening for "high-risk" groups in particular, such as patients with obesity, type 2 diabetes, metabolic syndrome and with abnormal liver enzymes[31, 32, 36]. Whereas, the American Association for the Study of Liver Diseases (AASLD) claims that there is no evidence of cost-effectiveness for NAFLD screening in adults; even for patients with metabolic risk factors ("high-risk" groups), and suggest vigilance for those cases[35].

2.5 Diagnostic workup

Initial diagnostic workup includes a noninvasive imaging examination for steatosis confirmation along with general liver biochemistry[46]. Abdominal ultrasound is commonly used as the first-line examination in patients with increased liver enzymes or suspected NAFLD, in daily clinical practice, due to its broad availability and low cost[46].

Magnetic resonance imaging (MRI), is the gold standard to assess and quantify hepatic steatosis, detecting the fat liver amount as low as 5%-10%[46]. Still, its use in the clinical practice is limited, due to high cost and a long time of execution.

EASL and Italian guidelines suggest the use of noninvasive serum scores ((NAFLD fibrosis score, Fibrosis 4 calculator, AST/ALT ratio index) along with transient

elastography performed for every patient with NAFLD, in order to exclude the presence of significant fibrosis[31, 34]. Hence, if advanced fibrosis is suspected, liver biopsy should be performed for final diagnosis[31, 34]. In addition, NICE guidelines suggest that patients with an incidental finding of NAFLD should be screened for advanced fibrosis by enhanced liver fibrosis blood test[36].

2.6 Follow up

For patients with NAFLD, normal liver enzymes and low risk of advanced fibrosis there is the suggestion of a clinical, laboratory and instrumental follow up every two years[31, 34]. While, for patients with evidence of NASH or fibrosis an annual screening is suggested; and for those with cirrhosis every six months, in concern to hepatocellular carcinoma's surveillance[31, 34].

According to NICE guidelines for patients with an incidental finding of NAFLD but negative for advanced fibrosis by blood test, screening should be repeated every three years for adults. For patients with type 2 diabetes mellitus or metabolic syndrome, but without steatosis at ultrasound examination, evaluation should be performed every three years[36].

2.7 Treatment

First- line therapeutic approach for NAFLD is an adequate lifestyle change, focusing on weight los[46]. Lifestyle modification is advised to those patients targeting mainly on physical activity and healthy diet. For more advanced disease (bridging fibrosis and cirrhosis) pharmacological treatment is recommended[46]. Medicines considered for NAFLD treatment and discussed with the patient are: metformin, pioglitazone, Vitamin E, Glucagon-like peptide-1 analogues and statins[46].

Moreover, bariatric surgery is another option for patients not responding to lifestyle modifications. It can favor weight loss, metabolic complications and improve liver histology, but it is related with peri-operative mortality[46]. Finally, liver transplantation remains an option for patients with end-stage liver disease, although there is a high risk for post-transplant complications and increased graft loss due to morbidities of obesity, sarcopenia, cardiovascular disease and chronic kidney disease[47, 48].

GDM is associated with obesity, insulin resistance, dyslipidaemia, and type 2 diabetes; hence, it is plausible that the risk of developing NAFLD is increased in women with GDM. Our hypothesis is that women with GDM are at increased risk of incident NAFLD following delivery. This relationship has not been examined widely in the literature.

To examine our hypothesis, we conducted a large population-based matched-controlled cohort study aimed at examining the relationship between GDM and incident NAFLD taking into account the potential confounders. In addition, we conducted a systematic review and a meta-analysis of the available literature examining the rismaternal age >37k of NAFLD development in women with GDM.

Fig. IIa NAFLD Pathogenesis

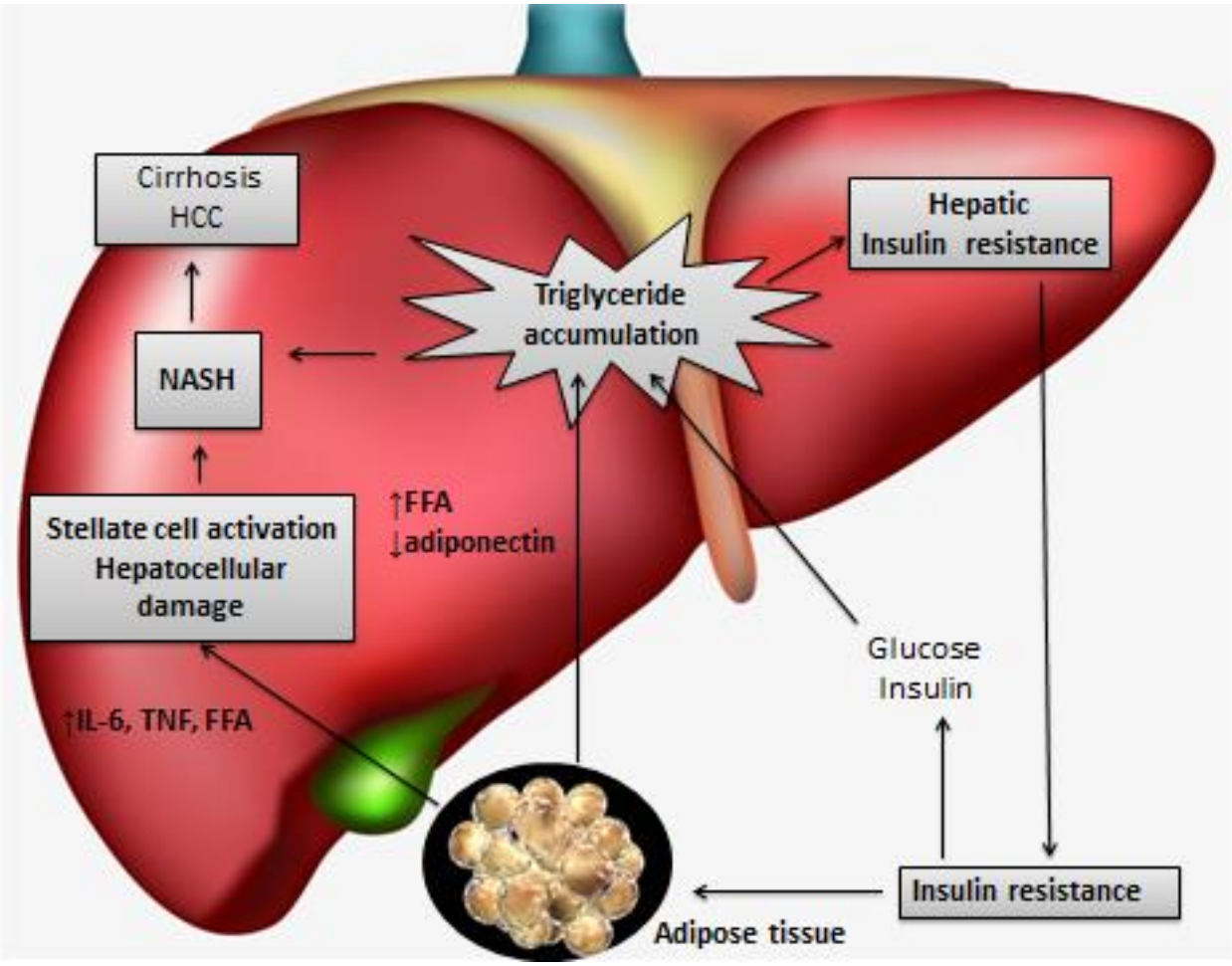
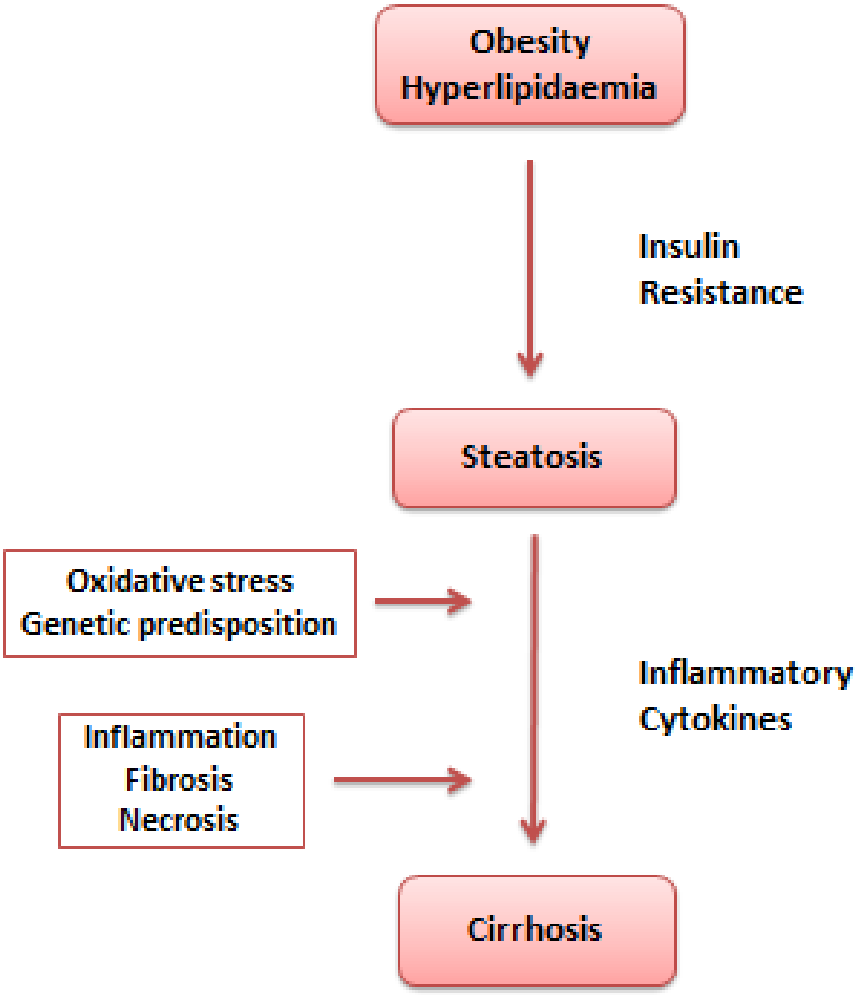


Fig. IIb NAFLD Pathogenesis



SPECIAL SECTION

Methods

The Population-Based Study

Research design:

We conducted a population-based retrospective matched-controlled cohort study utilising The Health Improvement Network (THIN) database, which our group has utilised previously to conduct studies in the fields of GDM and NAFLD [27, 49].

Data Source: The Health Improvement Network

The THIN database is a primary care database, representative of the United Kingdom population (in terms of demographics, mortality rates and major health conditions' prevalence) [50] and contains the electronic medical records of approximately 14 million patients from over 698 general practices in 2016 [51]. Details of medical care such as history, examination, investigations, diagnoses and prescriptions are recorded utilising the Vision patient record software [52] in a hierarchical system known as Read codes [53].

For primary care practices to be eligible for inclusion in the study they had to have used the electronic medical record (EMR) system for one year and have an acceptable mortality recording date. These conditions ensure the accuracy of data recording and that the practices included in the study were making full use of the EMR system.

Study population, inclusion and exclusion criteria:

Incident cases of GDM diagnosed between 01 January 1990 to 31 May 2016 were identified by using the Read codes **(Figure 1A & Table 1).** The date of diagnosis of GDM was assigned as the index date (i.e. study start date) of GDM cases. The control group in this study were women without GDM by the time of delivery. The date at

which pregnancy was first recorded was taken to be the index date for patients in the control arm.

Codes for Exposure - GDM	
L180811	Gestational diabetes mellitus
L180900	Gestational diabetes mellitus
Codes for Outcome - NAFLD and NASH	
J61y100	Non-alcoholic fatty liver
J61y800	Nonalcoholic steatohepatitis

Table 1: Read codes used to identify incident cases of GDM - (Exposure) and NAFLD/NASH development - (outcome).

Women were eligible for inclusion in the study following at least one year of registration with their primary care provider to ensure accurate co-morbidity recording. Women with GDM were matched to controls up to a ratio 1:4, for age (±1 year), time of pregnancy (± 90 days) and BMI ((± 2 kg/m^2). Patients with type 1 or type 2 diabetes mellitus prior to pregnancy or a history of alcohol excess at any point during the study were excluded. A follow chart for the above process is presented in **Figure 1A.**

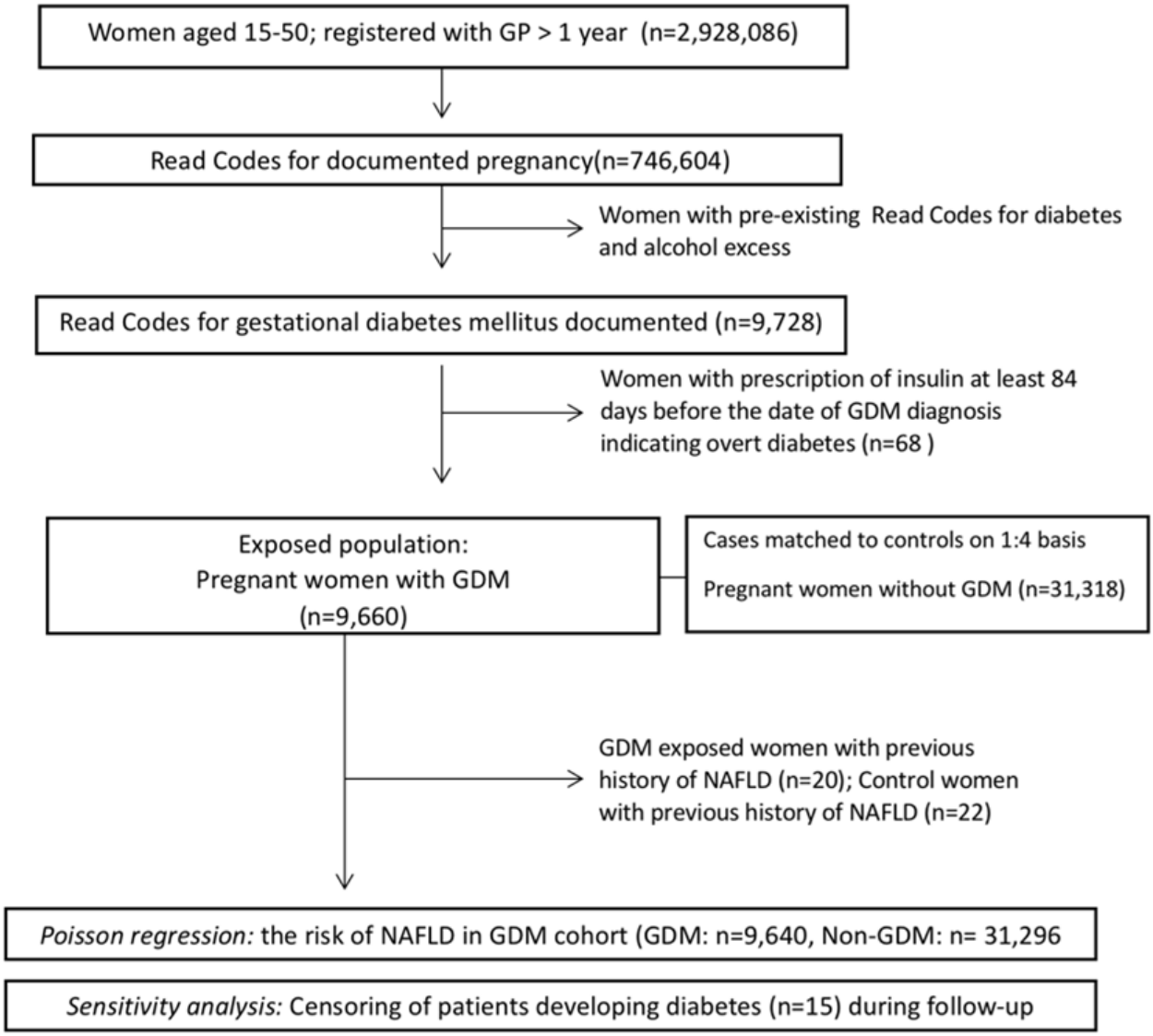


Figure 1A: Retrospective cohort study flow diagram for case identification and analysis

Study outcomes and follow-up:

The primary outcome of this study was NAFLD incidence following delivery during the follow up period. A secondary outcome was to identify covariates contributing to NAFLD development in women with GDM. A sensitivity analysis was performed to assess the effect of incident type 2 diabetes on the relationship between GDM and

incident NAFLD. This was done by censoring the pregnant women when they developed type 2 diabetes. Women who developed type 2 diabetes mellitus during follow-up were censored from analysis **(Figure 1A).** Co-variates were identified at baseline and outcomes during the follow up period both using the Read codes **(Table 1).** The follow-up period began from the index date until the earliest of the following events (exit date); Diagnosis of NAFLD, death, subject left the practice, or last data collection from practice.

Statistical Analysis

Categorical variables were presented as frequencies and continuous variables were presented as mean and standard deviation (SD). The p values for the comparison in baseline characteristics between women with and without GDM were not calculated as per the guidelines for observational studies [54, 55].

NAFLD incidence was compared between the exposed and control groups using Poisson regression and incidence rate ratios (IRR) and 95% confidence intervals (95%CI). The following variables were adjusted for: age, smoking, BMI, Townsend deprivation score[56], hypertension (ever-diagnosis), metformin use, polycystic ovarian syndrome (PCOS) (ever-diagnosis), hypothyroidism (ever-diagnosis) and lipid modifying medication use. These variables were chosen based on biological plausibility to affect the relationship between predictors and NAFLD. In this study, a $p < 0.05$ was considered significant. Statistical analysis was performed on Stata v14.0 software [57].

Ethical approval:

The THIN data collection scheme received multi-center research ethics committee (MREC) approval in 2003 with Scientific Review Committee approval (SRC Reference Number: 17THIN001) of this particular study in January 4 2018 from 'IQVIA' (data provider).

The Systematic Review and Meta-analysis

Aims

The primary aim was to assess the risk of NAFLD in patients with GDM. A secondary aim was to identify any co-variates that increased the risk of NAFLD in women with GDM.

Search Strategy and Selection Criteria

This systematic review was conducted according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines. A systematic literature search was conducted on Medline (1946 to March 2018) and Embase (1974 to March 2018) for studies assessing the risk of developing NAFLD subsequent to a diagnosis of GDM. The detailed search strategy was developed with the assistance of an information specialist and is presented in **Figure 2** of the online supplement.

Strategy - Medline:

# ▲	Searches	Results	Type	Actions	Annotations
1	NAFLD.ti,ab.	8673	Advanced	Display Results | More	
2	fatty liver.ti,ab.	19809	Advanced	Display Results | More	
3	non alcoholic steatohepatitis.ti,ab.	2649	Advanced	Display Results | More	
4	exp Fatty Liver/	26727	Advanced	Display Results | More	
5	exp non-alcoholic fatty liver disease/	7486	Advanced	Display Results | More	
6	NASH.ti,ab.	5523	Advanced	Display Results | More	
7	or/1-6	35318	Advanced	Display Results | More	
8	gestational diabetes.ti,ab.	10990	Advanced	Display Results | More	
9	exp Diabetes, Gestational/	9996	Advanced	Display Results | More	
10	GDM.ti,ab.	5126	Advanced	Display Results | More	
11	((pregnan$ or pregnant$ or gestation$) adj3 (diabetes or diabetic or glucose intolerance or impaired glucose tolerance)).ti,ab.	16951	Advanced	Display Results | More	
12	or/8-11	19640	Advanced	Display Results | More	
13	7 and 12	46	Advanced	Display Results | More	
14	limit 13 to humans	37	Advanced	Display Results | More	
15	(longitudinal or prospective).mp. or cohort.ti,ab. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1152131	Advanced	Display Results | More	
16	14 and 15	11	Advanced	Display Results | More	
17	14 not 16	26	Advanced	Display Results | More	

Strategy - Embase:

# ▲	Searches	Results	Type	Actions	Annotations
1	NAFLD.ti,ab.	16896	Advanced	Display Results | More	
2	fatty liver.ti,ab.	31728	Advanced	Display Results | More	
3	non alcoholic steatohepatitis.ti,ab.	4934	Advanced	Display Results | More	
4	exp Fatty Liver/	60191	Advanced	Display Results | More	
5	exp non-alcoholic fatty liver disease/	28389	Advanced	Display Results | More	
6	NASH.ti,ab.	11429	Advanced	Display Results | More	
7	or/1-6	66794	Advanced	Display Results | More	
8	gestational diabetes.ti,ab.	18056	Advanced	Display Results | More	
9	exp Diabetes, Gestational/	29312	Advanced	Display Results | More	
10	GDM.ti,ab.	8897	Advanced	Display Results | More	
11	((pregnan$ or pregnant$ or gestation$) adj3 (diabetes or diabetic or glucose intolerance or impaired glucose tolerance)).ti,ab.	25855	Advanced	Display Results | More	
12	or/8-11	36000	Advanced	Display Results | More	
13	7 and 12	208	Advanced	Display Results | More	
14	limit 13 to humans	187	Advanced	Display Results | More	
15	(longitudinal or prospective).mp. or cohort.ti,ab. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]	1582264	Advanced	Display Results | More	
16	14 and 15	27	Advanced	Display Results | More	
17	14 not 16	160	Advanced	Display Results | More	

Figure 2: Detailed search strategy on Medline and Embase for studies assessing the risk of developing NAFLD subsequent to a diagnosis of GDM.

The reference lists of all relevant articles were also included in the literature research. Two reviewers (T.T and A.L) independently screened the initial search results for abstract and titles pertaining to the research question.

Randomised controlled trials or cohort studies were suitable for inclusion if they reported any of the following: the raw number of patients, risk ratio in the form of odds ratio or hazard ratio, or incidence rate in person years describing the occurrence of NAFLD in patients with a previous history of GDM. Randomised controlled trials

or cohort studies assessing risk factors associated with the development of NAFLD in the GDM cohort were also eligible for inclusion.

Studies were excluded if they did not report original data or if their investigation was a laboratory-based investigation. Discrepancies between the reviews were resolved in conjunction with third party experts: K.N. and A.T. The flow chart for the above process is presented in **Figure 1B**.

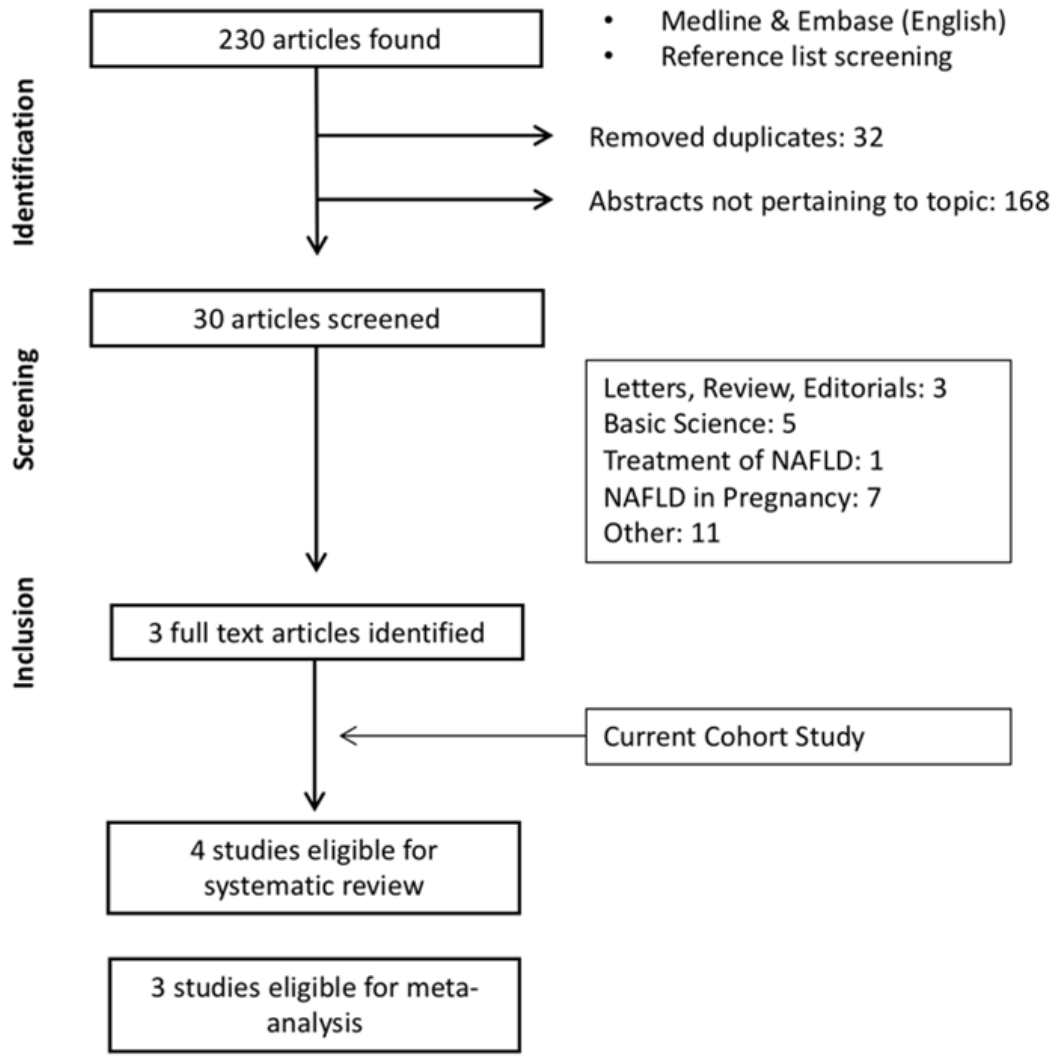


Figure 1B: Systematic review flow diagram for study identification and selection

Data Extraction and Analysis

The data extracted included: the first author, study design, study setting, study period, maternal characteristics, follow-up duration, definition of exposure and modalities of determining primary outcome (**Table 2**).

Table 2: Data extracted from the studies included in the systematic review

Study ID	Participants	Maternal Characteristics	Follow up	Exposures	Primary Outcome
Author: Forbes et al	European women with and without previous GDM were retrospectively identified via NHS antenatal care databases.	**GDM** **Age at exposure:** 33±1 years **Age at scan:** 39±1 years **Parity:** 3±0 **Primiparous:** 67 subjects **Multiparous:** 43 subjects **BMI at term:** 27.8±0.6	**Enrolment Time:** Patients who had live births from 1 to 9 years previous to the study start date retrospective identified and collected from antenatal care database	**Definition of GDM:** 2h 75g OGTT at 24-28 weeks' gestation and WHO criteria: fasting venous plasma glucose >7mmol/l or 2h venous plasma glucose 7.8mmol/l	Hepatic Steatosis (assessed by USS)
Study design: Retrospective Cohort	**Eligibility Criteria:** - Women who had live births from 1-9 years prior to study start date -Women who were more than 1 year but less than 10 years post-partum	**No GDM** **Age at exposure:** 33±1 years **Age at scan:** 39±1 years **Parity:** 2±0 **Primiparous:** Unknown **Multiparous:** Unknown **BMI at term:** 26.8±0.7	**Length:** 6±0 vs. 7±0 years following their index pregnancy (GDM vs. No GDM)	**Severity of GDM:** Diet controlled: 95 Insulin controlled: 15	
Language: English	**Exclusion Criteria:** - Breast Feeding Women - Non-diabetic glucose tolerance - Women with positive antibodies and abnormal liver function		**Methods:** Antenatal healthcare records and clinic visit		
Location: UK	**Sample Size:** 223 subjects - n=110 (previous GDM) - n=113 (no previous GDM)		**Data Collection:** - Antenatal health care records - Laboratory and biochemistry measurements Ultrasound Scanning		

Study ID	Participants	Maternal Characteristics	Follow up	Exposures	Primary Outcome
Author: Ajmera et al	Subjects were recruited from 4 US cities (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA) in 1985–1986. Subjects were not selected based on risk factors for metabolic disease and were recruited by random-digit dialing from total communities, census tract information, or from their health-care	**GDM** **Age at baseline visit, median (IQR):** 26(8) years **Age at scan, median (IQR):** 51 (8) years **Parity > 2 at 25 years:** 40 (32%) **BMI at baseline visit, median (IQR) kg/m^2:** 23.8 (8.8) **Waist circumference, median (IQR):** 74 (17) cm	**Enrolment Time:** Recruited by random-digit dialling from total communities, census tract information, or from their health-care plan in 1985-1986.	**Definition of GDM:** GDM was defined by self-reporting* among those without overt diabetes before pregnancy based on CARDIA laboratory tests	Hepatic Steatosis (assessed by non-contrast abdominal CT Scan)
Study design: Longitudinal cohort	**Eligibility Criteria:** -18-30 years of age -Women who delivered one or two more births -No diagnosis of diabetes prior to pregnancy	**No GDM** **Age at baseline visit, median (IQR):** 25(6) years **Age at scan, median (IQR):** 50 (6) years **Parity > 2 at 25 years:** 238 (24%) **BMI at baseline visit, median (IQR) kg/m^2:** 22.9 (6.2) **Waist circumference, median (IQR):** 71.3 (12.5) cm	**Length:** Patients followed up until 25-year point	**Severity of GDM:** Not reported	
Language: English	**Exclusion Criteria:** Women with other causes of hepatic steatosis including: • Alcohol use >2drinks/day • Self-reported HIV/hepatitis/ medication use of (amiodarone, methotrexate, valproic acid, tamoxifen, steroids, diltiazem, hormone replacement therapy)		**Methods:** Clinic Visit, standardized surveys		
Location: USA	**Sample Size:** 1,115 subjects - n=124 (previous GDM) - n=991 (no previous GDM)		**Data Collection:** -Survey answers -Laboratory and biochemistry results -CT Scan		

Study ID	Participants	Maternal Characteristics	Follow up	Exposures	Primary Outcome
Author: Foghsgaard et al	Subjects were recruited through an invitation letter sent to all women who were diagnosed at either the Center for pregnant Women with Diabetes, Rigshospitalet, Copenhagen, Denmark or the Department of Gynecology-Obstetrics, Copenhagen University Hospital Herley, Denmark within 10 years prior to study start date	**NAFLD in GDM** **Age at time of study, median (IQR):** 36.9 (5.6) years **BMI, median (IQR):** 34.6 (4.7) kg/m^2 **Waist circumference, median (IQR):** 109 (17) cm **Pregnancies:** 2.0 (0.0) **Time from pregnancy:** 4.5 (2.6) years	**Enrolment Time:** Patients who were diagnosed with GDM at the study centres within 10 years prior to study start date	**Definition of GDM:** GDM according to the current Danish guidelines, plasma glucose(PG) concentration at 120 min after 75g oral glucose tolerance test (OGTT) >9 mmol/L during pregnancy	Hepatic Steatosis (assessed by USS)
Study design: Randomised, placebo-controlled, double-blind	**Eligibility Criteria:** • Women with previous GDM as per current Danish Guidelines • Age > 18 years • Normal glucose tolerance, impaired fasting glucose and/or impaired glucose tolerance • Use of safe contraception or sterilization • Negative pregnancy test	**Non-NAFLD in GDM** **Age at time of study, median (IQR):** 39.0 (5.6) years **BMI, median (IQR):** 29.9 (4.7) kg/m^2 **Waist circumference, median (IQR):** 101 (16) cm **Pregnancies:** 2.0 (1.5) **Time from pregnancy:** 4.8 (4.2) years	**Methods:** Screening clinic visit	**Severity of GDM:** Not reported	
Language: English	**Exclusion Criteria:** • Women with established liver disease (based on patient history, biochemical and ultrasonic assessment) • Increased liver enzymes • Ongoing alcoholic abuse • Pregnant or breastfeeding		**Data Collection:** • Laboratory and biochemistry measurements • Ultrasonography • Transient elastography • DXA Audit questionnaire		
Location: Denmark	**Sample Size:** 111 subjects • n=11 (Healthy controls) • n=76 (pGDM non-NAFLD) • n=24 (pGDM NAFLD)				

Metabolic and anthropometric measurements collected over the course of each study were also extracted **(Table 3).** The raw number of subjects in the exposed and non-exposed cohort in each study as well as odds ratio (OR) and 95% CIs with and without adjustment for confounding factors were also extracted. Data on whether each study adjusted for the following variables were also collected: age, parity, baseline BMI, waist circumference, HOMA –IR, high density lipoprotein (HDL), low density lipoprotein (LPL), triglycerides (TG), hypertension, Townsend deprivation scale, smoking, lipid controlling drugs, use of metformin, PCOS, hypothyroidism, and incident diabetes mellitus.

Table 3: Baseline Characteristics and Statistical Analysis Summary of the studies included in the systematic review.

Forbes et al. 2011 Variables	Previous GDM	No Previous GDM	P-value	Statistical Analysis Summary
BMI (kg/m^2)	28.9±0.6	28.9±0.6	0.12	**Statistical Software:** Stata 8
Waist Circumference (cm)	89±1	84±1	0.002	**Baseline Comparison:** Univariate analysis comparing women with and without a previous history of GDM using the unpaired Student's t test and Mann-Whitney U test, as appropriate.
Hip Circumference (cm)	107±1	105±1	0.29	
Fat mass (kg)	107±1	107±1	0.001	Multiple logistic regression with a significant p value < 0.05 was performed with a number of variables to establish which of these were independently associated with NAFLD.
Fasting plasma glucose (mmol/l)	5.3±0.1	5.1±0.1	0.02	
2h plasma glucose (mmol/l)	6.8±0.2	5.8±0.3	0.02	
NGT (%)	82	88	0.04	
IFG (%)	18	6	0.04	
IGT (%)	6	11	0.04	
IFG (%) + IGT (%)	6	4	0.04	
Fasting insulin (pmol/l)	57 (40–114)	34 (24–49)	<0.001	
HOMA%B	97 (79–126)	64 (61–81)	<0.001	
HOMA%S	89 (47–137)	154 (103–228)	<0.001	
Plasma ALT (U/l) (NR 10–50)	27 (15–30)	21 (16–28)	0.41	
Plasma γGT (U/l) (NR 5–35)	19 (11–27)	17 (12–29)	0.61	
Fasting plasma TG (mmol/l) (NR 0.8–2.1)	1.3 (0.9–1.6)	1.0 (0.7–1.7)	0.03	
Fasting plasma cholesterol (mmol/l)	5.3±0.1	5.2±0.1	0.88	
Fasting plasma HDL-cholesterol (mmol/l)	1.3 (1.2–1.6)	1.8 (1.5–1.9)	<0.001	
Fasting plasma LDL-cholesterol (mmol/l)	3.3±0.1	2.8±0.1	0.001	
Fasting plasma NEFA (μmol/l)	666±19	649±13	0.49	

Ajmera et al. 2016 Variables	Previous GDM	No Previous GDM	P-value	Statistical Analysis Summary
BMI, median (IQR) kg/m2	31.1 (12.3)	30.0 (10.7)	0.13	**Statistical Software:** Stata 13.1 **Baseline Comparison:** Mann Whitney U test for continuous variables, chi squared for categorical variables. Logistic regression was used to evaluate the association between previous GDM and NAFLD at year 25. Significance level was set at 0.05 Bivariate models assessed the association between variables chosen beforehand for clinical relevance and known association with the outcome of NAFLD. These covariates included age, race, and baseline covariates (BMI, waist circumference, fasting LDL, HDL, triglycerides, and insulin resistance (HOMA-IR)). Variables were selected for the final multivariate model by backwards elimination with p-value < 0.05 used as the threshold for variable inclusion.
Waist circumference, median (IQR)	93.5 (26.3)	90 (22.3)	0.11	
HOMA-IR, median (IQR)	2.6 (2.9)	2.0 (2.2)	0.04	
Diabetes mellitus, n (%)	61 (49)	75 (7.6)	< 0.01	
Total cholesterol, median (IQR) mg/dL	188 (50.5)	192 (48)	0.13	
LDL, median (IQR) mg/dL	107 (45)	109.5 (43)	0.26	
HDL, median (IQR) mg/dL	57 (22.5)	60 (22)	0.09	
Triglycerides, median (IQR) mg/dL	87 (57.5)	83 (56)	0.12	

Foghsgaard et al. 2017 Variables	Non-NAFLD Previous GDM	NAFLD Previous GDM	P-value	Statistical Analysis Summary
BMI (kg/m^2)	29.9 (4.7)	34.6 (4.7)	0.0002	**Statistical Software:** GraphPad Prism version 6.0 RStudio version 0.98.1083 **Baseline comparison:** Assessment of categorical variables were analyzed using x2 test. Differences with P< 0.05 were considered significant. Logistic regression analysis of the significant variables in the univariate regression analysis were used to identify clinically relevant variables associated with the presence of NAFLD.
Waist Circumference (cm)	101 (16)	109 (17)	0.0003	
Waist-to-hip ratio	0.9 (0.0)	0.9 (0.1)	0.9999	
Fat mass (%)	43.7 (7.5)	46.4 (6.9)	0.1846	
HOMA2IR	1.5 (0.8)	2.4 (1.2)	0.0001	
Total cholesterol (mmol/L)	4.7 (1.2)	5.0 (0.9)	0.328	
HDL cholesterol (mmol/L)	1.2 (0.3)	1.1 (0.4)	0.0081	
LDL cholesterol (mmol/L)	3.2 (1.2)	3.3 (0.7)	0.5165	
VLDL cholesterol (mmol/L)	0.5 (0.2)	0.6 (0.5)	0.36	
Triglycerides (mmol/L)	1.0 (0.6)	1.3 (1.0)	0.164	
Metabolic syndrome	35 (46)	15 (63)	0.0131	

Risk of bias assessment of studies included was performed using a modified version of the Cochrane Collaboration endorsed Newcastle-Ottawa Quality Assessment Scale **(Table 4).**

Table 4: Newcastle-Ottawa Quality Assessment Scale

Study ID	Selection				Comparability		Outcome			Overall Score
	A1	A2	A3	A4	B1	B2	C1	C2	C3	
Forbes et al.	1	1	1	0	1	1	1	1	1	8
Ajmera et al.	1	1	1	0	1	1	1	1	1	8
Foghsgaard et al.	1	1	1	0	1	1	1	1	1	8
Current Study	1	1	1	1	1	1	1	1	1	9

The primary outcome of the meta-analysis was the risk of NAFLD in patients with a previous diagnosis of GDM. Pooled ORs and 95% CIs were derived using the random effects model described by DerSimonian and Laird[58]. Adjusted ORs were used in the meta-analysis to incorporate confounding variables. Heterogeneity was assessed through the I_2 statistic with values >50% indicative of significant heterogeneity. The secondary outcome was to screen for potential risk factors that were associated with NAFLD development in the GDM cohort.

Results

The Population-Based Study

Study population characteristics:

There were 9,640 subjects diagnosed with GDM matched to 31,296 controls within the THIN database (**Table 5**). The median follow-up duration in exposed and control groups was similar; 2.95 (1.21-6.01) and 2.85 (1.14-5.75) years respectively. The study population consisted mainly of young women below the age of 40 who were overweight or had grade 1 obesity. The GDM cohort had a higher proportion of subjects with PCOS (3.56% vs 1.89%) compared to controls. The control population had a higher proportion of current smokers (19.26% vs 15.81%) in comparison to the subjects with GDM. Only a minority of the study population (< 3%) were prescribed metformin, or lipid-lowering treatment. Study population characteristics are presented in **Table 5**.

Table 5: The Health Improvement Network gestational diabetes mellitus cohort and matched control group characteristics

		Gestational Diabetes Mellitus	Control
Number of subjects		9,640	31,296
Person years of follow-up (median, IQR)		2.95 (1.21-6.01)	2.85 (1.14-5.75)
Age		32.87 (5.58)	32.55 (5.27)
Body mass index (median, IQR)		29 (24.4-34.2)	27.6 (23.7-32.00)
Smoking Status			
	Current	1,524 (15.81%)	6,029 (19.26%)
	Former	1,816 (18.84%)	5,854 (18.71%)
	Never	6,099 (63.27%)	18,994 (60.69%)
	Missing	201 (2.09%)	419 (1.34%)
Alcohol Intake			
	No Intake	2,905 (30.13%)	7,137 (22.80%)
	Active Intake	5,198 (53.92%)	19,946 (63.73%)
	Missing	1,537 (15.94%)	4,213 (13.46%)
Lipid Lowering Drugs		6 (0.06%)	30 (0.10%)
Current Metformin Use		249 (2.58%)	11 (0.04%)
Hypertension		553 (5.74%)	1,000 (3.20%)
Polycystic Ovarian Syndrome		809 (8.39%)	1578 (5.04%)
Hypothyroidism		562 (5.83%)	1,301 (4.16%)
Townsend Index			
	1	1,638 (16.99%)	5,741 (18.34%)
	2	1,504 (15.60%)	5,356 (17.11%)
	3	1,898 (19.69%)	6,173 (19.72%)
	4	1,873 (19.43%)	5,603 (17.90%)
	5	1,521 (15.78%)	4,102 (13.11%)
	Not available	1,206 (12.51%)	4,321 (13.81%)

GDM and incident NAFLD

Women in the exposed group (i.e. with GDM) had a greater risk of incident NAFLD (IRR: 3.28, 95%CI 2.14-5.02, p<0.0001) (**Table 6**), which remained significant after adjustment for potential confounders (IRR: 2.70, 95% CI 1.74- 4.19, p<0.0001) (**Table 6).** NAFLD also occurred earlier during the follow up in the GDM group compared to the control group (median (IQR): 3.64 (1.44-6.46) years vs. 5.12 (2.68-9.58), p = 0.0505).

Table 6: The risk of developing non-alcoholic fatty liver disease in the gestational diabetes cohort

(Poisson Regression model with adjustment for confounding variables)

	Gestational Diabetes mellitus	Control
Number of outcomes	44 (0.46%)	41(0.13%)
Person-years	40,718	12,452
Incidence Rate (per 100,000 person-years)	108.06	32.93
Incidence Rate Ratio (95% CI) (**Unadjusted**)	3.28 (2.14-5.02)	
p-value	<0.0001	
Incidence Rate (95% CI) (**Adjusted**)*	2.70 (1.74- 4.19)	
p-value	<0.0001	

*adjusted for age, smoking, BMI, Townsend deprivation score, hypertension, metformin use, polycystic ovarian syndrome and hypothyroidism.

Risk factors for NAFLD in the GDM cohort

In women with GDM: older age (IRR 1.06 (95% CI 1.00-1.12), p=0.0384), obesity (IRR 16.28 (95% CI 2.20-120.57), p=0.006), hypothyroidism (IRR 2.94 (95% CI 1.43-6.08), p=0.004) and PCOS (IRR 3.24 (95% CI 1.60-6.56), p=0.001) predicted

incident NAFLD during the follow-up. Use of lipid-lowering drugs, and use of metformin were not predictors of incident NAFLD in women with GDM **(Table 7).**

Table 7: The risk factors for developing non-alcoholic fatty liver disease in the gestational diabetes mellitus cohort

(Poisson Regression model with adjustment for confounding variables)

		IRR	Lower 95% CI	Upper 95% CI	P value
Age*		1.06	1.00	1.12	0.038
Townsend					
	1	ref	Ref	ref	ref
	2	3.12	0.81	12.12	0.100
	3	3.99	1.09	14.58	0.036
	4	3.74	1.02	13.69	0.046
	5	3.85	1.01	14.69	0.048
	Missing	3.70	0.92	14.85	0.066
Smoking					
	Non-Smoker	Ref	Ref	Ref	Ref
	Ex-Smoker	0.42	0.15	1.19	0.103
	Smoker	0.93	0.42	2.05	0.850
	Missing	1.23	0.25	6.14	0.800
BMI					
	<25	ref	Ref	ref	ref
	25-30	7.19	0.90	57.62	0.063
	30-75*	16.28	2.20	120.57	0.006
	Missing	7.61	0.80	72.17	0.077
Lipid Controlling Drugs		3.13	0.41	23.96	0.271
Current Metformin Use		1.35	0.18	9.95	0.770
Hypertension		0.78	0.31	1.93	0.588
Polycystic Ovarian Syndrome*		3.24	1.60	6.56	0.001
Hypothyroidism*		2.95	1.43	6.08	0.004
Incident Diagnosis of Diabetes		1.29	0.64	2.61	0.473

Sensitivity Analysis:

Women with GDM remained at a higher risk of NAFLD compared to the control population (IRR 2.46; 95% CI 1.51-4.00, p<0.0001) despite censoring of patients who developed type 2 diabetes mellitus during follow-up. Out of the 44 women who developed NAFLD only 12 had preceding diagnosis diabetes **(Figure 1A).**

Systematic Review and Meta-analysis

Search Results

Out of 198 unique studies identified by the search strategy, only 3 studies fulfilled the inclusion criteria for the systematic review [59-61]. The current retrospective cohort study was subsequently included, resulting in a total of four studies for the systematic review. Three studies provided sufficient data including odds ratio to be included in the meta-analysis to assess the risk of developing NAFLD subsequent to a diagnosis of GDM (including the current study)[59, 60]. Foghsgaard et al.[61] was not included in the meta-analysis as it did not compare the risk of developing NAFLD in the GDM cohort to a non-GDM cohort. In summary, a total of 103 cases of NAFLD were diagnosed in 9,874 subjects with a previous history of GDM compared to118 cases of NAFLD in 32,400 control subjects.

Characteristics of Included Studies

The characteristics of all included studies are presented in **(Table 8)**. Forbes et al[60] and Ajmera et al[59] were both cohort studies. Forbes et al. comprised of patients with GDM that were retrospectively identified through use of the National Health Service

(NHS) antenatal database[60]. Ajmera et al selected participants from the pre-existing Coronary Artery Risk Development in Young Adults (CARDIA) cohort [59]. Patients were recruited to this cohort from four cities across the United States of America between 1985 to 1986. Subjects with at least one delivery and no history of diabetes prior to the delivery were included in this study. Both Forbes et al. and Ajmera et al. utilised imaging; ultrasonography (US) and computed tomography (CT) respectively, to identify the outcome of hepatic steatosis. Foghsgaard et al.[61] compared the baseline characteristics of NAFLD and non-NAFLD patients in the GDM cohort. These patients were sourced from a randomised, placebo-controlled, double blind intervention trial assessing the effect of a glucagon-like peptide-1 receptor agonist on glucose tolerance in women with previous GDM[62].

Table 8: Characteristics of Studies assessing the risk of development of non-alcoholic fatty liver disease in the gestational diabetes mellitus cohort.

Study ID	Design	Country	Sample Size		Effect Measures	Effect Size	Lower 95% CI	Upper 95% CI	P-value	Variables Adjusted For
			GDM	Non-GDM						
Forbes et al	Retrospective cohort	UK	NAFLD: 42 No NAFLD: 68	NAFLD: 19 No NALFD: 94	OR	2.77	1.43	5.37	0.002	3
Ajmera et al	Longitudinal cohort	USA	NAFLD: 17 No NAFLD: 107	NAFLD: 58 No NAFLD: 933	OR	2.29	1.23	4.27	0.01	1-11
Current study	Retrospective cohort	UK	NAFLD: 44 No NAFLD: 9,596	NAFLD: 41 No NAFLD: 31,255	OR	2.40	1.51	3.82	<0.0001	1,3,11-18

Confounding factors:
1: age, 2: parity, 3: baseline BMI, 4: waist circumference, 5: HOMA-IR, 6: HOMA-IR, 7: Total cholesterol, 8: LDL, 9: HDL, 10: TG, 11: Hypertension, 12: Townsend, 13: smoking, 14: lipid controlling drugs, 15: use of metformin, 16: polycystic ovarian syndrome, 17: hypothyroidism, 18: incident diabetes mellitus

Quality of Included Studies

The median Newcastle-Ottawa quality score for the included studies was 8 (range, 8-9); all studies were considered of high quality **(Table 4).** Adjustment for confounders

was heterogenous across the included studies. However, all studies adjusted for BMI. Both Ajmera et al and the current study adjusted for age and hypertension.

The risk of non-alcoholic fatty liver disease in GDM

All three studies included in the meta-analysis showed a significantly increased risk of developing NAFLD subsequent to a diagnosis of GDM. The meta-analysis showed that women with GDM are at a higher risk of developing NAFLD in comparison to patients without a previous diagnosis of GDM; OR 2.60 (95% CI 1.90-3.57), (p<0.0001) (**Figure 3**). The results showed minimal heterogeneity (I^2 = 0%). This was also reflected in the crude analysis; OR 3.11 (95% CI 2.30-4.20), p<0.0001 (**Figure 4**). A multivariable logistic regression model was computed for the current retrospective cohort study to produce an odds ratio. The IRR previously calculated and odds ratio were similar (IRR 2.70 (95% CI 1.74 - 4.19), p<0.0001 compared to OR 2.60 (95% CI 1.90-3.57), p<0.0001). The odds ratio analysis was used in the meta-analysis.

Figure 3: Overall meta-analysis of adjusted odds ratio assessing the risk of non-alcoholic fatty liver disease in the gestational diabetes mellitus cohort.

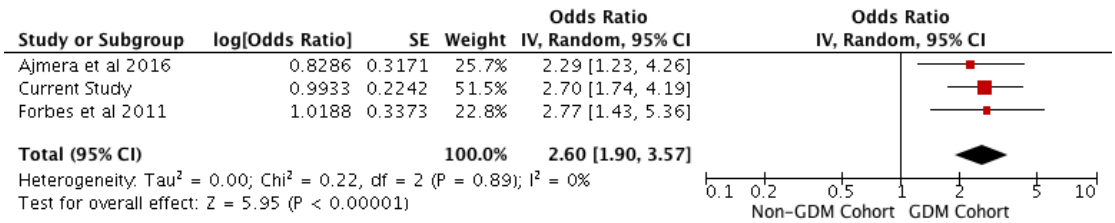
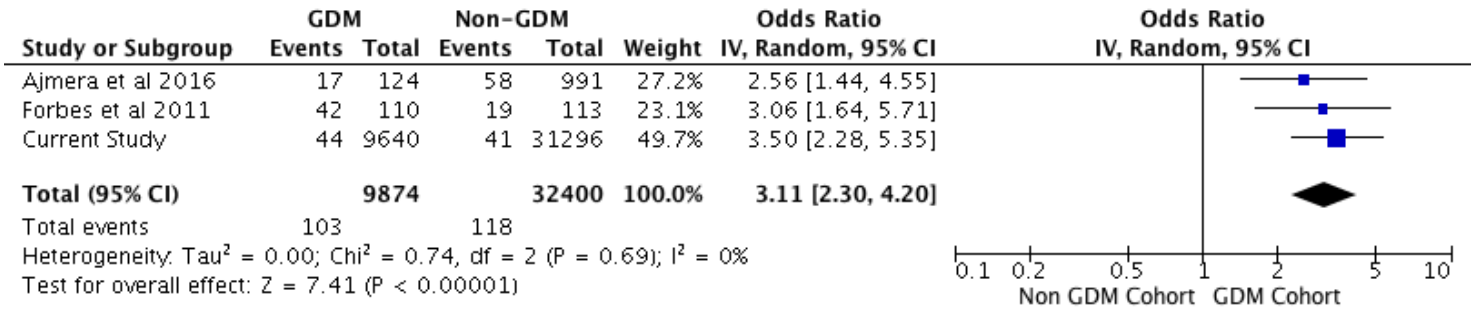


Figure 4: Overall meta-analysis of unadjusted odds ratio assessing the risk of non-alcoholic fatty liver disease in the gestational diabetes mellitus cohort.



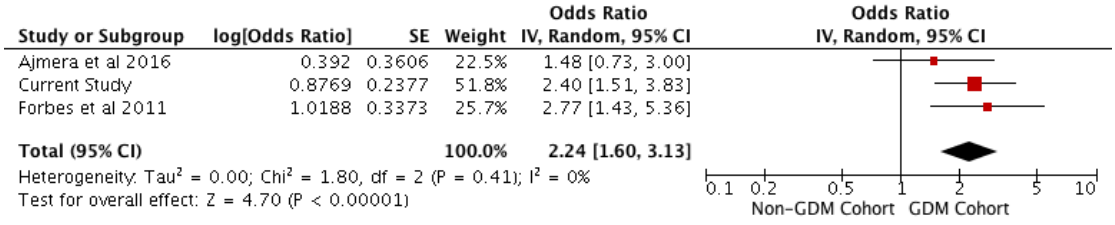
Risk factors for NAFLD in women with GDM

In addition to our current study, another study by Foghsgaard et al.[61] presented a significant univariate association between increase in BMI and development of NAFLD in the GDM cohort; OR 1.24 (95% CI 1.11-1.41), p=0.0005. However, this did not remain statistically significant following multivariate logistic regression including: weight, waist circumference, HDL cholesterol, VLDL cholesterol, triglycerides, visceral fat mass, android to gynoid fat ratio, total fat mass, ALT, AST, Matsuda Index, $HOMA2_{IR}$, FLI and glucagon tAUC. Our retrospective cohort study showed that increasing age, obesity, hypothyroidism, and PCOS confers an increased risk of NAFLD within the GDM cohort. Other studies pooled GDM and non-GDM patients in assessment of NAFLD and hence did not seek to investigate the role of BMI in development of NAFLD in the GDM cohort.

Sensitivity Analysis:

We conducted a sensitivity analysis for incident diabetes mellitus of the three included studies in the meta-analysis, which showed that the GDM cohort remained at a higher risk of NAFLD compared to the non-GDM cohort (OR 2.24; 95% CI 1.60-3.13) **(Figure 5).**

Figure 5: Sensitivity analysis with incident diabetes mellitus



Conclusions

In this paper we present the findings of a large population-based study that assessed the risk of postpartum NAFLD development in women with GDM. This study found that women with GDM were at significantly increased risk of incident NAFLD during the follow up period independent of potential confounders and the occurrence of type 2 diabetes. In addition, NAFLD manifested earlier in women with GDM compared to women without GDM. Finally, we identified additional risk factors for NALFD development within the GDM cohort; previous diagnosis of PCOS or hypothyroidism. This is consistent with previous findings in the literature however this relationship has not been previously shown in the GDM cohort [63, 64].

Our systematic review showed that only two other published studies provided extractable evidence to answer our research question [59, 60]. Our cohort study results were consistent with the previous two studies and the meta-analysis confirmed that women with GDM were at an increased risk of NAFLD compared to women without GDM. However, our study was much larger than the other two reported studies **(Table 8)** and more generalisable since we utilised a population-based primary care database. THIN data is representative of the UK population demographics (age and sex structure), co-morbidities and mortality rates [50]. This dataset has been previously used for studies involving GDM[27] and NAFLD[49]. In addition, the current study population were very well characterized which allowed us to adjust for several confounders that were not considered in previous studies. A previous study utilised self-reporting to identify GDM diagnosis making it prone to recall bias, in contrast this study has identified GDM diagnosis through reporting by physicians [59].

There are several potential mechanisms linking GDM to NAFLD development. GDM results from the inability to adapt to complex metabolic needs during gestation and leads to an increased risk of metabolic syndrome[65] and type 2 diabetes development in later life[66, 67]. GDM has been linked to subsequent lipid abnormalities, hyperinsulineamia/ insulin resistance and increased systematic inflammation especially in overweight or obese women [68-70]. These factors might play an important role in explaining the observed increased risk of NAFLD in women with GDM vs women without GDM. In contrast to a previous study [59], GDM conferred an increased risk of NAFLD independent of the development of type 2 diabetes during follow up in this study. Nonetheless, the fact that obesity, PCOS and hypothyroidism were independent predictors of NAFLD in women with GDM supports the role of insulin resistance, hyperlipidaemia and inflammation in developing NAFLD.

There are currently no established screening strategies in women with GDM to identify NAFLD. Developing such strategies is beyond the scope of the current study but we have identified age, obesity and a previous diagnosis of PCOS or hypothyroidism as independent risk factors of incident NAFLD. Hence, women with GDM who have any of these risk factors are particularly at increased risk of NAFLD development and clinicians should have low threshold for examining for NAFLD in these cohorts.

The findings of this study need to be interpreted in light of its limitations. The diagnosis of GDM in our study is based on physician diagnosis. We acknowledge that GDM diagnoses might be under-recorded in primary care; however, our estimates in our previous analysis for cardiovascular risk in the GDM cohort [27] suggested there were no systematic differences. The criteria for diagnosing GDM might vary between centres and would have changed during the course of the study as we included women

with GDM over a long period of time (1990 to 2016). GDM screening strategy in the UK is based on screening high risk individuals, hence some patients in the control group might have had undiagnosed GDM. To account for this, we used a 1:4 matching ratio to reduce the impact of undiagnosed GDM and our results were consistent with the 2 previous studies published in literature. Our study also had shorter follow-up period in comparison to previous studies [59, 60]. Our study is based on routine clinical diagnosis in contrast to the previous two studies that used systematic screening for NAFLD, and consequently this study could potentially have underestimated the risk of NAFLD development.

This is the largest study to-date that has examined the impact of GDM on development of incident NAFLD. It is also the first population-based study, representative of the UK population in a primary care setting that has examined incident NAFLD in women with GDM. Finally, this study adjusted for a large number of potential confounders.

In conclusion, women with GDM are at an increased risk of developing NAFLD compared to women without GDM independent of subsequent diagnosis of type 2 diabetes. It was also observed that the development of NAFLD occurred earlier in women with GDM compared to women without GDM. Age, obesity and history of PCOS or hypothyroidism were newly identified as independent predictors of the development of NAFLD within the GDM cohort. Clinicians need to be aware of the increased risk of NAFLD in women with GDM and have a lower threshold to investigate for NAFLD, particularly in women with GDM and obesity, PCOS or hypothyroidism. Further studies to develop appropriate screening and preventative strategies in this cohort are needed.

Acknowledgements

Funding. This study was funded by the Master's Program: ''Research in Female Reproduction'' of Athens Medical School, National and Kapodistrian University of Athens.

Duality of interest. No potential conflicts of interest relevant to this article were reported.

AAT is a Clinician Scientist supported by the National Institute for Health Research (NIHR) in the UK. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health. AAT has no conflicts of interest

Prior Presentation. Parts of this work were presented at the 20th European Congress of Endocrinology, Barcelona Spain, 19-22 May 2018 and at the 54th EASD Annual Meeting, Berlin, 1- 5 October 2018.

References

1. Gardner DG & Shoback DM. *Greenspan's Basic and Clinical Endocrinology, Tenth Edition*. McGraw-Hill Education, 2017.

2. Lawrence JM, Contreras R, Chen W & Sacks DA. Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999-2005. *Diabetes Care* 2008 **31** 899-904.

3. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018 **41** S13-s27.

4. Eades CE, Cameron DM & Evans JMM. Prevalence of gestational diabetes mellitus in Europe: A meta-analysis. *Diabetes Res Clin Pract* 2017 **129** 173-181.

5. Li KT, Naik S, Alexander M & Mathad JS. Screening and diagnosis of gestational diabetes in India: a systematic review and meta-analysis. *Acta Diabetol* 2018 **55** 613-625.

6. Ferrara A. Increasing Prevalence of Gestational Diabetes Mellitus. *A public health perspective* 2007 **30** S141-S146.

7. Ovesen PG, Fuglsang J, Andersen MB, Wolff C, Petersen OB, #x00F8, rn & David McIntyre H. Temporal Trends in Gestational Diabetes Prevalence, Treatment, and Outcomes at Aarhus University Hospital, Skejby, between 2004 and 2016. *J Diabetes Res* 2018 **2018** 6.

8. Kruse AR, Darling MS, Hansen MK, Markman MJ, Lauszus FF & Wielandt HB. Recurrence of gestational diabetes in primiparous women. *Acta Obstet Gynecol Scand* 2015 **94** 1367-1372.

9. Moses RG. The recurrence rate of gestational diabetes in subsequent pregnancies. *Diabetes Care* 1996 **19** 1348-1350.

10. Getahun D, Fassett MJ & Jacobsen SJ. Gestational diabetes: risk of recurrence in subsequent pregnancies. *Am J Obstet Gynecol* 2010 **203** 467.e461-466.

11. MacNeill S, Dodds L, Hamilton DC, Armson BA & VandenHof M. Rates and Risk Factors for Recurrence of Gestational Diabetes. *Diabetes Care* 2001 **24** 659-662.

12. Schwartz N, Nachum Z & Green MS. Risk factors of gestational diabetes mellitus recurrence: a meta-analysis. *Endocrine* 2016 **53** 662-671.

13. Schwartz N, Nachum Z & Green MS. The prevalence of gestational diabetes mellitus recurrence--effect of ethnicity and parity: a metaanalysis. *Am J Obstet Gynecol* 2015 **213** 310-317.

14. Schwartz N, Green MS, Yefet E & Nachum Z. Modifiable risk factors for gestational diabetes recurrence. *Endocrine* 2016 **54** 714-722.

15. England L, Kotelchuck M, Wilson HG, Diop H, Oppedisano P, Kim SY, Cui X & Shapiro-Mendoza CK. Estimating the Recurrence Rate of Gestational Diabetes Mellitus (GDM) in Massachusetts 1998-2007: Methods and Findings. *Matern Child Health J* 2015 **19** 2303-2313.

16. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS & Sacks DA. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008 **358** 1991-2002.

17. Metzger BE, Buchanan TA, Coustan DR, de Leiva A, Dunger DB, Hadden DR, Hod M, Kitzmiller JL, Kjos SL, Oats JN, Pettitt DJ, Sacks DA & Zoupas C. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes Care* 2007 **30 Suppl 2** S251-260.

18. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS & Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005 **352** 2477-2486.

19. Wass J & Owen K. *Oxford Handbook of Endocrinology and Diabetes*. OUP Oxford, 2014.

20. Plows JF, Stanley JL, Baker PN, Reynolds CM & Vickers MH. The Pathophysiology of Gestational Diabetes Mellitus. *Int J Mol Sci* 2018 **19**.

21. NICE. Diabetes in pregnancy: management from preconception to the postnatal period. In *NICE guidelines*: , 2015.

22. Carpenter MW & Coustan DR. Criteria for screening tests for gestational diabetes. *Am J Obstet Gynecol* 1982 **144** 768-773.

23. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 1979 **28** 1039-1057.

24. Gestational Diabetes Mellitus. *Diabetes Care* 2004 **27** s88-s90.

25. Bernstein JA, Quinn E, Ameli O, Craig M, Heeren T, Lee-Parritz A, Iverson R, Jack B & McCloskey L. Follow-up after gestational diabetes: a fixable gap in women's preventive healthcare. *BMJ Open Diabetes Res Care* 2017 **5** e000445.

26. Feig DS, Zinman B, Wang X & Hux JE. Risk of development of diabetes mellitus after diagnosis of gestational diabetes. *Cmaj* 2008 **179** 229-234.

27. Daly B, Toulis KA, Thomas N, Gokhale K, Martin J, Webber J, Keerthy D, Jolly K, Saravanan P & Nirantharakumar K. Increased risk of ischemic heart disease, hypertension, and type 2 diabetes in women with previous gestational diabetes mellitus, a target group in general practice for preventive interventions: A population-based cohort study. *PLoS Med* 2018 **15** e1002488.

28. Tobias DK, Hu FB, Forman JP, Chavarro J & Zhang C. Increased risk of hypertension after gestational diabetes mellitus: findings from a large prospective cohort study. *Diabetes Care* 2011 **34** 1582-1584.

29. Tobias DK, Stuart JJ, Li S & et al. Association of history of gestational diabetes with long-term cardiovascular disease risk in a large prospective cohort of us women. *JAMA Internal Medicine* 2017.

30. Samuele De M, Chris D & Gianluca S-B. From NAFLD to NASH and HCC: Pathogenetic Mechanisms and Therapeutic Insights. *Current Pharmaceutical Design* 2013 **19** 5239-5249.

31. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016 **64** 1388-1402.

32. Wong VW, Chan WK, Chitturi S, Chawla Y, Dan YY, Duseja A, Fan J, Goh KL, Hamaguchi M, Hashimoto E, Kim SU, Lesmana LA, Lin YC, Liu CJ, Ni YH, Sollano J, Wong SK, Wong GL, Chan HL & Farrell G. Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017-Part 1: Definition, risk factors and assessment. *J Gastroenterol Hepatol* 2018 **33** 70-85.

33. Chitturi S, Wong VW, Chan WK, Wong GL, Wong SK, Sollano J, Ni YH, Liu CJ, Lin YC, Lesmana LA, Kim SU, Hashimoto E, Hamaguchi M, Goh KL, Fan J, Duseja A, Dan YY, Chawla Y, Farrell G & Chan HL. The Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017-Part 2: Management and special groups. *J Gastroenterol Hepatol* 2018 **33** 86-98.

34. AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions. *Dig Liver Dis* 2017 **49** 471-483.

35. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM & Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018 **67** 328-357.

36. (NICE) NIfHaCE. Non-alcoholic fatty liver disease (NAFLD): assessment and management. In *NICE guideline [NG49]*. UK, Published date: July 2016

37. Dai W, Ye L, Liu A, Wen SW, Deng J, Wu X & Lai Z. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: A meta-analysis. *Medicine (Baltimore)* 2017 **96** e8179.

38. Satapathy SK & Sanyal AJ. Epidemiology and Natural History of Nonalcoholic Fatty Liver Disease. *Semin Liver Dis* 2015 **35** 221-235.

39. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M & Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012 **55** 2005-2023.

40. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G & Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001 **50** 1844-1850.

41. Ofosu A, Ramai D & Reddy M. Non-alcoholic fatty liver disease: controlling an emerging epidemic, challenges, and future directions. *Annals of gastroenterology* 2018 **31** 288.

42. Estes C, Razavi H, Loomba R, Younossi Z & Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2017.

43. Wong RJ, Cheung R & Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014 **59** 2188-2195.

44. Sporea I, Popescu A, Dumitrascu D, Brisc C, Nedelcu L, Trifan A, Gheorghe L & Fierbinteanu Braticevici C. Nonalcoholic Fatty Liver Disease: Status Quo. *J Gastrointestin Liver Dis* 2018 **27** 439-448.

45. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J & Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018 **15** 11-20.

46. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S & Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. *World J Gastroenterol* 2018 **24** 3361-3373.

47. Nair S, Verma S & Thuluvath PJ. Obesity and its effect on survival in patients undergoing orthotopic liver transplantation in the United States. *Hepatology* 2002 **35** 105-109.

48. Tandon P, Ney M, Irwin I, Ma MM, Gramlich L, Bain VG, Esfandiari N, Baracos V, Montano-Loza AJ & Myers RP. Severe muscle depletion in patients on the liver transplant wait list: its prevalence and independent prognostic value. *Liver Transpl* 2012 **18** 1209-1216.

49. Kumarendran B, O'Reilly MW, Manolopoulos KN, Toulis KA, Gokhale KM, Sitch AJ, Wijeyaratne CN, Coomarasamy A, Arlt W & Nirantharakumar K. Polycystic ovary syndrome, androgen excess, and the risk of nonalcoholic fatty liver disease in women: A longitudinal study based on a United Kingdom primary care database. *PLoS Med* 2018 **15** e1002542.

50. Blak B, Thompson M, Dattani H & Bourke A. Generalisability of The Health Improvement Network (THIN) database: demographics, chronic disease prevalence and mortality rates. *Journal of Innovation in Health Informatics* 2011 **19** 251-255.

51. University College London. THIN database. London: University College London; 2018 [cited 2018 Jan 3]. Available from: http://www.ucl.ac.uk/pcph/research/thin-database.

52. Vision. Available at: https://www.visionhealth.co.uk/. Accessed Jan 3, 2018.

53. Booth N. What are the Read codes? *Health libraries review* 1994 **11** 177-182.

54. Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM & Committee RW. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement. *PLoS Med* 2015 **12** e1001885.

55. Bland JM & Altman DG. Multiple significance tests: the Bonferroni method. *Bmj* 1995 **310** 170.

56. Townsend P, Phillimore P & Beattie A. Health and Deprivation: Inequality and the North (Croom Helm, London) Google Scholar. 1988.

57. StataCorp. Stata Statistical Software. Release 14. College Station (Texas) StataCorp; 2015.

58. DerSimonian R & Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986 **7** 177-188.

59. Ajmera VH, Gunderson EP, VanWagner LB, Lewis CE, Carr JJ & Terrault NA. Gestational Diabetes Mellitus Is Strongly Associated With Non-Alcoholic Fatty Liver Disease. *Am J Gastroenterol* 2016 **111** 658-664.

60. Forbes S, Taylor-Robinson SD, Patel N, Allan P, Walker BR & Johnston DG. Increased prevalence of non-alcoholic fatty liver disease in European women with a history of gestational diabetes. *Diabetologia* 2011 **54** 641-647.

61. Foghsgaard S, Andreasen C, Vedtofte L, Andersen ES, Bahne E, Strandberg C, Buhl T, Holst JJ, Svare JA & Clausen TD. Nonalcoholic fatty liver disease is prevalent in women with prior gestational diabetes mellitus and independently associated with insulin resistance and waist circumference. *Diabetes Care* 2017 **40** 109-116.

62. Foghsgaard S, Vedtofte L, Mathiesen ER, Svare JA, Gluud LL, Holst JJ, Damm P, Knop FK & Vilsbøll T. The effect of a glucagon-like peptide-1 receptor agonist on glucose tolerance in women with previous gestational diabetes mellitus: protocol for an investigator-initiated, randomised, placebo-controlled, double-blinded, parallel intervention trial. *BMJ open* 2013 **3** e003834.

63. Ramezani-Binabaj M, Motalebi M, Karimi-sari H, Rezaee-Zavareh MS & Alavian SM. Are women with polycystic ovarian syndrome at a high risk of non-alcoholic Fatty liver disease; a meta-analysis. *Hepatitis monthly* 2014 **14**.

64. Eshraghian A & Jahromi AH. Non-alcoholic fatty liver disease and thyroid dysfunction: a systematic review. *World journal of gastroenterology: WJG* 2014 **20** 8102.

65. Noussitou P, Monbaron D, Vial Y, Gaillard RC & Ruiz J. Gestational diabetes mellitus and the risk of metabolic syndrome: a population-based study in Lausanne, Switzerland. *Diabetes Metab* 2005 **31** 361-369.

66. Huvinen E, Eriksson JG, Koivusalo SB, Grotenfelt N, Tiitinen A, Stach-Lempinen B & Rono K. Heterogeneity of gestational diabetes (GDM) and long-term risk of diabetes and metabolic syndrome: findings from the RADIEL study follow-up. *Acta Diabetol* 2018 **55** 493-501.

67. Bellamy L, Casas JP, Hingorani AD & Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet* 2009 **373** 1773-1779.

68. Mirghani Dirar A & Doupis J. Gestational diabetes from A to Z. *World J Diabetes* 2017 **8** 489-511.

69. Aktulay A, Engin-Ustun Y, Ozkan MS, Erkaya S, Kara M, Kaymak O & Danisman N. GESTATIONAL DIABETES MELLITUS SEEMS TO BE ASSOCIATED WITH INFLAMMATION. *Acta Clin Croat* 2015 **54** 475-478.

70. Prikoszovich T, Winzer C, Schmid AI, Szendroedi J, Chmelik M, Pacini G, Krššák M, Moser E, Funahashi T & Waldhäusl W. Body and liver fat mass rather than muscle mitochondrial function determine glucose metabolism in women with a history of gestational diabetes mellitus. *Diabetes Care* 2011 **34** 430-436.

Appendix A - figures

Figure 1 A & B

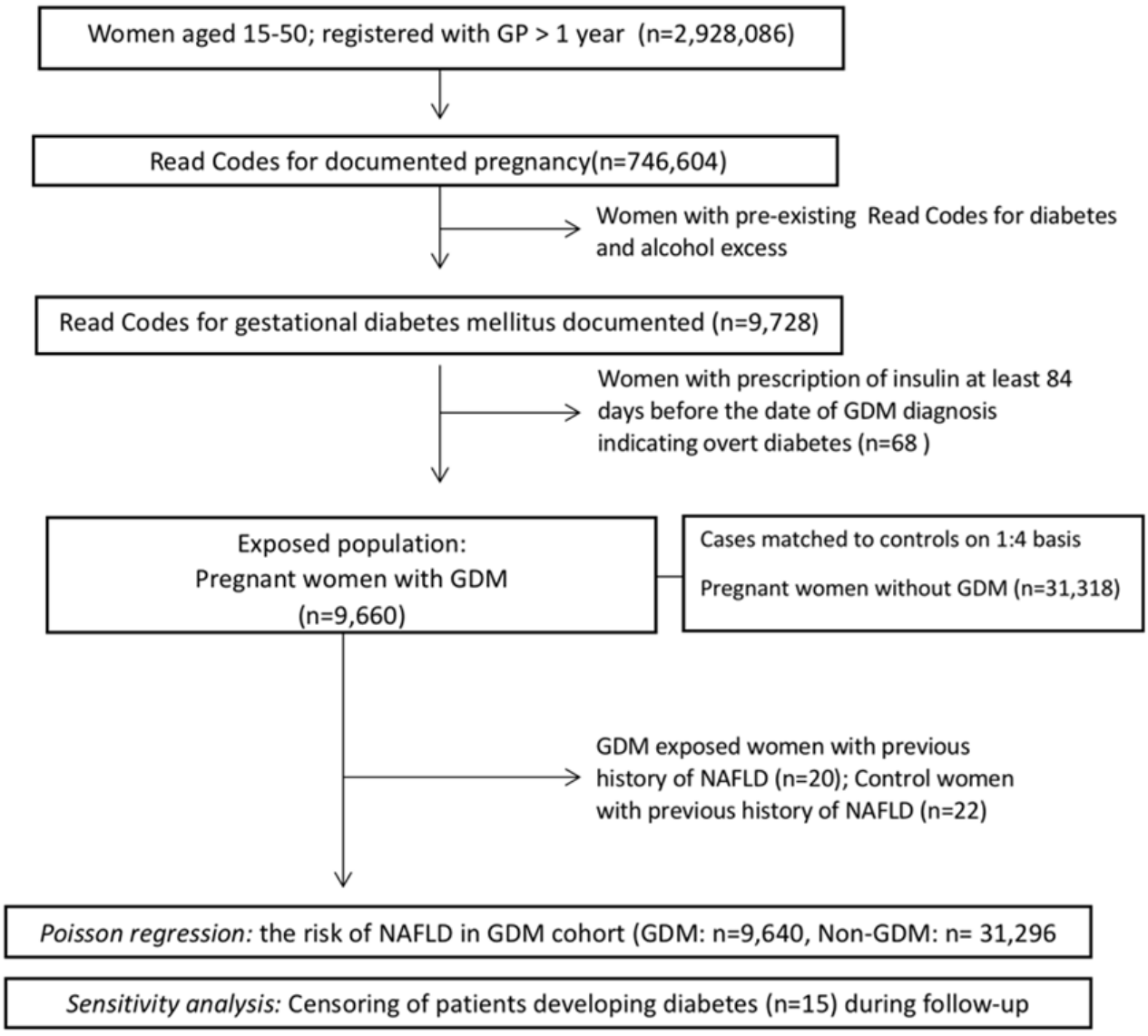


Figure 1A: Retrospective cohort study flow diagram for case identification and analysis

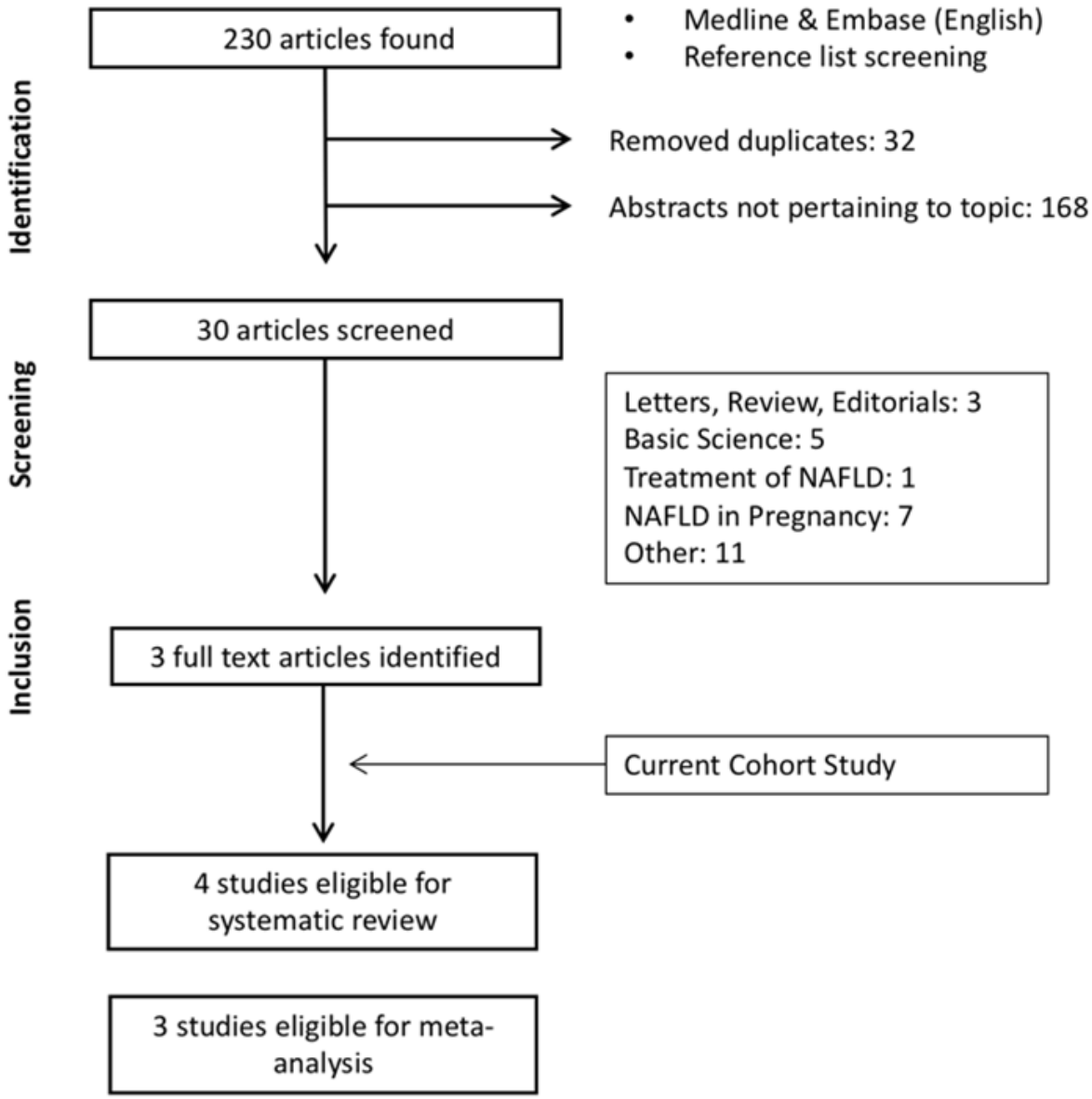


Figure 1B: Systematic review flow diagram for study identification and selection

Figure 2. Detailed search strategy on Medline and Embase for studies assessing the risk of developing NAFLD subsequent to a diagnosis of GDM.

Strategy - Medline:

# ▲	Searches	Results	Type	Actions	Annotations
1	NAFLD.ti,ab.	8673	Advanced	Display Results | More ▾	
2	fatty liver.ti,ab.	19809	Advanced	Display Results | More ▾	
3	non alcoholic steatohepatitis.ti,ab.	2649	Advanced	Display Results | More ▾	
4	exp Fatty Liver/	26727	Advanced	Display Results | More ▾	
5	exp non-alcoholic fatty liver disease/	7486	Advanced	Display Results | More ▾	
6	NASH.ti,ab.	5523	Advanced	Display Results | More ▾	
7	or/1-6	35318	Advanced	Display Results | More ▾	
8	gestational diabetes.ti,ab.	10990	Advanced	Display Results | More ▾	
9	exp Diabetes, Gestational/	9996	Advanced	Display Results | More ▾	
10	GDM.ti,ab.	5126	Advanced	Display Results | More ▾	
11	((pregnan$ or pregnant$ or gestation$) adj3 (diabetes or diabetic or glucose intolerance or impaired glucose tolerance)).ti,ab.	16951	Advanced	Display Results | More ▾	
12	or/8-11	19640	Advanced	Display Results | More ▾	
13	7 and 12	46	Advanced	Display Results | More ▾	
14	limit 13 to humans	37	Advanced	Display Results | More ▾	
15	(longitudinal or prospective).mp. or cohort.ti,ab. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1152131	Advanced	Display Results | More ▾	
16	14 and 15	11	Advanced	Display Results | More ▾	
17	14 not 16	26	Advanced	Display Results | More ▾	

Strategy - Embase:

# ▲	Searches	Results	Type	Actions	Annotations
1	NAFLD.ti,ab.	16896	Advanced	Display Results More ▾	
2	fatty liver.ti,ab.	31728	Advanced	Display Results More ▾	
3	non alcoholic steatohepatitis.ti,ab.	4934	Advanced	Display Results More ▾	
4	exp Fatty Liver/	60191	Advanced	Display Results More ▾	
5	exp non-alcoholic fatty liver disease/	28389	Advanced	Display Results More ▾	
6	NASH.ti,ab.	11429	Advanced	Display Results More ▾	
7	or/1-6	66794	Advanced	Display Results More ▾	
8	gestational diabetes.ti,ab.	18056	Advanced	Display Results More ▾	
9	exp Diabetes, Gestational/	29312	Advanced	Display Results More ▾	
10	GDM.ti,ab.	8897	Advanced	Display Results More ▾	
11	((pregnan$ or pregnant$ or gestation$) adj3 (diabetes or diabetic or glucose intolerance or impaired glucose tolerance)).ti,ab.	25855	Advanced	Display Results More ▾	
12	or/8-11	36000	Advanced	Display Results More ▾	
13	7 and 12	208	Advanced	Display Results More ▾	
14	limit 13 to humans	187	Advanced	Display Results More ▾	
15	(longitudinal or prospective).mp. or cohort.ti,ab. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]	1582264	Advanced	Display Results More ▾	
16	14 and 15	27	Advanced	Display Results More ▾	
17	14 not 16	160	Advanced	Display Results More ▾	

Figure 3: Overall meta-analysis of adjusted odds ratio assessing the risk of non-alcoholic fatty liver disease in the gestational diabetes mellitus cohort

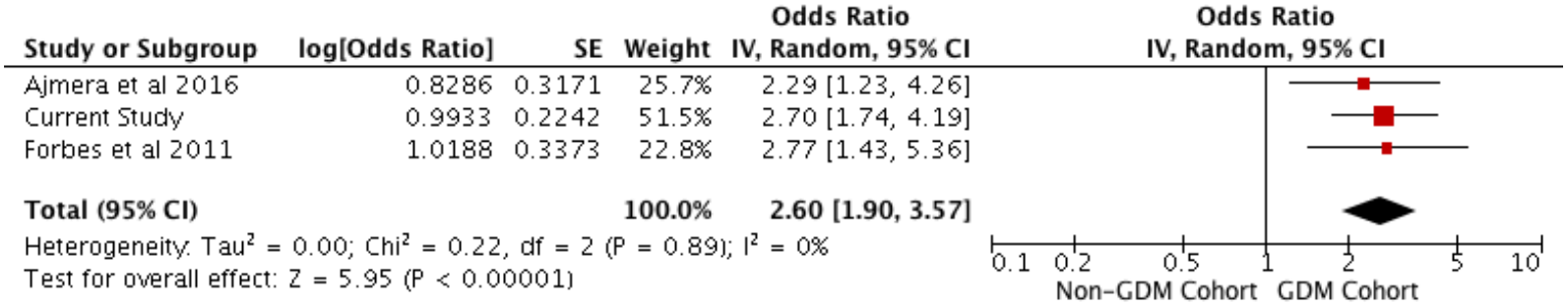


Figure 4: Overall meta-analysis of unadjusted odds ratio assessing the risk of non-alcoholic fatty liver disease in the gestational diabetes mellitus cohort

	GDM		Non-GDM			Odds Ratio
Study or Subgroup	**Events**	**Total**	**Events**	**Total**	**Weight**	**IV, Random, 95% CI**
Ajmera et al 2016	17	124	58	991	27.2%	2.56 [1.44, 4.55]
Forbes et al 2011	42	110	19	113	23.1%	3.06 [1.64, 5.71]
Current Study	44	9640	41	31296	49.7%	3.50 [2.28, 5.35]
Total (95% CI)		**9874**		**32400**	**100.0%**	**3.11 [2.30, 4.20]**
Total events	103		118			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.74$, df = 2 (P = 0.69); $I^2 = 0\%$

Test for overall effect: Z = 7.41 (P < 0.00001)

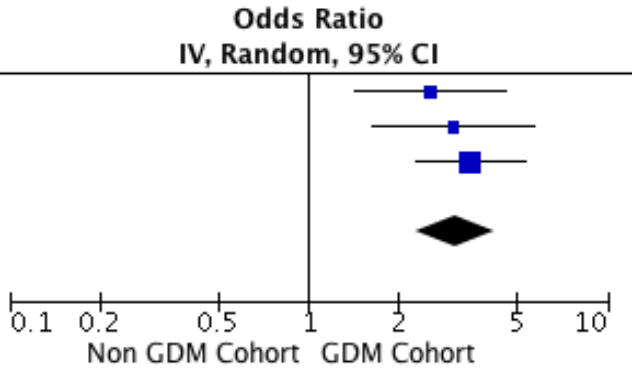
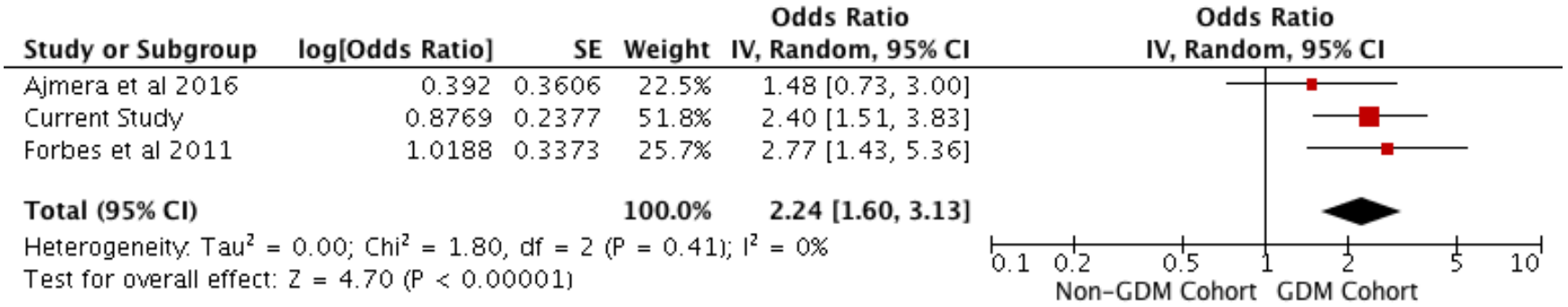


Figure 5: Sensitivity analysis with incident diabetes mellitus



Appendix B - tables

Table 1: Read codes used to identify incident cases of GDM - (Exposure) and NAFLD/NASH development - (outcome).

Codes for Exposure - GDM	
L180811	Gestational diabetes mellitus
L180900	Gestational diabetes mellitus
Codes for Outcome - NAFLD and NASH	
J61y100	Non-alcoholic fatty liver
J61y800	Nonalcoholic steatohepatitis

Table 2: Data extracted from the studies included in the systematic review

Study ID	Participants	Maternal Characteristics	Follow up	Exposures	Primary Outcome
Author: Forbes et al	European women with and without previous GDM were retrospectively identified via NHS antenatal care databases.	**GDM** **Age at exposure:** 33±1 years **Age at scan:** 39±1 years **Parity:** 3±0 **Primiparous:** 67 subjects **Multiparous:** 43 subjects **BMI at term:** 27.8±0.6	**Enrolment Time:** Patients who had live births from 1 to 9 years previous to the study start date retrospective identified and collected from antenatal care database	**Definition of GDM:** 2h 75g OGTT at 24-28 weeks' gestation and WHO criteria: fasting venous plasma glucose >7mmol/l or 2h venous plasma glucose 7.8mmol/l	Hepatic Steatosis (assessed by USS)
Study design: Retrospective Cohort	**Eligibility Criteria:** - Women who had live births from 1-9 years prior to study start date -Women who were more than 1 year but less than 10 years post-partum	**No GDM** **Age at exposure:** 33±1 years **Age at scan:** 39±1 years **Parity:** 2±0 **Primiparous:** Unknown **Multiparous:** Unknown **BMI at term:** 26.8±0.7	**Length:** 6±0 vs. 7±0 years following their index pregnancy (GDM vs. No GDM)	**Severity of GDM:** Diet controlled: 95 Insulin controlled: 15	
Language: English	**Exclusion Criteria:** - Breast Feeding Women - Non-diabetic glucose tolerance - Women with positive antibodies and abnormal liver function		**Methods:** Antenatal healthcare records and clinic visit		
Location: UK	**Sample Size:** 223 subjects - n=110 (previous GDM) - n=113 (no previous GDM)		**Data Collection:** - Antenatal health care records - Laboratory and biochemistry measurements Ultrasound Scanning		

Study ID	Participants	Maternal Characteristics	Follow up	Exposures	Primary Outcome
Author: Ajmera et al	Subjects were recruited from 4 US cities (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA) in 1985–1986. Subjects were not selected based on risk factors for metabolic disease and were recruited by random-digit dialing from total communities, census tract information, or from their health-care	**GDM** **Age at baseline visit, median (IQR):** 26(8) years **Age at scan, median (IQR):** 51 (8) years **Parity > 2 at 25 years:** 40 (32%) **BMI at baseline visit, median (IQR) kg/m²:** 23.8 (8.8) **Waist circumference, median (IQR):** 74 (17) cm	**Enrolment Time:** Recruited by random-digit dialling from total communities, census tract information, or from their health-care plan in 1985-1986.	**Definition of GDM:** GDM was defined by self-reporting* among those without overt diabetes before pregnancy based on CARDIA laboratory tests	Hepatic Steatosis (assessed by non-contrast abdominal CT Scan)
Study design: Longitudinal cohort	**Eligibility Criteria:** -18-30 years of age -Women who delivered one or two more births -No diagnosis of diabetes prior to pregnancy	**No GDM** **Age at baseline visit, median (IQR):** 25(6) years **Age at scan, median (IQR):** 50 (6) years **Parity > 2 at 25 years:** 238 (24%) **BMI at baseline visit, median (IQR) kg/m²:** 22.9 (6.2) **Waist circumference, median (IQR):** 71.3 (12.5) cm	**Length:** Patients followed up until 25-year point	**Severity of GDM:** Not reported	
Language: English	**Exclusion Criteria:** Women with other causes of hepatic steatosis including: • Alcohol use >2drinks/day • Self-reported HIV/hepatitis/ medication use of (amiodarone, methotrexate, valproic acid, tamoxifen, steroids, diltiazem, hormone replacement therapy)		**Methods:** Clinic Visit, standardized surveys		
Location: USA	**Sample Size:** 1,115 subjects - n=124 (previous GDM) - n=991 (no previous GDM)		**Data Collection:** -Survey answers -Lab, & biochemistry results -CT Scan		

Study ID	Participants	Maternal Characteristics	Follow up	Exposures	Primary Outcome
Author: Foghsgaard et al	Subjects were recruited through an invitation letter sent to all women who were diagnosed at either the Center for pregnant Women with Diabetes, Rigshospitalet, Copenhagen, Denmark or the Department of Gynecology-Obstetrics, Copenhagen University Hospital Herley, Denmark within 10 years prior to study start date	**NAFLD in GDM** **Age at time of study, median (IQR):** 36.9 (5.6) years **BMI, median (IQR):** 34.6 (4.7) kg/m^2 **Waist circumference, median (IQR):** 109 (17) cm **Pregnancies:** 2.0 (0.0) **Time from pregnancy:** 4.5 (2.6) years	**Enrolment Time:** Patients who were diagnosed with GDM at the study centres within 10 years prior to study start date	**Definition of GDM:** GDM according to the current Danish guidelines, plasma glucose(PG) concentration at 120 min after 75g oral glucose tolerance test (OGTT) >9 mmol/L during pregnancy	Hepatic Steatosis (assessed by USS)
Study design: Randomised, placebo-controlled, double-blind	**Eligibility Criteria:** • Women with previous GDM as per current Danish Guidelines • Age > 18 years • Normal glucose tolerance, impaired fasting glucose and/or impaired glucose tolerance • Use of safe contraception or sterilization • Negative pregnancy test	**Non-NAFLD in GDM** **Age at time of study, median (IQR):** 39.0 (5.6) years **BMI, median (IQR):** 29.9 (4.7) kg/m^2 **Waist circumference, median (IQR):** 101 (16) cm **Pregnancies:** 2.0 (1.5) **Time from pregnancy:** 4.8 (4.2) years	**Methods:** Screening clinic visit	**Severity of GDM:** Not reported	
Language: English	**Exclusion Criteria:** • Women with established liver disease (based on patient history, biochemical and ultrasonic assessment) • Increased liver enzymes • Ongoing alcoholic abuse • Pregnant or breastfeeding		**Data Collection:** Lab. and biochemistry measurements/ Ultrasonography/ Transient elastography/ DXA Audit questionnaire		
Location: Denmark	**Sample Size:** 111 subjects • n=11 (Healthy controls) • n=76 (pGDM non-NAFLD) • n=24 (pGDM NAFLD)				

Table 3: Baseline Characteristics and Statistical Analysis Summary of the studies included in the systematic review.

Forbes et al. 2011 Variables	Previous GDM	No Previous GDM	P-value	Statistical Analysis Summary
BMI (kg/m²)	28.9±0.6	28.9±0.6	0.12	
Waist Circumference (cm)	89±1	84±1	0.002	
Hip Circumference (cm)	107±1	105±1	0.29	
Fat mass (kg)	107±1	107±1	0.001	
Fasting plasma glucose (mmol/l)	5.3±0.1	5.1±0.1	0.02	
2h plasma glucose (mmol/l)	6.8±0.2	5.8±0.3	0.02	**Statistical Software:** Stata 8
NGT (%)	82	88	0.04	**Baseline Comparison:** Univariate analysis comparing women with and without a previous history of GDM using the unpaired Student's t test and Mann-Whitney U test, as appropriate.
IFG (%)	18	6	0.04	
IGT (%)	6	11	0.04	
IFG (%) + IGT (%)	6	4	0.04	
Fasting insulin (pmol/l)	57 (40–114)	34 (24–49)	<0.001	
HOMA%B	97 (79–126)	64 (61–81)	<0.001	Multiple logistic regression with a significant p value < 0.05 was performed with a number of variables to establish which of these were independently associated with NAFLD.
HOMA%S	89 (47–137)	154 (103–228)	<0.001	
Plasma ALT (U/l) (NR 10–50)	27 (15–30)	21 (16–28)	0.41	
Plasma γGT (U/l) (NR 5–35)	19 (11–27)	17 (12–29)	0.61	
Fasting plasma TG (mmol/l) (NR 0.8–2.1)	1.3 (0.9–1.6)	1.0 (0.7–1.7)	0.03	
Fasting plasma cholesterol (mmol/l)	5.3±0.1	5.2±0.1	0.88	
Fasting plasma HDL-cholesterol (mmol/l)	1.3 (1.2–1.6)	1.8 (1.5–1.9)	<0.001	
Fasting plasma LDL-cholesterol (mmol/l)	3.3±0.1	2.8±0.1	0.001	
Fasting plasma NEFA (μmol/l)	666±19	649±13	0.49	

Ajmera et al. 2016 Variables	Previous GDM	No Previous GDM	P-value	Statistical Analysis Summary
BMI, median (IQR) kg/m2	31.1 (12.3)	30.0 (10.7)	0.13	
Waist circumference, median (IQR)	93.5 (26.3)	90 (22.3)	0.11	**Statistical Software:** Stata 13.1 **Baseline Comparison:** Mann Whitney U test for continuous variables, chi squared for categorical variables. Logistic regression was used to evaluate the association between previous GDM and NAFLD at year 25. Significance level was set at 0.05 Bivariate models assessed the association between variables chosen beforehand for clinical relevance and known association with the outcome of NAFLD. These covariates included age, race, and baseline covariates (BMI, waist circumference, fasting LDL, HDL, triglycerides, and insulin resistance (HOMA-IR)). Variables were selected for the final multivariate model by backwards elimination with p-value < 0.05 used as the threshold for variable inclusion.
HOMA-IR, median (IQR)	2.6 (2.9)	2.0 (2.2)	0.04	
Diabetes mellitus, n (%)	61 (49)	75 (7.6)	< 0.01	
Total cholesterol, median (IQR) mg/dL	188 (50.5)	192 (48)	0.13	
LDL, median (IQR) mg/dL	107 (45)	109.5 (43)	0.26	
HDL, median (IQR) mg/dL	57 (22.5)	60 (22)	0.09	
Triglycerides, median (IQR) mg/dL	87 (57.5)	83 (56)	0.12	

Foghsgaard et al. 2017 Variables	Non-NAFLD Previous GDM	NAFLD Previous GDM	P-value	Statistical Analysis Summary
BMI (kg/m^2)	29.9 (4.7)	34.6 (4.7)	0.0002	**Statistical Software:** GraphPad Prism version 6.0 RStudio version 0.98.1083 **Baseline comparison:** Assessment of categorical variables were analyzed using x2 test. Differences with P< 0.05 were considered significant. Logistic regression analysis of the significant variables in the univariate regression analysis were used to identify clinically relevant variables associated with the presence of NAFLD.
Waist Circumference (cm)	101 (16)	109 (17)	0.0003	
Waist-to-hip ratio	0.9 (0.0)	0.9 (0.1)	0.9999	
Fat mass (%)	43.7 (7.5)	46.4 (6.9)	0.1846	
HOMA2IR	1.5 (0.8)	2.4 (1.2)	0.0001	
Total cholesterol (mmol/L)	4.7 (1.2)	5.0 (0.9)	0.328	
HDL cholesterol (mmol/L)	1.2 (0.3)	1.1 (0.4)	0.0081	
LDL cholesterol (mmol/L)	3.2 (1.2)	3.3 (0.7)	0.5165	
VLDL cholesterol (mmol/L)	0.5 (0.2)	0.6 (0.5)	0.36	
Triglycerides (mmol/L)	1.0 (0.6)	1.3 (1.0)	0.164	
Metabolic syndrome	35 (46)	15 (63)	0.0131	

Table 4: Newcastle-Ottawa Quality Assessment Scale

Study ID	Selection				Comparability		Outcome			Overall Score
	A1	A2	A3	A4	B1	B2	C1	C2	C3	
Forbes et al.	1	1	1	0	1	1	1	1	1	8
Ajmera et al.	1	1	1	0	1	1	1	1	1	8
Foghsgaard et al.	1	1	1	0	1	1	1	1	1	8
Current Study	1	1	1	1	1	1	1	1	1	9

Table 5: The Health Improvement Network gestational diabetes mellitus cohort and matched control group characteristics

		Gestational Diabetes Mellitus	Control
Number of subjects		9,640	31,296
Person years of follow-up (median, IQR)		2.95 (1.21-6.01)	2.85 (1.14-5.75)
Age		32.87 (5.58)	32.55 (5.27)
Body mass index (median, IQR)		29 (24.4-34.2)	27.6 (23.7-32.00)
Smoking Status	Current	1,524 (15.81%)	6,029 (19.26%)
	Former	1,816 (18.84%)	5,854 (18.71%)
	Never	6,099 (63.27%)	18,994 (60.69%)
	Missing	201 (2.09%)	419 (1.34%)
Alcohol Intake	No Intake	2,905 (30.13%)	7,137 (22.80%)
	Active Intake	5,198 (53.92%)	19,946 (63.73%)
	Missing	1,537 (15.94%)	4,213 (13.46%)
Lipid Lowering Drugs		6 (0.06%)	30 (0.10%)
Current Metformin Use		249 (2.58%)	11 (0.04%)
Hypertension		553 (5.74%)	1,000 (3.20%)
Polycystic Ovarian Syndrome		809 (8.39%)	1578 (5.04%)
Hypothyroidism		562 (5.83%)	1,301 (4.16%)
Townsend Index	1	1,638 (16.99%)	5,741 (18.34%)
	2	1,504 (15.60%)	5,356 (17.11%)
	3	1,898 (19.69%)	6,173 (19.72%)
	4	1,873 (19.43%)	5,603 (17.90%)
	5	1,521 (15.78%)	4,102 (13.11%)
	Not available	1,206 (12.51%)	4,321 (13.81%)

Table 6: The risk of developing non-alcoholic fatty liver disease in the gestational diabetes cohort (Poisson Regression model with adjustment for confounding variables)

	Gestational Diabetes mellitus	Control
Number of outcomes	44 (0.46%)	41(0.13%)
Person-years	40,718	12,452
Incidence Rate (per 100,000 person-years)	108.6	32.93
Incidence Rate Ratio (95% CI) (**Unadjusted**)	3.28 (2.14-5.02)	
p-value	<0.0001	
Incidence Rate (95% CI) (**Adjusted**)*	2.70 (1.74- 4.19)	
p-value	<0.0001	

*adjusted for age, smoking, BMI, Townsend deprivation score, hypertension, metformin use, polycystic ovarian syndrome and hypothyroidism.

Table 7: The risk factors for developing non-alcoholic fatty liver disease in the gestational diabetes mellitus cohort

(Poisson Regression model with adjustment for confounding variables)

		IRR	Lower 95% CI	Upper 95% CI	P value
Age*		1.06	1.00	1.12	0.038
Townsend					
	1	ref	Ref	ref	ref
	2	3.12	0.81	12.12	0.100
	3	3.99	1.09	14.58	0.036
	4	3.74	1.02	13.69	0.046
	5	3.85	1.01	14.69	0.048
	Missing	3.70	0.92	14.85	0.066
Smoking					
	Non-Smoker	Ref	Ref	Ref	Ref
	Ex-Smoker	0.42	0.15	1.19	0.103
	Smoker	0.93	0.42	2.05	0.850
	Missing	1.23	0.25	6.14	0.800
BMI					
	<25	ref	Ref	ref	ref
	25-30	7.19	0.90	57.62	0.063
	30-75*	16.28	2.20	120.57	0.006
	Missing	7.61	0.80	72.17	0.077
Lipid Controlling Drugs		3.13	0.41	23.96	0.271
Current Metformin Use		1.35	0.18	9.95	0.770
Hypertension		0.78	0.31	1.93	0.588
Polycystic Ovarian Syndrome*		3.24	1.60	6.56	0.001
Hypothyroidism*		2.95	1.43	6.08	0.004
Incident Diagnosis of Diabetes		1.29	0.64	2.61	0.473

Table 8: Characteristics of Studies assessing the risk of development of non-alcoholic fatty liver disease in the gestational diabetes mellitus cohort

Study ID	Design	Country	Sample Size		Effect Measures	Effect Size	Lower 95% CI	Upper 95% CI	P-value	Variables Adjusted For
			GDM	Non-GDM						
Forbes et al	Retrospective cohort	UK	NAFLD: 42 No NAFLD: 68	NAFLD: 19 No NALFD: 94	OR	2.77	1.43	5.37	0.002	3
Ajmera et al	Longitudinal cohort	USA	NAFLD: 17 No NAFLD: 107	NAFLD: 58 No NAFLD: 933	OR	2.29	1.23	4.27	0.01	1-11
Current study	Retrospective cohort	UK	NAFLD: 44 No NAFLD: 9,596	NAFLD: 41 No NAFLD: 31,255	OR	2.40	1.51	3.82	<0.0001	1,3,11-18

Confounding factors:

1: age, 2: parity, 3: baseline BMI, 4: waist circumference, 5: HOMA-IR, 6: HOMA-IR, 7: Total cholesterol, 8: LDL, 9: HDL, 10: TG, 11: Hypertension, 12: Townsend, 13: smoking, 14: lipid controlling drugs, 15: use of metformin, 16: polycystic ovarian syndrome.