Universitat Autònoma de Barcelona

Risk factors for hyperglycemia in pregnancy, and vitamin D as a prevention strategy in the DALI study

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Doctoral thesis

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Glossary of abbreviations

ADA	American Diabetes Association
ADIPS	Australasian Diabetes in Pregnancy Society
AGT	Abnormal glucose tolerance
AUC	Area under the curve
AUROC	Area under receiving operation curves
BMI	Body mass index
FIGO	International Federation of Gynecology and Obstetrics
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
GWG	Gestational weight gain
HAPO	Hyperglycemia And Pregnancy Outcomes
HE	Healthy eating
HIP	Hyperglycemia in pregnancy
HOMA-IR	Homeostasis Model Assessment-Insulin Resistance
LGA	Large for gestational age
NC	Neck circumference
NDDG	National Diabetes Data Group
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health and Care Excellence
OECD	Organization for Economic Co-operation and Development
OGTT	Oral glucose tolerance test
PA	Physical activity
PAF	Population attributable fraction
PCOS	Polycystic ovarian syndrome
PG	Plasma glucose
PTH	Parathyroid hormone
RHR	Resting heart rate
T2DM	Type 2 diabetes mellitus
VDR	Vitamin D receptor
25(OH)D	25-hydroxy-vitamin D

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Summary

The present doctoral thesis focuses on the study of risk factors and the use of vitamin D as a prevention strategy for gestational diabetes mellitus (GDM) in a high-risk population (pregnant overweight/obese women) enrolled in the DALI (Vitamin D And Lifestyle Intervention for GDM prevention) study.

In the first article, the DALI vitamin D randomized controlled trial for GDM prevention is reported.

Low vitamin D concentrations have been associated with both obesity and hyperglycemia, in and outside pregnancy. Although the relationship between hypovitaminosis D and impaired glucose metabolism is well-established, supplementation trials aiming to prevent hyperglycemia have shown diverging results.

The DALI vitamin D randomized controlled trial for GDM prevention tested vitamin D supplementation with 1600 IU/day, with or without combined lifestyle intervention in a high-risk population for GDM, starting at <20 weeks' gestation until delivery. The primary study outcomes were the GDM surrogates, fasting plasma glucose (FPG), HOMA-IR and gestational weight gain (GWG). There was a small improvement in FPG (-0.14 mmol/l; 95%CI -0.28, -0.00) at 35-37 weeks' gestation, but no improvement in any primary outcome was observed at 24-28 weeks' gestation, when testing for GDM usually takes place. A *post hoc* analysis identified as independent variables for vitamin D sufficiency: European ethnicity (OR 19.84, CI95 5.87-67.08), season of measurement (OR summer vs. spring 17.0, CI 95 1.84-157.5, ns for other seasons) and taking vitamins (OR 11.1, CI 95 3.01-41.2).

In the second article risk factors for hyperglycemia in pregnancy (HiP) in the DALI population, at different pregnancy periods and oral glucose tolerance test (OGTT) time points are described.

Many risk factors for HiP have been described, but most of them have been evaluated around 24-28 weeks' gestation. The contribution of abnormal plasma glucose (PG)

values at different OGTT time points to GDM diagnosis varies in different populations. Furthermore, abnormal PG values at different OGTT time points have been associated with different adverse pregnancy outcomes.
We conducted an observational sub-analysis of the DALI study, including 971 women, who underwent an OGTT at <20, 24–28 and 35–37 weeks (IADPSG/WHO_{2013} criteria). A multivariate logistic regression selected independent variables (including baseline maternal and current pregnancy characteristics) for HiP.

Clinical characteristics independently associated with HiP were: at <20 weeks, previous abnormal glucose tolerance (odds ratio (OR): 3.11; 95%CI: 1.41–6.85), previous GDM (OR: 2.22; 95%CI: 1.20–4.11), neck circumference (NC) (OR: 1.58; 95%CI: 1.06–2.36 for the upper tertile), resting heart rate (RHR, OR: 1.99; 95%CI: 1.31–3.00 for the upper tertile) and recruitment site; at 24–28 weeks, previous stillbirth (OR: 2.92; 95%CI: 1.18-7.22), RHR (OR: 3.32; 95%CI: 1.70-6.49 for the upper tertile) and recruitment site; at 35–37 weeks, maternal height (OR: 0.41; 95%CI: 0.20–0.87 for upper tertile). Clinical characteristics independently associated with GDM/overt diabetes differed by OGTT time point (for example, NC was associated with abnormal fasting glucose at <20 weeks, while RHR was associated with post-challenge glucose at <20 weeks and with both, fasting and post-challenge glucose at 24-28 weeks).

In conclusion, in overweight/obese women enrolled in the DALI study, vitamin D supplementation did not substantially improve surrogate GDM measurements defined as primary outcomes (FPG, HOMA-IR, GWG) and did not modify secondary outcomes. Average vitamin D concentrations at baseline were higher than expected and major vitamin D sufficiency predictors were European ethnicity and multivitamin intake. In this population, risk factors for HiP differed by pregnancy period and OGTT time point and could assist in defining criteria for selective screening or participants of prevention trials.

Resumen

La presente tesis doctoral se centra en el estudio de los factores de riesgo y el uso de la vitamina D como estrategia de prevención de la diabetes mellitus gestacional (DMG) en una población de alto riesgo (embarazadas con sobrepeso/obesidad) participantes en el estudio DALI (Intervención de estilo de vida y vitamina D para la prevención de DMG)

En el primer artículo, se describe el ensayo controlado aleatorizado DALI de vitamina D para la prevención de DMG.

Las concentraciones bajas de vitamina D se han asociado tanto con obesidad como con hiperglucemia, dentro y fuera del embarazo. Aunque la relación entre hipovitaminosis D y alteración del metabolismo de la glucosa está bien establecida, los ensayos de suplementación para prevención de la hiperglucemia han mostrado resultados divergentes.

El ensayo controlado aleatorizado DALI de vitamina D para la prevención de DMG evaluó la suplementación de vitamina D con 1600 UI/día, con o sin intervención combinada de estilo de vida, en una población de alto riesgo de DMG, desde <20 semanas de gestación hasta el parto. Los resultados primarios del estudio fueron las medidas subrogadas de DMG: glucemia plasmática en ayunas (GPA), HOMA-IR y aumento de peso intragestación. Hubo una pequeña mejoría en la GPA (-0,14 mmol/l; IC 95%: -0,28, -0,00) a las 35-37 semanas de gestación, pero no se observó mejora en ningún resultado primario a las 24-28 semanas, cuando habitualmente se realiza el diagnóstico de DMG. Un análisis *post hoc* identificó como variables independientes para la suficiencia de vitamina D: la etnia europea (OR 19,84, CI95 5,87-67,08), la estación del año de la extracción (OR verano vs. primavera 17,0, IC 95 1,84-157,5, ns para otras estaciones) y la toma de vitaminas (OR 11,1, IC 95 3,01-41,2).

En el segundo artículo se describen los factores de riesgo de hiperglucemia gestacional (HG) en la población DALI, en diferentes periodos de la gestación y puntos de la sobrecarga oral de glucosa (SOG).

Se han descrito muchos factores de riesgo de HG, pero la mayoría de ellos se han evaluado alrededor de las 24-28 semanas de gestación. La contribución de los

valores alterados de glucosa plasmática (GP) en diferentes puntos de la SOG al diagnóstico de DMG varía en diferentes poblaciones. Además, los valores alterados de GP en diferentes puntos de la SOG se han asociado con diferentes resultados perinatales adversos.

Realizamos un sub-análisis observacional del estudio DALI, que incluyó a 971 mujeres, que se sometieron a una SOG a las <20, 24-28 y 35-37 semanas (criterios IADPSG/WHO_{2013}). Una regresión logística multivariante seleccionó variables independientes (incluyendo características basales maternas y de la gestación actual) para HG.

Las características clínicas asociadas de forma independiente con HG fueron: <20 semanas, intolerancia a la glucosa previa (odds ratio (OR): 3,11; IC 95%: 1,41-6,85), DMG previa (OR: 2,22; IC 95%: 1,20-4,11), circunferencia cervical (CC) (OR: 1,58; IC 95%: 1,06–2,36 para el tercil superior), frecuencia cardíaca en reposo (FCR, OR: 1,99; IC 95%: 1,31–3,00 para el tercil superior) y centro de reclutamiento; a las 24-28 semanas, mortinato previo (OR: 2,92; IC 95%: 1,18-7,22), FCR (OR: 3,32; IC 95%: 1,70-6,49 para el tercil superior) y centro de reclutamiento; a las 35-37 semanas, talla materna (OR: 0,41; IC 95%: 0,20-0,87 para el tercil superior). Las características clínicas asociadas de forma independiente con DMG/diabetes franca, diferían según el punto de tiempo de la SOG (por ejemplo, la CC se asoció con glucosa alterada en ayunas a <20 semanas, mientras que la FCR se asoció con la glucosa postsobrecarga a <20 semanas y, tanto con la glucosa en ayunas como con la postsobrecarga a las 24-28 semanas).

En conclusión, en mujeres con sobrepeso / obesidad participantes en el estudio DALI, la suplementación con vitamina D no mejoró sustancialmente las medidas subrogadas de DMG definidas como resultados primarios (GPA, HOMA-IR, aumento de peso intragestación) y no modificó los resultados secundarios. Las concentraciones promedio de vitamina D al inicio del estudio fueron más altas de lo esperado y los principales predictores de la suficiencia de vitamina D fueron la etnia europea y la ingesta de multivitaminas. En esta población, los factores de riesgo de HG diferían según el período de la gestación y el punto de la SOG, y podrían ayudar a definir los criterios para la detección selectiva o los participantes de ensayos de prevención.

1. Introduction

In a healthy pregnancy, metabolic changes occur to ensure an adequate glucose supply to the developing fetus (1).

During the first half of gestation, metabolic changes lead to an anabolic state, aiming to store nutrients to cope with future energy demands. In this period, increased food intake and insulin-dependent lipogenesis lead to fat depots expansion (2–4). Maternal FPG decreases throughout pregnancy (Fig. 1), with lower concentrations observed since the 5th week (5,6), and a further decrease due to increased uptake by the maternal-fetal unit as pregnancy progresses (5–9). An increase in insulin concentrations from pre-pregnancy to 12-14 weeks gestation has been observed (7,10). In parallel, insulin sensitivity increases from pre-pregnancy to 12-14 weeks gestation (10) (Fig. 1).

As pregnancy progresses, metabolic changes continue, leading to a catabolic state that characterizes the third trimester. In this phase, marked oscillations between the fasted and fed states are present (11).

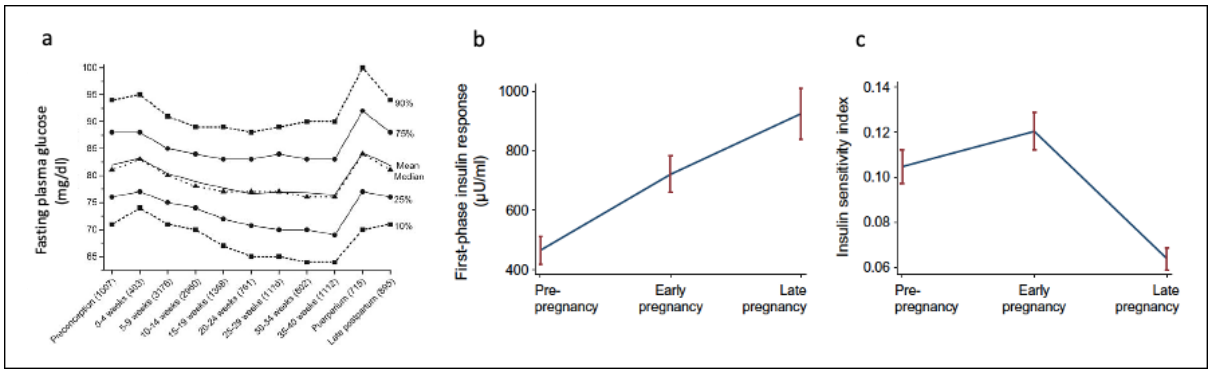

Figure 1. Longitudinal changes in FPG (a) (retrospective data), *Source: Riskin-Mashiah et al., J. Perinat. Med. 2011*(5)*;* Longitudinal changes in (b) 1st phase insulin response from prepregnancy, at 12-14 and 34-36 weeks (c), insulin sensitivity pre-pregnancy, at 12-14 and 34-36 weeks. *Source: Powe et al., Diabetologia. 2019 (b)*(10).

The main metabolic adaptation that takes place in the last part of pregnancy is the striking decrease in insulin sensitivity (or increase insulin resistance), up to 50-80% by 34-36 weeks' gestation (12,13) (Fig. 1). Under these conditions, hepatic glucose output increases (14,15). Adipose tissue lipolysis, under fasting conditions, is increased, facilitating a rise in plasma triglycerides (16–18) and promoting a greater activation of ketogenesis (19). Postprandial facilitated anabolism/fasting accelerated catabolism coexist (11). As previously mentioned, maternal FPG is decreased, but,

in the fed state, increased insulin resistance promotes a greater rise in glucose (11,20), guaranteeing fetal energy supply.

Maternal euglycemia is maintained by an increase in both, first and second phase insulin secretion (21,22) due to beta cell adaptation (23,24).

Table 1. Metabolic changes during pregnancy

	Early pregnancy (~12-14 weeks)	Late pregnancy (~34-36 weeks)
Basal metabolism		
Fasting glucose	Decreased	Decreased
Fasting insulin	Unchanged	Increased
Hepatic metabolism		
Basal hepatic glucose production	Unchanged	Increased
Hepatic insulin sensitivity		Decreased
Glucose suppression	Decreased	Decreased
Insulin metabolism		
Insulin secretion		
First phase	Increased	Increased
Second phase	Increased	Increased
Insulin sensitivity	Increased	Decreased

Adapted from Lain KY, Catalano PM. Clin Obstet Gynecol. 2007(25) and Powe et al. Diabetologia. 2019 (10)

1.2. Obesity-related metabolic changes in pregnancy

Obesity has an impact on the metabolic changes that occur during pregnancy. Compared to normal-weight women, obese women do not show a drop in FPG levels in the first trimester of pregnancy (6).

In the third trimester, a similar decrease in insulin sensitivity in lean, overweight and obese women (~50-60%) from pre-pregnancy is observed (25). However, overall, the obese women were more insulin resistant than overweight and lean women, particularly pre-gravid and in early gestation. Glucose production increases with maternal body weight (26), with obese women displaying higher FPG and postprandial glucose (27). Obese women display higher fasting plasma triglyceride and VLDL concentrations compared to their lean counterparts (28). Leptin concentrations as much as 2-fold higher in obese women have been observed in this period (28,29). Interestingly, leptin concentration increases throughout pregnancy both in lean and obese women, but the increase percentage is lower in the latter group

(30). Thus, the severity of insulin resistance observed in obese women in the third trimester of pregnancy seem to be a reflection of the pre-gravid condition.

Higher insulin resistance exhibited by obese women in the third trimester, beyond the physiological degree expected for a healthy pregnancy, can lead to excessive metabolic fuels availability, increasing the risk of adverse pregnancy outcomes (31). These obesity-related changes support the definition of “fuel mediated teratogenesis” coined by Freinkel over 40 years ago, and goes beyond abnormal glucose metabolism. In fact, obesity has showed to have a greater impact compared to gestational hyperglycemia in pregnancy outcomes, such as LGA (32).

1.3. Hyperglycemia in pregnancy

The 1980 Banting lecture was dedicated to the effects of hyperglycemia during pregnancy on both mother and fetus (11). In this report, Freinkel dated back to the late 19th century, the first description of what later came to be known as GDM. In 1920, Cron draw attention to glycosuria that was observed in pregnancy but not necessarily afterwards (33). These observations led to several reports investigating the “prediabetic state of pregnancy” (34–37). In the second half of the 20th century, efforts to improve perinatal outcomes sparked interest to identify these pre-diabetic women. works from Wilkerson and O’Sullivan on pregnant women, screening according to risk factors or elevated glucose after a 50 gr. glucose challenge, helped to determine the blood glucose behavior during an OGTT in pregnancy, confirmed that adverse outcomes were more common in these women, and evaluated the effect of insulin treatment on outcomes (38,39). Results from these works led to the definition of GDM, understood as diabetes first recognized in pregnancy, and to the first GDM diagnostic criteria after a 100 g 3h OGTT in 1964 (40) (Table 2). Two noteworthy aspects of these diagnostic criteria should be highlighted: 1) the criteria for GDM were validated for prediction of subsequent maternal diabetes, and 2) glucose values were measured in whole blood using the Somogyi-Nelson method. In 1979, the National Diabetes Data Group (NDDG) recommended glucose cut-off points based on the conversion from whole blood to plasma (41), and in 1982 Carpenter and Coustan further corrected methodological aspects of the conversion resulting in modified cutoffs (42). It became standard practice to use the 50 g glucose challenge as a screening test

and refer for the 100 gr 3 h OGTT those women who exceeded the recommended threshold. This 2-step approach was generally accepted when using either NDDG or Carpenter and Coustan cut-offs. When the NDDG endorsed the 2-h 75g OGTT for the diagnosis of diabetes and glucose intolerance in the general population (41), the WHO extended this recommendation to pregnant women (43). Controversy surrounding GDM screening and diagnosis has been going on for years, as there is not consensus on diagnostic criteria and whether to recommend routine or selective screening based on risk factors, in addition to the fact that criteria had not been developed to predict pregnancy outcomes (44–46).

Table 2. Commonly used diagnostic criteria for gestational diabetes mellitus

Criteria	Glucose val Fasting	ue (mmol/l) 1h	2h	3h
O'Sullivan and Mahan[a]	³ 5.0	³ 9.2	³ 8.1	³ 6.9
NDDG[a]	³ 5.8	³ 10.6	³ 9.2	³ 8.1
Carpenter and Coustan[a]	³ 5.3	³ 10.0	³ 8.6	³ 7.8
NICE - UK[b]	³ 5.6		³ 7.8	
IADPSG/WHO_{2013}[b]	³ 5.1	³ 10.0	³ 8.5	

a. 100-g oral glucose tolerance test. Diagnosis requires 2 abnormal glucose values.
b. 75-g oral glucose tolerance test. Diagnosis requires 1 abnormal glucose value.

The HAPO study published in 2008 was designed to clarify the risks of adverse pregnancy outcomes associated with various degrees of maternal glucose intolerance less severe than that in overt diabetes mellitus (47). This multicenter study, included ~25.000 women, who underwent a 75 gr OGTT at 24-32 weeks. Results showed a continuous relationship between glucose concentrations (FPG, 1h and 2h) and adverse pregnancy outcomes (LGA, cesarean section, neonatal hypoglycemia and cord C-peptide >90th percentile). As there was not a clear-cut threshold separating healthy pregnancies from those with complications, new diagnostic criteria were derived after consensus in a 2010 IADPSG meeting (48). IADPS diagnostic criteria are displayed in table 2. The IADPSG consensus also considered the increase in prevalence of T2DM in women of reproductive age, and advocated for screening for overt diabetes in the first antenatal visit (48) considering that women with "unknown diabetes" may have worse pregnancy outcomes than women aware of a Type 2 diabetes diagnosis (49). In accordance with this, since 2016 the ADA has defined GDM as diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes (50).

IADPSG criteria have been endorsed by the WHO in 2013, and later on by the ADIPS, FIGO and ADA (46,51,52).

1.4. Prevalence of GDM

The real prevalence of GDM is difficult to estimate due to lack of uniformity of screening and diagnostic protocols. A meta-analysis of over 17000 participants in 40 European studies, reported an average prevalence of GDM of 5.4% (53), while data from the National Health and Nutrition Examination Survey (NHANES) 2007-2014, reported a prevalence of GDM of 7.6% in the US (54). Higher rates, ~12%, have been observed in Asia and Southeast Asia (55). Differences in GDM incidence according to population characteristics have long been described, even within the same country or region (56).

In 2019, the International Diabetes Federation (IDF) estimated a global worldwide prevalence of HiP of 15.8%, with 12.8% attributed to GDM and the remaining 2.6% to overt diabetes in pregnancy (57). Regional differences were also observed in the IDF report, with HiP ranging from a 27% in South-East Asia to 7.5% in the Middle-East and North Africa region (58).

Using IADPSG criteria, a considerable increase in GDM prevalence would be expected, as observed in different reports, including the HAPO and DALI studies (59,60), and confirmed in a meta-analysis including data from 51 population-based studies (61). In Spain IADPSG criteria have not been universally adopted. GDM prevalence has been reported at 6.5%, using data from hospital admission for single births in Catalonia using NDDG criteria (62), while a report from a single-center study, using IADPSG criteria, described a GDM prevalence of 35.5% vs. 10.6% prevalence in a historic cohort where Carpenter and Coustan criteria were used (63).

1.5. Adverse pregnancy outcomes and long-term complications of GDM and obesity

GDM has been associated with an increased risk of adverse perinatal outcomes for both mother and fetus, including pre-eclampsia, increased frequency of C-section,

polyhydramnios, pre-term delivery, macrosomia, LGA, shoulder dystocia, neonatal hypoglycemia, hyperbilirrubinemia and a higher rate of neonatal intensive care admission and perinatal mortality (47,64–67).

GDM has also been associated with long term maternal and offspring morbidity. GDM is nowadays the best-known risk factor for T2DM, with women with previous GDM being ~10 times more likely to develop the condition (68). It has been reported that previous GDM increases ~4-fold the risk of developing metabolic syndrome (69). However, the magnitude of the association may differ according to the population studied (70). A 2 to 3-fold increase in cardiovascular events later in life, independent of the presence of T2DM has been observed (71,72). GDM has also been associated with the later development of chronic kidney disease (73,74), especially among black women (75). The association between GDM and cancer is more controversial (76,77), and has predominantly been described among Asian women (76,78).

As for the offspring of women with GDM, follow-up studies have shown a ~8-fold greater risk of pre-diabetes and T2DM (79), and they are about twice as likely to be overweight or obese (80,81) and 4 times more likely to develop metabolic syndrome (80,81). Besides the adverse metabolic outcomes, higher rates of neuropsychiatric disease, including autistic spectrum disorder have been described in offspring of GDM mothers (82).

The prevalence of obesity has risen in the past decade reaching epidemic proportions worldwide. Data from the 2017-2018 NHANES in the US, estimated the prevalence of obesity among childbearing age women at 39.7% (83). IIn the 2017 OECD update, global obesity prevalence was reported at 19.5% with a wide variation between member countries (84). In this report, obesity prevalence in Europe oscillated between 9.8% and 30%. Spain’s obesity prevalence was in the mid-range, estimated at 16.7%.

Overweight/obese women are 2-5.6 times more likely to develop GDM (85), which is why increased BMI is commonly used a criteria for selective screening strategies. Beyond its well-established relationship with GDM, obesity has independently been associated with adverse pregnancy outcomes (i.e. LGA, high cord C-peptide levels, C-section, shoulder dystocia, pre-eclampsia) (32,86). Not only these outcomes are

common with GDM but, in fact, obesity seems to confer a higher risk of pre-eclampsia and C-section delivery compared to hyperglycemia alone (32,86). Obese women have also shown an increased risk of congenital anomalies compared to women with normal weight (87), also in those with GDM (88).

Both obesity and hyperglycemia confer great risk to the mother and child, and when combined, the effect is enhanced leading to a ~4-fold increase in LGA, fetal adiposity and >90% centile cord blood C-peptide concentration, as well as a ~6-fold increase in pre-eclampsia (86).

As for long term effects, maternal obesity has been associated with greater risk of offspring obesity in adulthood (89), as well as an increased risk of T2DM and cardiovascular disease (90).

Gestational weight gain (GWG), especially during the first trimester of pregnancy has been associated with an increased risk of GDM, and this association is stronger in overweight/obese women (91). The Institute of Medicine (IOM) has published recommendations for GWG, according to pre-pregnancy BMI (92), aiming to limit GWG in overweight/obese women at greater risk of adverse pregnancy outcomes. Furthermore, similar to pre-pregnancy obesity, excessive GWG has been associated with a higher risk of offspring obesity in adolescence (93).

1.6. The DALI Vitamin D and lifestyle intervention for gestational diabetes mellitus prevention

Outside pregnancy, there is compelling evidence supporting the beneficial effects of lifestyle interventions aiming at weight loss to improve glycemic control in people with T2DM (94,95). Furthermore, lifestyle intervention strategies have shown positive results in trials aiming to prevent T2DM in overweight/obese population (96).

In line with this, many lifestyle intervention trials aiming to limit GWG, improve glucose tolerance and/or prevent GDM have been conducted. In 2017, the Cochrane library published a meta-analysis, including 19 RCTs that evaluated combined diet and exercise strategies for GDM prevention stated there was a *possible* risk reduction of

GDM in the intervention group (97). Later, in 2018, Guo et al. observed a *significant* risk reduction in GDM (RR 0.77) in a metaanalysis of 47 RCTs of lifestyle interventions (diet, exercise or both). Moreover, they reported that lifestyle interventions were more effective when initiated early, applied in a high-risk population, with adequate frequency and intensity of exercise, and when GWG was limited (98).

The DALI *Vitamin D and Lifestyle Intervention for gestational diabetes mellitus prevention* study was a European multicenter project, testing different approaches aiming to reduce the risk of GDM. The project is comprised of 3 trials: 1) the DALI lifestyle pilot published in 2015 (99), 2) the DALI lifestyle study comparing healthy eating (HE), physical activity (PA) and combined healthy eating and physical activity (HE&PA) interventions with a control group, reported in 2017 (100), and 3) the DALI vitamin D study comparing vitamin D supplementation with and without HE&PA vs. placebo, published in 2019 (101) and included in the present thesis.

The full protocol was published in 2013 (102). In summary: Participants were eligible if pre-pregnancy BMI ≥29 kg/ m2, ≤19 + 6 weeks of gestation, singleton pregnancy and age ≥18 years. Exclusion criteria were being unable to walk at least 100 m safely or to speak the language of the recruitment site, having complex diet requirements or chronic medical or psychiatric conditions. Additionally, in the vitamin D trial, women with a current or past abnormal calcium metabolism (hypo/hyperparathyroidism, nephrolithiasis, hypercalciuria), or having hypercalcemia detected at baseline measurement according to trimester cut-offs, or hypercalciuria, were excluded.
Women were recruited in 9 European countries at 11 different sites. Baseline assessment was immediately followed by randomization to either lifestyle counseling (HE and PA) & placebo, HE & PA & vitamin D supplementation, placebo alone, or vitamin D alone using a computerized random number generator, pre-stratified for site. The outcome of the allocation was reported by the lifestyle coach to the participant, but those involved with measurements were kept blinded. Clinical assessments were made at four time points: ≤19+6 weeks of gestation (baseline), 24–28 weeks, 35–37 weeks' gestation and after delivery. At these time points, blood samples were collected, anthropometric measurements were performed and participants were asked to complete different questionnaires. In each trimester,

women undertook a 75 g OGTT, unless GDM or overt diabetes had been diagnosed in an earlier assessment. IADPSG/WHO_{2013} criteria (48,103) were used.

The present thesis includes two DALI articles: 1) the report of the DALI vitamin D study, and 2) a sub-analysis reporting risk factors for Hyperglycemia in Pregnancy at different pregnancy periods and OGTT time points.

1.7. Vitamin D and glucose metabolism in pregnancy

1.7.1. Vitamin D, mineral metabolism and non-skeletal effects

Vitamin D, a cholesterol-derived hormone, is obtained from dietary sources (D3 and D2) or by skin synthesis from 7-dehydrocholesterol under the influence of UV light (D3). Dietary sources of vitamin D2 (ergocalciferol) are plant or fungi and fortified foods with added vitamin D2. As for vitamin D3 (cholecalciferol), it can be obtained from different dietary sources, mainly oil-rich fish. In the circulation, vitamin D travels bound to the vitamin D binding protein, which transports it to the liver where it is metabolized to 25(OH)D, the main circulating form. Although 25(OH)D is used in clinical practice to determine vitamin D status, it is an inactive form. 25(OH)D is transformed to its active form, 1,25-$(OH)_2$D in the kidney, with a tight regulation by plasma parathyroid hormone (PTH), and serum calcium and phosphorus. 1,25-$(OH)_2$D is the ligand for the vitamin D receptor (VDR), a transcription factor, binding to sites in the DNA called vitamin D response elements (VDR) (104). VDR have a wide distribution in a variety of organs and tissues (105) , including pancreas and insulin-responsive tissues, such as skeletal muscle and adipose tissue (106,107).

Classical vitamin D functions involve the regulation of calcium and phosphorus metabolism, and its deficiency leads to the development of rickets. Vitamin D increases calcium and phosphorus intestinal absorption, stimulates osteoclast for bone resorption, and interacts with PTH to increase distal renal tubular calcium reabsorption (108). Various vitamin D non-skeletal effects have been described including modulation of the immune response (109), regulation of a variety of genes that control cell proliferation, differentiation and apoptosis (110), PTH inhibition, and regulation of glucose metabolism (111). Regarding glucose metabolism, vitamin D has been associated with both, insulin secretion and insulin resistance (112–114)

1.7.2. Vitamin D deficiency, definition, prevalence, clinical relevance

There is debate regarding what should be considered optimal vitamin D concentrations.

The IOM (115) and the Endocrine Society Task Force (116) have proposed serum 25(OH)D cut-offs based on primarily bone health outcomes (table 3).

Whether either of these cut-offs apply for other known vitamin D pleiotropic effects is yet to be elucidated. In 2018, the 2nd International Conference on Controversies in Vitamin D was held and the definition of vitamin D nutritional status was revisited. The expert consensus recommended that 25(OH)D values below 30 nmol/L should be considered to be associated with an increased risk of rickets/osteomalacia (117), whereas 25(OH)D concentrations between 50 and 125 nmol/L appear to be safe and sufficient in the general population for skeletal health (117,118).

Table 3. Recommended 25(OH)D concentrations (130, 131)

IOM 2011	Deficiency	<30 nmol/L
	Inadequacy	<50 nmol/L
	Adequacy	≥50 nmol/L
	Upper safety limit	125 nmol/L
Endocrine Society Task Force 2011	Deficiency	<50 nmol/L
	Insufficiency	50-74 nmol/L
	Sufficiency	≥75 nmol/L
Controversies in vitamin D international conference 2017	Insufficient (rickets risk)	<50 nmol/L
	Sufficient	≥50-125 nmol/L

Regardless of the concentration cut-off employed, vitamin D deficiency is a common problem, with an estimated prevalence ~1 billion people worldwide (119). In Europe, vitamin D deficiency prevalence has been estimated at 13% (for 30 nmol/l cutoff) and 44% if a cutoff of 50 nmol/l was considered (120).

Vitamin D status can be influenced by individual characteristics such as skin pigmentation, age, style of clothing, use of sunscreen, outdoor activity and sun exposure (121). Diet can also be an important determinant of vitamin D status. Some high-risk groups have been identified, including the elderly, young children, people with darker skin and pregnant and lactating women (122). However, whether screening for vitamin D deficiency in these groups should be advised is not clear (117,123).

Various epidemiological reports have linked vitamin D deficiency with an increased risk of numerous diseases (i.e. autoimmune diseases, cancer, and cardiovascular disease, diabetes), in addition to its known deleterious effect on bone metabolism (118,124).

1.7.3. Vitamin D, obesity and diabetes

1.7.3.1 Data from observational studies

There is strong evidence indicating an inverse relationship between **adiposity** and vitamin D concentrations (125,126). Prevalence ratio (PR) of vitamin D deficiency in overweight and obese subjects compared to lean subjects has been reported at 1.24 (CI 1.14-1.34) and 1.35 (CI 1.21-1.50) respectively (127). It has been generally accepted that lower vitamin D concentration among obese subjects is caused by vitamin D sequestration in the adipose tissue (128) and/or volumetric dilution (129). Conversely, some authors have wondered if low vitamin D concentrations could stimulate weight gain or inhibit weight loss. Effects of vitamin D on genes related to adipocyte differentiation, lipolysis and lipogenesis in *in vitro* studies, suggest that vitamin D may have a protective effect against obesity (130). Hence, low vitamin D levels might have a contrary obesogenic effect.

Observational studies have shown an inverse association between vitamin D levels and **FPG, HbA1c** (131–133) and between vitamin D deficiency, obesity and **abnormal glucose tolerance** (134,135).

A meta-analysis including 21 studies showed a 38% lower risk of developing T2DM in subjects in the highest tertiles of 25(OH)D compared to the lowest tertile (RR 0.62; CI 0.54-0.70) (136). Post-challenge PG has not been addressed in these reports.

1.7.3.2 Mechanisms relating vitamin D and glucose tolerance

As to mechanisms involved, vitamin D has been associated with **insulin sensitivity, insulin secretion and chronic inflammation** (137). Vitamin D has been described to stimulate the expression of insulin receptors (138,139), and modulate cytokine expression and activity, hence improving insulin sensitivity. As for insulin secretion,

vitamin D deficiency has shown to impair glucose-mediated **insulin secretion** (112,140) which in animal models, is restored after vitamin D supplementation (141).

1.7.3.3 Vitamin D supplementation trials

Several **vitamin D supplementation trials** focusing on both obesity (142) and impaired glucose metabolism/T2DM (143), have been conducted, but no robust results have been obtained.

The effect of vitamin D on FPG, 2h post-challenge PG and insulin sensitivity and secretion indices has been investigated in several trials. An improvement in insulin sensitivity (144,145) and disposition index (145) after vitamin D supplementation in subjects with pre-diabetes has been observed. A study, aiming to test vitamin D for T2DM prevention observed no significant changes in glucose variables after supplementation, but reported a small effect on HOMA-IR (146). Other studies conducted in patients with pre-diabetes or T2DM have reported no effect after vitamin D supplementation on any of the above-mentioned glucose variables (147–149) . A meta-analysis conducted in 2018, reported no overall benefit on FPG or HOMA-IR after vitamin D supplementation, but subgroup analysis showed a decrease in FPG in those with BMI <25 and in those with vitamin D insufficiency at baseline. As for HOMA-IR, it was lower after vitamin D supplementation within the subgroup that was initially vitamin D sufficient (150).

A large RCT published in 2019, involving >2000 participants with prediabetes, supplemented with 4000 IU/d of vitamin D3, showed no significant differences between the intervention and control groups in the incidence of T2DM after 2.5 years of median follow-up (HR 0.88; CI 0.75-1.04; P = 0.12) (151). Similar observations had been previously reported in a trial including participants with pre-diabetes supplemented with 20.000 IU of vitamin D a week (152). Both trials had common limitations: the studies might have been underpowered and average baseline vitamin D concentration among participants was >50 nmol/L, which may have limited the supplementation effect (151,152).

Two recent meta-analyses on vitamin D supplementation for T2DM prevention have been reported (153,154). In a meta-analysis of 9 RCTs, including large supplementation trials, Barbarawi et al. did not find a protective effect of vitamin D supplementation. However, sensitivity analysis excluding 2 trials where vitamin D doses <1000 IU/day were used, displayed a significant risk reduction in the incidence of T2DM (RR 0.88, 95%CI, 0.79-0.99) (153). The trials excluded in the sensitivity analysis were conducted in participants with an average risk of T2DM and its incidence was investigated *post hoc*. On the other hand, the 7 trials included in the sensitivity analysis were conducted in participants with prediabetes, and included diabetes-related variables or T2DM incidence, in their primary outcomes. Different subgroup analyses were performed and intriguingly, no differences were observed in vitamin D deficient patients at baseline. Another subgroup analysis from the same report, observed a significant risk reduction among participants with BMI <30 but not in those with BMI ≥30. A second metaanalysis published by Zhang et al. including 8 RCTs observed a risk reduction in the incidence of T2DM with vitamin D supplementation (RR 0.89; 95% CI 0.80–0.99) (154). In this report, only studies with T2DM prevention as primary objective were included. A sensitivity analysis showed that the effect was only observed in the subgroup with BMI <30 (RR 0.73; 95% CI 0.57–0.92), in agreement with Barbarawi's report. Furthermore, Zhang's metaanalysis showed that vitamin D supplementation was associated with reversion to normoglycemia in patients with prediabetes (RR 1.48; 95%CI 1.14–1.92). With this results in mind, it has been advocated that vitamin D supplementation trials should focus on vitamin D concentrations (baseline and desired target), to account for individual variability which may be one of the reasons why trials have shown heterogenous results (155).

Overall, recent evidence hints to a beneficial effect for vitamin D supplementation in the prevention of T2DM, but data interpretation is complex.

A new systematic review and meta-analysis is ongoing, including RCTs testing vitamin D supplementation vs. placebo in subjects with pre-diabetes (156). Individual participant data meta-analyses to evaluate heterogeneity of treatment effects across participant subgroups have been proposed to provide clearer data regarding the benefit of vitamin D supplementation for T2DM prevention (157), but so far, no

individual participant data meta-analysis on this subject has been published (*last checked at Prospero 23rd June 2020*)

1.7.4. Vitamin D deficiency in pregnancy

Estimating vitamin D deficiency in pregnancy is difficult, in view of the ongoing debate about sufficiency cutoffs, and due to the lack of specific data from pregnant populations. Therefore, general population cutoffs are used in pregnancy epidemiological studies and clinical trials. Using these cutoffs, a high prevalence of vitamin D deficiency in pregnant women has been reported, making them an at-risk group, in whom supplementation has been advised by some guidelines (i.e. NICE, ACOG) (122,158).

Using the IOM cutoffs, 7% of pregnant women in the US met criteria for vitamin D deficiency (<30 nmol/L) and an additional 21% for inadequacy (<50 nmol/L) (159). In Europe, the prevalence of vitamin D deficiency in pregnancy varies widely among countries. In the Mediterranean region, reported prevalence ranged from 22.7 to 90.3%, according to a meta-analysis that included studies with different deficiency definitions (160), while reports from Northern Europe reported a prevalence ranging from 43 to 74% for vitamin D deficiency cut-off of <50 nmol/l and raising up to 88% if <75 nmol/l was used (161–163).

Newborns derive their vitamin D entirely from maternal vitamin D supply (164); therefore, it is not surprising that vitamin D deficiency during pregnancy is associated with rickets, neonatal hypocalcemia and poor bone mineralization in childhood (165). Other adverse perinatal outcomes, including preeclampsia, GDM, low birth weight, preterm delivery, cesarean section, and infectious diseases have been associated with vitamin D deficiency in pregnancy (166).

In its 2011 report, the IOM recommended a DRI of 600 IU per day of 25-(OH)D for pregnant women to support bone metabolism, and advised against doses higher than 4000 IU per day (115). Although these recommendations have been adopted by some obstetrics societies, including the ACOG, there is no evidence that supplementations

have a beneficial effect in other adverse pregnancy outcomes, different from infant rickets.

1.7.5. Vitamin D deficiency in pregnancy, obesity and GDM

The well-known inverse relationship between BMI and serum 25(OH)D concentrations, is also observed in pregnancy (167). The association between low 25(OH)D concentration and increased risk of gestational hyperglycemia, has been described in early (168) and mid pregnancy (169). In 2012, a meta-analysis described an association between low 25(OH)D concentration and increased risk of GDM (170). These observations are confirmed in a recent meta-analysis of observational studies where low vitamin D was associated with approximately double the risk of GDM (171).

Based on evidence from observational trials, several RCTs aiming to address vitamin D supplementation as a prevention strategy for GDM have been undertaken, including the DALI vitamin D trial reported in this thesis.

When DALI took off, only one article from Rudnicki addressing the effect of 1,25 OHD supplementation on glucose-related variables had been published (172). Since then, some clinical trials using vitamin D supplements during pregnancy have shown to increase insulin sensitivity (173,174), reduce FPG (174) and also reduce the incidence of GDM (175). However, other studies have not observed metabolic benefits after vitamin D supplementation (176). Before the publication of the DALI vitamin D trial, other trials have reported on GDM after vitamin D supplementation, seven of which have included GDM as a primary outcome (175–181). Trials aiming to test vitamin D supplementation for GDM prevention as a main outcome are summarized in Table 4.

Table 4. Published randomized clinical trials with vitamin D supplementation for GDM prevention

Reference & year	Country	n, population	Gestational age at entry	Baseline VD nmol/L	Supplement	Dosage	GDM criteria	Outcome (GDM)
Yap, 2014(193)	Australia	179 Healthy	<20 w	50	Vit D3	5.000 IU/d vs. 400 IU/d	ADIPS 1998	OR 0.56 (95CI 0.21-1.50), NS
Hossain, 2014	Pakistan	Healthy	<20 w	<25	Vit D3	4000 UI vs. calcium + Fe	GCT	NS
Sablok, 2015(194)	India	180 Healthy	14-20 w	<25 (n=53) 25–50 (n=27) >50 (n=28)	Vit D3	120.000 IU (x4) 120.000 IU (x2) 60.000 IU* vs. placebo	-	OR 0.52 (95CI 0.03-8.53), NS
Mojibian, 2015(192)	Iran	500 Vit D deficient	12-16 w	36.1	Vit D3	50.000 IU /15d vs. 400IU/d	Carpenter and Coustan	OR 0.46 (95CI 0.24-0.88), p<0.001
Shagheibi 2016(198)	Iran	90 High-risk for GDM	1st trimester	33.75	-	5.000 IU/w vs. placebo	-	OR 0.24 (95CI 0.08-0.74), p<0.01
Tehrani 2017(196)	Iran	210 Vit D deficient	14-16 w	<25	-	50.000 IU/15 d vs. placebo	Local criteria	OR 0.86 (95CI 0.29-2.52), NS
Rostami, 2018(197)	Iran	800 Vit D deficient	<14 w	27.5	Vit D3	50.000 IU orally/w to 300.000 IU I.M/3w*	IADPSG	OR 0.50 (95CI 0.34-0.88) p=0.02

* In the trials of Rostami et al. and Sablok et al. different supplementation doses were given according to baseline 25(OH) concentrations. Reported OR combined results from participants in all intervention groups vs. no intervention.

1.8. Risk factors for Hyperglycemia in Pregnancy

Many risk factors for GDM have been identified and some of them are used for selective screening strategies. Increased BMI (surrogate for overweight/obesity), maternal age, previous GDM, macrosomia, non-Caucasian ethnicity and first-degree family history of diabetes are among the best documented ones, but other risk factors have also been described (Table 5).

1.8.1. Risk factors for Hyperglycemia in Pregnancy in different pregnancy periods

As GDM, or even overt diabetes in pregnancy, rarely presents with symptoms, diagnosis has to be performed by screening. In general, testing for GDM at 24-28 weeks' gestation is widely accepted, and most of the evidence regarding risk factors for GDM has been assessed at this pregnancy period (Table 5).

Selective screening in early pregnancy has been adopted by various scientific societies (44,46,182) Clinical risk factors are used to identify women for selective screening in early pregnancy (44,46,182,183) or when this approach is used at 24–28 weeks (44). As treatment for GDM has proven to improve pregnancy outcomes (184,185); when universal screening is not employed, it is important to use adequate clinical risk factors for selective screening.

Various reports have described clinical factors in the **first trimester of pregnancy** that can help identify women at risk of GDM at 24-28 weeks, including maternal age, weight, BMI, ethnicity, family history of diabetes, previous GDM and previous LGA (186,187). However, the possibility of differences in risk factors for early vs. later screening has not been widely addressed in the literature.

Sweeting et al. reported a GDM prediction model combining clinical risk factors (maternal age, first trimester BMI, ethnicity, parity, previous GDM and family history of DM), with an overall AUC of 0.88 (95%CI 0.85–0.92). Interestingly, the same prediction model performed best in women with *early* GDM (AUC 0.96; 95%CI 0.940.98) (188). However, it should be noted that women tested for *early* GDM underwent selective screening based on risk factors, while universal testing was

performed at 2428 weeks. Thus, the prediction model has not been tested in the general pregnant population, which limits the impact of identifying risk factors to predict GDM before 24 weeks gestation.

As for hyperglycemia diagnosed **after 28 weeks' gestation,** data are scarce, but there is limited evidence suggesting that in obese women, it may be associated with excessive GWG (189).

Identified risk factors for GDM at different pregnancy periods are summarized in table 5.

Obesity is commonly defined after BMI. However, BMI and obesity are not straightforward equivalents, and other adiposity measurements have been proposed in order to assess obesity and its associated risk. In pregnancy, waist and neck circumference have shown to be good predictors of GDM at 20-28 weeks (190–192). Moreover, a report found that the neck/thigh ratio measured at 15-18 weeks, surpassed BMI as GDM predictor (193).

Other less well-known risk factors associated with GDM, may need further investigation in order to design better screening strategies. For instance, studies have shown that the site of residence or even the country of birth, can be associated with a greater risk of GDM (56,59). Though ethnicity could partly explain this observation, other factors seem to be involved (i.e. season, socio-economic condition) (194,195) but not all of them are entirely identified. Another factor would be resting heart rate (RHR). Outside pregnancy, RHR has been associated with the incidence of T2DM (196,197) but it has not been addressed in pregnancy.

Table 5. Risk factors for GDM in different pregnancy periods.

Risk factors	<24 weeks (203,215–217)	24-28 weeks	>2
Pre-pregnancy BMI[85]*	+	+	
• Overweight /Obesity /Morbid obesity		OR 1.9/ 3.0 / 5.5	
Gestational weight gain (Excessive vs. nonexcessive) [198]*		OR 1.4	
Advanced maternal age (35 years)[199]*	+	OR 2.8	
Previous GDM[200]†	OR 3.2	OR 13.2	
Previous macrosomia[201]†		OR 6.1	
Previous stillbirth[202]†		OR 4.52	
Family history of DM[201]†	OR 3.1	OR 2.0	
Ethnicity (vs. Caucasian) [203,204] †	+	OR 1.8 to 11.3	
PCOS[205]*		OR: 2.8	
Multiparity [206]†	+	OR: 1.5	
Smoking [207,208]*		OR: 0.98 / 1.38	
Unhealthy dietary habits			
• Sugar beverages[209]§, High fat [210]§, High animal protein [211]§, Low fiber diet[212]§		RR 1.22 / 1.88/ 1.49 / 1.60 (lowest vs. highest Q)	
Sedentary lifestyle[213]§		RR: 2.3	
Male fetus[214]†		OR: 1.39	
Maternal height[215]†		OR 0.34 (highest vs. lowest Q)	
Genetic factors[216]¶		OR 1.15 – 1.44	
Environmental contaminants[217]§		OR: 1.86	
Vitamin D deficiency[171]*		OR: 1.86	

Quality of evidence: † Retrospective study, § Prospective study, ¶ Systematic review, * Systematic review and meta-analysis, + Descriptive association between risk factors and GDM. No association measurement calculated in the reports.

1.8.2. Contribution of glucose concentration at different OGTT time points to GDM diagnosis and adverse pregnancy outcomes

In line with the HAPO report of a continuous relationship between maternal glucose concentrations and adverse pregnancy outcomes, there is compelling evidence suggesting that women with 1 abnormal glucose value in a 3h OGTT have a higher risk of adverse pregnancy outcomes compared to normoglycemic women, and similar to women diagnosed of GDM (218). The IADPSG/WHO_{2013} criteria require a single abnormal glucose value to make the diagnosis of GDM. Although simpler, these recommendations imply that all glucose values are equally relevant for adverse outcome development.

In the HAPO study, glucose values at different OGTT time points where associated with adverse pregnancy outcomes. Nonetheless, the individual glucose measures from different OGTT time points were not highly correlated, and even though all three glucose values showed similar predictive value for LGA and C-section, after adjustment, FPG was a better predictor of cord blood serum C-peptide values, and 1h PG was the only value associated with neonatal hypoglycemia (47). Other reports have found differences between abnormal glucose values at different OGTT time points and adverse pregnancy outcomes. FPG seems to be a better predictor of LGA and shoulder dystocia, while preterm delivery, gestational hypertension, and hyperbilirubinemia display a stronger association with postchallenge PG (219).

Furthermore, the contribution of different OGTT time points to GDM diagnosis significantly differed across centers in the HAPO study, with most participants from Western countries being diagnosed by FPG (>60% of all diagnosis in US, Canada, UK and Australia), while some countries like Thailand and China had a higher proportion of women diagnosed by 1-hour PG (64 and 45% respectively) (59).

This begs the question of whether different OGTT time points, with different discriminatory capacity, amongst different populations may have different risk factors. Based on this knowledge, screening strategies adapted to the characteristics of each population according to their particular risk could be tested.

2. Hypothesis

In overweight/obese pregnant women,

- Clinical characteristics associated with GDM/overt diabetes may differ by pregnancy period and OGTT time point.

- Vitamin D supplementation may be able to improve GDM-related variables.

3. Objectives

In overweight/obese pregnant women enrolled in the DALI study,

Main objective:

- To test the effect of vitamin D supplementation as a strategy to reduce GDM risk evaluated after surrogate primary variables (FPG, insulin resistance and GWG).

Secondary objectives:

- To test the effect of vitamin D supplementation on secondary obstetric and metabolic variables.

- To assess clinical factors associated with GDM/overt diabetes according to pregnancy period and OGTT time point.

4. Original articles

4.1 Article 1

Corcoy R, Mendoza LC, Simmons D, Desoye G, Adelantado JM, Chico A, Devlieger R, van Assche A, Galjaard S, Timmerman D, Lapolla A, Dalfra MG, Bertolotto A, Harreiter J, Wender-Ozegowska E, Zawiejska A, Kautzky-Willer A, Dunne FP, Damm P, Mathiesen ER, Jensen DM, Andersen LLT, Tanvig M, Hill DJ, Jelsma JG, Snoek FJ, Köfeler H, Trötzmüller M, Lips P, van Poppel MNM. **The DALI vitamin D randomized controlled trial for gestational diabetes mellitus prevention: No major benefit shown besides vitamin D sufficiency.** Clin Nutr. 2020 Mar;39(3):976984. doi: 10.1016/j.clnu.2019.04.006.

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doi: 10.1016/j.clnu.2019.04.006.

doi: 10.1016/j.clnu.2019.04.006.

4.2 Article 2

Mendoza LC, Harreiter J, Simmons D, Desoye G, Adelantado JM, Juarez F, Chico A1, Devlieger R, van Assche A, Galjaard S, Damm P, Mathiesen ER, Jensen DM, Andersen LLT, Tanvig M, Lapolla A, Dalfra MG, Bertolotto A, Mantaj U, WenderOzegowska E, Zawiejska A, Hill D, Jelsma JG, Snoek FJ, van Poppel MNM, Worda C, Bancher-Todesca D, Kautzky-Willer A, Dunne FP, Corcoy R. **Risk factors for hyperglycemia in pregnancy in the DALI study differ by period of pregnancy and OGTT time point.** Eur J Endocrinol. 2018 Jul;179(1):39-49. doi: 10.1530/EJE18-0003.

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5. Overall summary of the results

Vitamin D supplementation for GDM prevention

One hundred and fifty-four eligible women were enrolled in the DALI vitamin D trial, 75 were randomized to placebo and 79 to vitamin D. Women allocated to vitamin D and placebo arms only differed in the rate of European ancestry. Baseline clinical characteristics and vitamin D metabolism-related variables are summarized in Tables 1 and 2 of the corresponding article. Baseline average concentrations of total serum 25(OH)D were 69.6 ± 26.8 nmol/l and 73.3 ± 26.8 nmol/l in the placebo and vitamin D arms, and were in the sufficiency range (>50 nmol/l) in 80.1% of the entire study group.

The multivariate logistic regression analysis identified as independent variables for sufficiency: European ethnicity (OR 19.84, CI95 5.87-67.08), season of measurement (OR summer vs. spring 17.0, CI 95 1.84-157.5, ns for other seasons) and taking vitamins (OR 11.1, CI 95 3.01-41.2). The area under the receiver-operating curve was 0.84 (CI 95 0.77-0.92). The corresponding PAFs were: 80.8% (CI 95 70.6-83.8) for European ethnicity, 19.6% (CI 95 9.5-20.7) for summer and 77.4% (CI 95 56.8-83.0) for taking vitamins.

During follow-up, total serum 25(OH)D concentrations (nmol/l) in the intervention arm were 81.9 ± 39.4 vs. 119.4 ± 35.5 in the placebo arm at 24-28 weeks, 84.5 ± 39.8 vs. 122.9 ± 38.8 at 35-37 weeks and 76.9 ± 34.8 vs. 101.2 ± 32.2 at delivery. Cord blood 25(OH)D concentrations were 52.8 ± 20.8 and 75.5 ± 21.9 nmol/l in intervention and placebo arms respectively. Vitamin D sufficiency in the placebo arm was ~70-80%, while it reached virtually 100% in the intervention group at all follow-up assessments. No significant calcium metabolism disturbances were observed in either arm.

Primary outcomes

A small but significant reduction in FPG (-0.14 mmol/l; 95% CI -0.28, -0.00) was observed in the vitamin D arm at 35-37 weeks without any additional significant improvement in HOMA-IR or GWG.

Secondary outcomes

No significant differences for pregnancy-related secondary outcomes between the intervention and placebo arm were observed.

Hyperglycemia in pregnancy in the DALI study

From the 971 women with available glucose data, 425 (43.8%) presented hyperglycemia in pregnancy (GDM/overt diabetes).

At baseline (≤ 19+6 weeks) the prevalence of HiP was 27.9%; at 24-28 weeks, HiP was diagnosed in 15.5% of the previous 580 glucose-tolerant participants; and at 35-37 weeks, GDM was diagnosed in 15.2% of the remaining 422 glucose-tolerant women.

As to GDM/overt diabetes risk factors in different periods of pregnancy:

At ≤19+6 weeks' gestation, previous AGT (OR 3.11, CI95% 1.41-6.85), previous GDM (OR 2.22, CI95% 1.2-4.11), NC (OR 1.15, CI95% 1.06-1.24), RHR (OR 1.03, CI95% 1.01-1.05) and recruitment site (OR 0.12 to 0.83), were significantly associated with GDM/overt diabetes. The direction of the association was positive for all risk factors with the exception of recruitment site.

At 24-28 weeks' gestation, stillbirth in a prior pregnancy (OR 2.92, CI95% 1.18-7.22), RHR (OR 1.05, CI95% 1.02-1.07) and recruitment site (ORs 0.09 to 0.78) were the independent variables associated with GDM/overt diabetes.

At 35-37 weeks' gestation, only maternal height was (negatively) associated with GDM/overt diabetes (OR 0.96, CI95% 0.92-0.99).

As to clinical risk factors for abnormal plasma glucose by OGTT time points, different risk factors were associated with glucose values over the cut-off at different OGTT time points:

At ≤19+6 weeks' gestation

FPG was associated with previous AGT (OR 2.47, CI95%1.07-5.7), NC (OR 1.13, CI95% 1.03-1.25) and recruitment site. Post-challenge 1h PG was associated with RHR (OR 1.05, CI95% 1.02-1.08) and 2h PG with RHR (OR 1.05, CI95% 1.02-1.09) and previous AGT (OR 2.89, CI95% 1.02-8.18).

At 24-28 weeks' gestation

Stillbirth in a prior pregnancy was associated with post-challenge 1h PG (OR 4.18, CI95% 1.68-10.38), while RHR was associated with both abnormal FPG (OR 1.04, CI95% 1.01-1.071) and post-challenge 1h PG (OR 1.04, CI95% 1.0-1.07). Recruitment site, predictive for overall GDM/overt DM was not associated with any individual OGTT time point glucose.

At 35-37 weeks' gestation

At this assessment, no predictive factors were associated with any individual OGTT time point glucose

6. Overall summary of the discussion

Vitamin D supplementation for GDM prevention

Vitamin D deficiency has been consistently linked to increased risk of GDM, but intervention studies have not reported conclusive results. In this randomized controlled trial, involving 154 overweight/obese pregnant women <20 weeks' gestation, vitamin D3 supplementation with 1600 IU/day, achieved sufficiency at term and showed a small reduction in FPG at 35-37 weeks' gestation, without having an impact in other investigated outcomes.

Baseline vitamin D sufficiency was unexpectedly high considering the characteristics of the study population. Therefore, *post hoc* we investigated clinical factors associated with sufficiency. Identified factors are in line with published literature (179,239), and population attributable fractions (PAFs) for Caucasian ethnicity (80.8%) and vitamin consumption (77.4%), indicate that these factors are important contributors for vitamin D sufficiency.

After the intervention started, vitamin D sufficiency was achieved in ~100% of the participants in the intervention arm, while ~20% of women in the placebo arm did not achieve sufficiency at term. As for vitamin D concentrations exceeding the desirable threshold (>135 nmol/l), they were observed in 4% of the participants at baseline and increased during follow-up in both groups but, with a significantly higher proportion in the intervention arm. This raises the question of whether supplementation can be universally advised in pregnancy without monitoring. The answer is probably no.

Primary outcomes in this trial were FPG, HOMA-IR and GWG at 24-28, and 35-37 weeks' gestation. We observed a small improvement in FPG at 35-37 weeks, but no other significant differences.

In 2018, Zhang et al., published a systematic review and meta-analysis showing that vitamin D supplementation during pregnancy significantly reduces FPG, fasting insulin levels and HOMA-IR (171). However, they did not observe a significant effect on GDM after vitamin D supplementation (RR 0.72; 95CI 0.39-1.31) (171), while including in

the meta-analysis four RCTs testing vitamin supplementation in pregnancy (175–178). In a letter to the editor that I signed as a second author, the DALI consortium reanalyzed the data, excluding the study by Hossain et al. where the outcome reported was not incident GDM but an abnormal glucose challenge test (178). The metaanalysis of the remaining three studies (n= 793) resulted in a GDM relative risk of 0.53, 95% CI 0.33–0.85, I 2 = 0%, P = 0.009 (220). It must be noted that average vitamin D concentration in women in these trials was <50 nmol/l, and vitamin D doses in the intervention arm were high, ranging from 3400 to 5000 IU/day (175–177). Our report is in line with a Cochrane review published in 2019 examining the effect of vitamin D supplementation. A GDM risk reduction was observed compared to women receiving no intervention, or placebo (RR 0.51, 95CI 0.27 – 0.97) (221) . Interestingly, average baseline vitamin D concentration in women included in this meta-analysis was also below 50 nmol/l. The DALI vitamin D trial showed a small effect on FPG in women with a high rate of vitamin D sufficiency at baseline. Furthermore, the intervention consisted in 1600 IU/day of vitamin D, which is in average lower than the doses used in the abovementioned studies.

Evidence from recent reports addressing T2DM prevention have shown a positive effect from vitamin D supplementation that seems to be limited to non-obese subjects (153,154). In vitamin D supplementation trials for GDM prevention displayed in table 4, average BMI was <30 in three reports (175,176,181), and not reported in the rest (177,179,180). If we extrapolate data from T2DM trials, our study results would be expected, considering that participants had a BMI ≥29.

The clinical relevance of the improvement in FPG observed in this study at 35-37 weeks is limited, as GDM is usually diagnosed earlier in pregnancy. There is some evidence suggesting that, in obese women, maternal hyperglycemia in late pregnancy might be associated with increased birth weight and higher C-peptide levels (189), so reducing FPG at this point could have some clinical relevance.

We did not observe any differences in GWG between study arms. Our observation concur with intervention studies outside pregnancy, that have not observed changes in adiposity measures (222,223) after vitamin D supplementation.

Overall, our interpretation is that the limited beneficial effects on glucose metabolism in this trial are attributable to the relatively high baseline vitamin D status that limited the effect of the supplementation.

Three comprehensive meta-analysis examining the effect of vitamin D supplementation on GDM prevention have been published in recent years. Table 6 displays a summary of methodological and outcome differences between them. Discordance between meta-analyses can be explained by the inclusion of different studies. The meta-analysis conducted by Zhang et al., included studies published up to May 2017. In the first Cochrane report, only trials comparing vitamin D supplementation with placebo or interventions different from vitamin D were included (174). A subsequent Cochrane meta-analysis, published in late 2019 by the same authors, investigated different vitamin D supplementation doses on pregnancy outcomes, excluding placebo-compared trials, and showed a GDM risk reduction (RR 0.54, 95CI 0.34 - 0.86) in women takin doses ≥601 IU/day compared to ≤600 IU/day (224). This meta-analysis included a total of 7 RCT, 4 of which did not include GDM related-variables in their main outcomes (225–228). Subgroup analysis by baseline vitamin D status did not modify the results. Furthermore, the study reported no benefits on GDM in women receiving vitamin D doses ≥4000 IU/day compared to ≤3999 IU/day.

Current evidence hints to a positive effect of vitamin D supplementation on GDM prevention but high-quality RCTs are still needed. The DALI vitamin D trial has not been included in the latest meta-analysis, and there are some ongoing registered trials focusing on vitamin D supplementation for GDM prevention in women with previous GDM (229), and for prevention of three main pregnancy outcomes: pre-eclampsia, fetal growth retardation and GDM (230).

Table 6. Methodological and outcome differences between recent meta-analysis evaluating the effect vitamin D supplementation on GDM.

	Zhang et al.(171)	**Palacios et al.**(221)	**Palacios et al.**(224)
Publication year	2018	2019	2019
Type of studies	RCTs	RCTs or quasi-randomized trials	RCTs or quasi-randomized trials
Type of intervention	Vit D supplementation (any regimen)	Vit D supplementation (any regime)	Vit D supplementation ≥601 vs. ≤600 IU/d ≥4000 vs. ≤3999 IU/d
Control group	Lower Vit D dose or placebo	Placebo	Lower Vit D dose (not placebo)
Primary outcomes	GDM and GDM-related variables	Pre-eclampsia	Pre-eclampsia
		GDM	GDM
		Adverse events	Adverse events
		Pre-term birth	Pre-term birth
		Low birth weight	Low birth weight
Information sources	Cochrane Central Register of Controlled Trials	Cochrane Central Register of Controlled Trials	Cochrane Pregnancy and Childbirth's Trials Register
	MEDLINE	MEDLINE	MEDLINE
	EMBASE	EMBASE	EMBASE
	CINAHL	CINAHL	CINAHL
		Handsearches	Handsearches

	China Biology Medicine (CBM) disc		
Quality evaluation	Cochrane Handbook for Systematic Reviews of Interventions	Cochrane Handbook for Systematic Reviews of Interventions	Cochrane Handbook for Systematic Reviews of Interventions
Statistical analyses	Comprehensive Meta Analysis software (Version 2.2.064)	Review Manager Software	Review Manager Software
	• Forest plot to display effect size and 95%CI • Heterogeneity I^2 • Sensitivity analysis: one study was omitted at each time to test the robustness of the results	• Forest plot to display effect size and 95%CI • Heterogeneity Tau^2, I^2, Chi^2 • Sensitivity analysis: not performed	• Forest plot to display effect size and 95%CI • Heterogeneity Tau^2, I^2, Chi^2 • Sensitivity analysis: based on study quality (high vs. low)
Trials included			
• Wagner 2013	X	X	✓
• Asemi 2013	X	✓	X
• Hashemipour 2014	X	X	✓
• Hossain 2014	✓	X	X
• Yap 2014	✓	X	✓
• Sablok 2015	✓	✓	X
• Mojibian 2015	✓	X	✓
• Stephensen 2016			
• Shagheibi 2016			
• Tehrani 2017			
• Rostami 2018			
• Roth 2010			

	X	X	✓
	X	✓	X
	X	✓	X
	X	X	✓
	X	X	✓
N of subjects	968	446	• 1846 (≥601 vs. ≤600) • 2276 (≥4000 vs. ≤3999) ≠ between studies
Average baseline vitamin D concentration (nmol/l)	<50 (except Yap, 2014)	<50	
Vitamin D supplementation doses	>4000 IU/d	From 400 to ~7150 IU/d	From 400 to ~7150 IU/d
Intervention started (gestational week)	12 to 20	1st trimester to 25	Pre-pregnancy to 26
Intervention duration	10 to ~28 weeks	1 dose to ~14 weeks	1 dose to throughout pregnancy
Reported risk ratio (95%CI)	0.72 (95CI 0.39 – 1.31)	0.51 (95CI 0.27 – 0.97)	0.54 (95CI 0.34 – 0.86) for ≥601 vs. ≤600 IU/d 0.89 (95CI 0.56 - 1.42) for ≥4000 vs. ≤3999 IU/d
Graded evidence	Not graded	Moderate-certainty evidence	Moderate-certainty evidence for ≥601 vs. ≤600 IU/d Low-certainty evidence for ≥4000 vs. ≤3999 IU/d

Hyperglycemia in Pregnancy in the DALI study

In this sub-analysis of the DALI study population, prevalence of HiP was high in all three pregnancy periods assessed, which is not surprising, considering that the study was conducted in a high-risk population, applying more sensitive IADPSG criteria. Yet, it was higher than rates reported in trials of similar characteristics (231,232), in which prevalence at ~28 weeks was reported at ~25%.

At baseline, **the identified risk factors for GDM/overt diabetes** were *previous AGT, previous GDM, NC, RHR and recruitment site*. The association of *previous GDM* with GDM diagnosed before 24 weeks of pregnancy has been described (188), and NICE guidelines consider it a major risk factor, recommending women to be tested for GDM ideally before 16 week gestation if they have a GDM history (44). As for the other identified risk factors, we are not aware of studies addressing NC, previous AGT, RHR or recruitment site at first assessment. *AGT* (either impaired FPG or impaired glucose tolerance), is associated with the incidence of T2DM (233), but in pregnancy it is rarely reported as a risk factor. One report observed a significant increase in the risk for GDM for every 1 mg/dl increase in pre-pregnancy FG concentration, but did not study the relationship with post-challenge glucose before pregnancy (234). *NC*, a proxy for central adiposity, has been reported to confer additional risk beyond BMI for glucose intolerance outside pregnancy (235). Our study showed that *NC* out-performs BMI itself as risk factor for GDM/overt diabetes, in women with pre-pregnancy BMI ≥29, and describes for the first time that this association at ≤19 +6 weeks. *RHR* has been validated as a proxy for fitness status (236), and outside pregnancy, has been associated with an increased risk of T2DM in women (197). To our knowledge this is the first report of *RHR* in pregnancy as a risk factor for GDM. In line with previous reports (59), we have observed a wide variation in the risk of GDM/overt diabetes between centers, and even *sites* of the same country, despite adjustment by ethnicity, which suggests that genetic, environmental and/or lifestyle factors.

At 24–28 weeks, the identified risk factors for GDM/overt diabetes were *previous stillbirth, RHR and recruitment site*. As previously discussed,

differences in GDM prevalence according to the geographic *site* have been described. *RHR* has not been previously reported as a GDM risk factor at any pregnancy period. Als for unexplained ***stillbirth,*** it has previously been reported as a risk factor for AGT/GDM in a subsequent pregnancy (OR: 4.52) (202).

At 35–37 weeks, ***maternal height*** was the only independent variables associated with GDM/overt diabetes. Maternal height has previously been described as a GDM risk factor (237). Outside pregnancy, height has been negatively associated with 2h PG and beta cell function (238). However, it had not been investigated in late pregnancy.

The impact of GDM, diagnosed at 24-28 weeks, on pregnancy is well-known. GDM diagnosed earlier in pregnancy (<24 weeks) has been associated with worse pregnancy outcomes (i.e. higher rates of hypertensive disorders, cesarean section and preterm delivery), compared with women with GDM diagnosed at a later period (239–242). Rates of macrosomia/LGA similar to those of women with preexisting diabetes (239) have been described, highlighting the importance of early GDM diagnosis. As for late onset HiP, data are scarce, but an association with macrosomia and increased adiposity later in life (189) has been suggested.

Despite obesity being a major risk factor for GDM, a recent meta-analysis reported that baseline BMI of participants was not associated with the effect size of the interventions (98), which implies that other clinical factors need to be taken into account. *NC* and *RHR* are modifiable risk factors associated with HiP <20 weeks' gestation. This suggests that, in overweight/obese women, lifestyle interventions (diet and exercise) aiming at weight control and physical condition improvement should be started very early in pregnancy, as supported by recent meta-analyses (98,243,244), or even before conception. Not surprisingly, a retrospective report in healthy pregnant women, observed that participants who exercised regularly during pregnancy (at least 30 min, 3 times a week), displayed lower *RHR* compared to a non-exercise group at 28 weeks' gestation (245). In this same report, maternal BMI was positively associated with HR, suggesting that HR may be a surrogate measurement for fitness in pregnancy.

As to abnormal plasma glucose at different OGTT time points, their relevance comes from their different impact on outcomes, their relative contribution to the GDM diagnosis itself (47), and from different pathophysiology since subjects with impaired FPG mainly display basal insulin resistance, while subjects with impaired glucose tolerance mainly display impaired insulin secretion (246). We have identified that distinct **clinical characteristics are associated with abnormal PG at different OGTT time point**s. In pregnancy, reports investigating risk factors for individual abnormal OGTT glucose values are scarce.

At < 19 +6 weeks gestation, we have observed that *NC* was associated with abnormal FPG, which is in line with observations outside pregnancy (235). Recruitment *site* was also associated with FPG, similar to observations reported in the HAPO study (at a later gestational age), with FPG showing differences among sites despite adjustment for other variables such as BMI (59). *Previous AGT* was associated with both abnormal FPG and 2h PG, which is not surprising, considering that the definition of AGT includes both impaired FPG and post-challenge PG. *RHR* was associated with abnormal post-challenge PG. Measures of cardiorespiratory fitness are mainly associated with post-challenge PG both outside and during pregnancy (247,248).

At 24–28 weeks' gestation, *RHR* was associated with both fasting and postchallenge PG. Our results indicate that in pregnant women, surrogates of exercise, display favorable associations with FPG but preferentially with post-challenge PG. *Previous stillbirth*, was associated with abnormal 1h PG, in agreement with the *post hoc* analysis of the aspart trial in women with type 1 DM, where spikes of high glucose values in the third trimester were associated with poor late pregnancy outcomes including stillbirth (249).

No significant associations between maternal characteristics and different OGTT time points PG values were observed *at 35-37 weeks' gestation*.

As above mentioned, the contribution of abnormal PG values at different OGTT time points to the GDM diagnosis varies according to the population studied (59). They also have been associated with different adverse pregnancy outcomes. Therefore,

identifying specific modifiable risk factors for fasting or post-challenge PG also could be relevant for preventions strategies. For instance, in populations where FPG contributes to most of GDM diagnosis, tackling adiposity may have a greater impact than in populations preferentially diagnosed by post-challenge PG where exercise could have a higher impact.

7. Conclusions

In women with pre-pregnancy BMI ≥29 enrolled in the DALI study:

- Vitamin D supplementation on top of prenatal multivitamins did not improve any primary outcomes (FPG, HOMA-IR, GWG) at 24-28 weeks' gestation, but showed a small improvement in FPG at 35-37 weeks' gestation.

- Vitamin D supplementation did not improve any secondary outcomes.

- Average vitamin D concentrations at baseline were higher than expected in a population with known risk factors for deficiency. European ethnicity and multivitamin intake were major vitamin D sufficiency predictors at baseline.

- Different maternal clinical characteristics, modifiable and non-modifiable, were associated with GDM/overt diabetes at different pregnancy periods and could assist in defining criteria for selective screening, especially before 24-28 weeks.

- Different maternal clinical characteristics, modifiable and non-modifiable, were associated with abnormal PG at different OGTT time points and could assist in selecting participants of prevention trials.

8. Future perspectives

HiP is a prevalent condition that leads to increased maternal and fetal morbidity. Adverse pregnancy outcomes associated with HiP can be mitigated by a prompt diagnosis and appropriate treatment, but effective preventive strategies should be the decisive goal.

Regarding vitamin D as a preventive GDM strategy:

- I would propose an RCTs addressing pregnant women with baseline vitamin D deficiency. Taking into account evidence from vitamin D supplementation trials for T2DM where BMI was a modifying factor for the response to the intervention, I would stratify the study group by pre-pregnancy BMI.

Regarding the identified risk factors for HiP at different pregnancy periods, I would propose:

- To test baseline *RHR* as risk factor for GDM at 24-28 weeks in a prospective observational study addressing the general obstetric population.

- If this is the case, design a GDM prevention RCT, aiming at improving *RHR* in high-risk women (i.e. previous GDM) initiated before pregnancy/in early pregnancy and stratified by baseline RHR.

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