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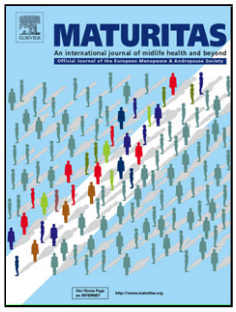

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Annes Ahmeidat, Sohinee Bhattacharya, Robert N. Luben, Kay-Tee Khaw, Phyo K. Myint

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Long-term effects of gestational diabetes on bone mineral density and fracture risk: Analysis of the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk) population-based study

Annes Ahmeidat[1], Sohinee Bhattacharya[1], Robert N Luben[2], Kay-Tee Khaw[2], Phyo K Myint[1]

1School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, Scotland, UK

2Clinical Gerontology Unit, University of Cambridge, Cambridge, UK

Annes Ahmeidat- Research Associate

Sohinee Bhattacharya- Senior Lecturer in Obstetric Epidemiology

Robert N Luben- Research Associate

Kay-Tee Khaw- Professor of Clinical Gerontology

Phyo Kyaw Myint- Professor of Medicine of Old Age

Corresponding author: Professor Phyo Kyaw Myint

Room 4.03, Polwarth Building, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland
Tel: +44 (0) 1224 437841
Mail to: phyo.myint@abdn.ac.uk

Highlights

- A total of 12,145 women were included for analysis from the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC).
- Gestational diabetes mellitus (GDM) was significantly associated with hip fractures after adjusting for potential confounding factors.
- No other study has investigated the link between gestational diabetes mellitus and fracture and further research is required to gain deeper insight into the relationship.

Abstract

Objectives

Gestational diabetes mellitus (GDM) is a common pregnancy complication. This study aims to investigate the association between a history of GDM and bone mineral density (BMD), fractures, and falls in later life.

Study design

We used data from the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk) where BMD at calcaneum was measured at second health check (1997-2000) using broadband ultrasound attenuation (BUA) and velocity of sound (VOS) in 7,515 women. Fractures and falls were documented from hospital admissions data via linkage with ENCORE (East Norfolk Commission Record) and history of GDM from health questionnaires at baseline. We examined the relationship between GDM and BUA/VOS using linear regression. Cox regression was used to estimate hazard ratios (HRs) for incident fractures and falls, controlling for age, BMI, smoking status, physical activity, area deprivation, self-reported stroke, use of diuretics, calcium and vitamin D supplements, social class and education, statin and total blood cholesterol, prevalent diabetes, hormone therapy and menopausal status.

Results

History of GDM (n=183) was not statistically significantly associated with BUA/VOS in fully adjusted linear regression models with unstandardised beta coefficients (standard error): -0.37 (1.40) and -5.41 (3.48). GDM was significantly ($p<0.05$) associated with risk of hip and all fractures, fully adjusted HRs(95%CI) 2.46(1.54-3.92) and 1.60(1.09-2.35), respectively. Median follow-up from first live birth to date of admission was 53 and 52 years, respectively.

Conclusion

There was an association between history of GDM and risk of any fracture as well as hip fracture specifically. Further research is required to confirm this.

Abbreviations

BMD: Bone Mineral Density

BUA: Broadband Ultrasound Attenuation

DEXA: Dual energy X-ray absorptiometry

DM: Diabetes Mellitus

ENCORE: East Norfolk Commission Record

EPIC: European Prospective Investigation into Cancer

FFQ: Food Frequency Questionnaires

GDM: Gestational Diabetes Mellitus

HR: Hazard Ratio

HT: Hormone Therapy

NHS: National Health Service

NICE: National Institute for health and Care Excellence

QUS: Quantitative UltraSound

SE: Standard Error

VOS: Velocity Of Sound

WHO: World Health Organization

Keywords: Gestational diabetes, fracture risk, bone mineral density, pregnancy

Introduction

Diabetes developed during pregnancy, gestational diabetes mellitus (GDM), has an estimated prevalence of around 8-24% in the UK according to a systematic review [1]. This has been attributed to several factors including increased incidence of obesity and diabetes mellitus (DM) among women of childbearing age as well as increasing maternal age at first pregnancy [2,3].

Indeed, GDM is amongst the commonest pregnancy complications along with gestational hypertension and preterm labour [4]. Furthermore, it is a recognised risk factor for development of T2DM, making it an important pregnancy related complication to identify and monitor in order to safeguard future health [5,6]. Examples of maternal and infant consequences of GDM previously reported include macrosomia in the baby and is also thought to contribute to BMD reduction [7,8]. However, to date future fracture and falls risk in women with GDM has not been investigated.

Dual energy X-ray absorptiometry (DEXA) is utilised in clinical practice as the gold standard for diagnosis of osteoporosis. However, it is expensive and not always accessible. BMD measurement can be performed using different modalities. Peripheral sites can be measured with quantitative ultrasound (QUS) which is fast, portable, and feasible in a large sample. QUS of the calcaneus was reported to predict total and hip fracture risk in men and women in a previous European Prospective Investigation into Cancer (EPIC)-Norfolk study [9].

Furthermore, some studies suggest usefulness of QUS in predicting osteoporosis in at-risk groups such as women with premature menopause [10].

Osteoporosis is an escalating health problem, with fragility fractures accounting for over 300,000 patients admitted to hospitals in the UK each year and 1.5 million in the US who are over 50 years of age [11,12]. A previous meta-analysis into the link between DM and BMD has demonstrated findings that type 1 DM decreases BMD while type 2 DM increases BMD [13]. Despite the difference in BMD there was still an increased hip fracture risk for both conditions [13]. However, to date there is a lack of evidence of long term sequalae of GDM regarding its association with future risk of osteoporosis, fractures, and falls.

The aim of our study was to investigate the association between GDM with BMD, fractures, and falls using data from the UK population-based cohort study, EPIC-Norfolk.

Methods

Study population

The study population was drawn from the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC) which consisted of 30,000 men and women aged 39-79 at the baseline who resided in Norfolk, UK, recruited between 1993 and 1997. Follow-up and data collection were extended to allow assessment of other chronic diseases and their determinants. The recruitment and study methods have been detailed elsewhere [14]. The EPIC-Norfolk study was approved by the Norwich Research Ethics committee.

Exposure assessment

At the baseline (1993-1997), participants completed a detailed health and lifestyle questionnaire and attended the first clinic visit where lifestyle determinants of health were measured. From this questionnaire, the participants' educational status, occupational social class, and physical activity were acquired. The main exposure of interest, GDM, was ascertained from the binary question of "Have you ever had diabetes during pregnancy?". Educational status was recorded as no qualification, O-level, A-level, and degree. Social class was classified according to the Registrar General's occupation-based classification scheme [15]. Physical activity was categorised into four groups as derived from the validated EPIC short physical activity questionnaire designed to evaluate combined work and leisure activity [16].

Other information collected from the questionnaire included menopausal status (premenopausal, perimenopausal <1yr, perimenopausal for 1-5 years, and postmenopausal) and hormone therapy (HT) in women (current, former, never). Trained nurses examined participants for the clinical assessments and anthropometric measurements using standardised protocol. Weight was measured with participants wearing light clothing and without shoes. Height was measured to the nearest 0.1cm using the stadiometer with shoes

removed. Body mass index (BMI) was calculated with the formula weight (Kg) divided by height squared (m^2). From non-fasting venous blood samples, full lipid assay was performed, and serum total cholesterol was measured with the RA 1000 (Bayer Diagnostics, Basingstoke, UK).

Nutritional status was derived from food frequency questionnaires (FFQ) answered at the baseline health check. Vitamin D, calcium, and protein were obtained and utilised from the FFQ in this study. Furthermore, vitamin D and calcium supplement use were recorded at the baseline health check.

At the second health check (1997-2000), individuals were invited to attend the second clinic visit. Approximately 15,000 responded of those mailed after excluding participants that had moved or died.

Medical history was ascertained on the health questionnaire repeated at the second health check (1997-2000) with the question "Has a doctor ever told you have any of the following?" followed by a list of conditions including stroke, cancer, and DM. Smoking status was determined from questions "Have you ever smoked as much as one cigarette a day for as long as a year?" and "Do you smoke cigarettes now?". For current medications at baseline, participants were asked to report the medications (such as diuretics and statins) they were on (name, frequency, dose, etc).

BMD outcomes

At the second health check (1997-2000) broadband ultrasound attenuation (decibel/megahertz) and velocity of sound (metres/second) were quantified at each calcaneum at least twice using the CUBA sonometer (McCue Ultrasonics, Winchester, UK). Mean of left and right ultrasound measurements were used for analysis. Coefficient of variation was 3.5%. The five CUBA machines were calibrated daily with a physical phantom and compared to one calcaneus.

Fracture and fall outcomes

Participants of EPIC-Norfolk were followed for health events to the present date. Participant fracture and fall admissions in England, Wales, and Norfolk hospitals were identified by linking via the unique national health service (NHS) number by data linkage with ENCORE (East Norfolk Commission Record) [17]. The fracture data for analysis was available up to 31st March 2016 and were captured after baseline. This method of fracture ascertainment has been previously validated [18]. Based on ICD-10 criteria the following hospital admissions for: hip fracture (S72.0), falls (W00-W19), and all fracture (S32, S62, S72, and S82) were extracted.

Grouping of variables for analysis

Categorical variables were grouped into dichotomous variables before univariate analysis: occupational social class as manual (non-skilled, semi-skilled, skilled manual) and non-manual

(skilled non-manual, manager, professional); educational status as O-level or less (no education, O-level) and A-level or more (A-level, degree). Additionally, social class and education were made into a single variable: non-manual and O-level or less; manual and O-level or less; non-manual and A-level or more; and manual and A-level or more. Calcium and vitamin D supplements as none, calcium only, vitamin D only, or both. Total blood cholesterol was categorised as ≥ 5mmol/L and < 5 mmol/L as defined by Institute for Quality and Efficiency in Health Care for hypercholesterolaemia [19]. Total cholesterol level was made into a single variable with statin use: no-statin and normal cholesterol; no-statin and hypercholesterolaemia; yes-statin and normal cholesterol; yes-statin and hypercholesterolaemia. This derivation of combined variables reduced the total number of variables and allow us to control for multiple variables. Age was categorised as <65 years and ≥65 years and BMI into tertiles: <23, 23-26, and >26 kg/m^2.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences for Windows version 26.0 (SPSS Inc, Chicago, IL, USA). Participants with missing data were excluded from the regression analysis. However, missing participants in the variable "have you ever had diabetes during pregnancy" were recoded into the "no" category due to the percentage missing being closer to the percentage no category in the "prevalent diabetes".

Linear regression tested strength of association between the main predictor: gestational diabetes (GDM) and the continuous outcomes of VOS and BUA. For linear regression, multiple models were built to better understand which factors - other than GDM - influenced BMD. The first model, model A, was built with the variables: GDM, age at baseline, BMI, smoking status, activity level, area deprivation index, self-reported stroke, diabetes mellitus prevalence, dietary vitamin D and calcium intake, diuretics for > 3months, calcium and vitamin D supplement, social class and education, statin and total blood cholesterol, hormone therapy, menopausal status. Next, model B, utilised significant variables at 10% significance (cut off p = 0.10) from univariate analysis for BUA which was the same as model A minus the variables deprivation index, prevalent diabetes mellitus, and dietary calcium. For VOS in model B, variables were same as those in BUA except smoking status which was non-significant at univariate model was removed. Unstandardised beta coefficient (B) was calculated with the standard of error (SE) and presented with the p-value for significance of the association.

Cox regression models were constructed to calculate the hazard ratios (HR) for the following outcomes: hip fracture, all fractures, and hospitalisations for falls. Other fracture variables which violated the proportional hazard assumption were not assessed (wrist fracture, spine fracture, and other fracture). Follow up time was calculated from the date of first live birth to date of hospital admission for first fracture or first hospitalisation with a fall, for respective outcomes. Date of first live birth represented a suitable date for when GDM would have manifested. With the same variables as model A from linear regression, suitable models were

constructed utilised for cox regression. HRs are presented with 95% confidence intervals and p-values for significance.

Results

A total of 10,526 women attended the baseline health examination (1993-1997) and 7,478 attended second health check where skeletal properties using calcaneum ultrasound was measured. Therefore, falls and fracture outcomes were assessed in 10,526 women and BUA/VOS analysis was based on 7,478 women.

Table 1 shows the comparison of characteristics of women with GDM to those without; they were more likely to be younger (56.7 ± 8.8 and 58.7 ± 9.2, p-value < 0.01) and less likely to have prevalent diabetes. Moreover, participants with GDM were less likely to be menopausal at the time of enrolment when compared to the control. Other factors such as: BMI, smoking status, alcohol intake, level of activity, deprivation index, social class, education, nutritional intake, self-reported stroke, and medication usage were not significantly different in the two exposure groups (Table 1). Whilst the number of trips, falls and stumbles were not significant between the groups, there were significant differences with regards to all fractures and hip fractures.

Gestational diabetes and bone mineral density

Table 2 shows the results of linear regression for the predictor GDM with the outcomes of calcaneus ultrasound VOS (n =7,478) and BUA (n=7,478) unadjusted demonstrated significance for VOS and no significance for BUA with a B (SE) of -9.61 (3.81), p=0.012 and -2.43 (1.57), and p=0.122 respectively (Table 2).

Gestational diabetes and fractures

The follow up time for all fracture, hip fractures, and falls respectively were: median of 52 years (SD, 10; 550,194 total person years); median of 53 years (SD, 10; 555,491 total person years); median of 52 years (SD, 9.9; 548,816 total person years). Number of women identified for each outcome was 1266, 582, and 1576 for all fracture, hip fracture, and falls respectively.

Table 3 shows the HRs and 95% CIs for all fractures, hip fractures, and falls hospitalisations in women with GDM using an unadjusted model and model A which controls for same variables as linear regression. The outcome all fractures when analysed unadjusted yielded a significant HR=1.6 (1.1-2.33) and remained significant after adjustment in model A HR=1.6 (1.09-2.35) as seen in Table 3. Hip fractures were significant for both unadjusted model and model A with HRs of 2.47 (1.57-3.91) and p<0.001, 2.46(1.54-3.92) and p<0.001, and 2.46 (1.54-3.92) and p<0.001. Trips, falls, stumbles did not reach statistical significance unadjusted or in model A as displayed in Table 3.

Discussion

Main findings

In the EPIC Norfolk cohort, gestational diabetes mellitus (GDM) was significantly associated with fractures, specifically hip fractures, after adjusting for potential confounding factors. Apart from GDM, BMI was the only other significant ($p<0.05$) variable on cox regression for hip fractures where B=-0.19 and SE=0.11. Trips and falls were not significantly associated with GDM.

There were no associations between BUA and VOS measurements and GDM. VOS was only significant when unadjusted suggesting other factors were responsible for the result. In multivariate analysis, it was noted that out of all the covariates adjusted, BMI (B=1.94, SE=0.04), HT (B=-10.6, SE=-0.5), and menopausal status (B=-12.6, SE=0.4) had the strongest influence ($p<0.05$). Calcaneal ultrasound measurements give insight into skeletal properties and not BMD. Hence, BMD changes may have occurred given the hip fracture significance. The hip and spine are the most sensitive markers of osteoporosis and fragility fractures and thus it was hypothesised osteoporosis played a role. It is possible that reduced bone mineral density would explain the increased fracture risk in women with history of GDM.

Strengths and Limitations

To the best of our knowledge, this is the first cohort study which examined the link between gestational diabetes during reproductive age and long-term bone mineral density and fracture and falls risk in women. The strength of this study lies in the long follow up period after development of GDM which allows for detection of any changes to BMD in the long term. The large sample size of the study together with population-based apparently healthy community living women where data were collected prospectively strengthen the relevance. The use of validated follow-up methods and our ability to adjust for wide range of confounders including menopausal status and hormone therapy also increase the robustness of our findings.

A limitation of this study was the measurements for QUS were made a few years after baseline measurements. Within this time period participants could have altered their lifestyles which may have influenced their BMD. One of the limitations of this study is the method of ascertaining GDM with the question of “have you ever had diabetes during pregnancy” which includes the possibility of patients with DM type 1 and 2 answering yes introducing reporting bias. However, the National Institute for Health and Care Excellence (NICE) in the UK estimates the majority of DM during pregnancy is mainly GDM, with an incidence of 87.5% [20]. In addition, prevalent diabetes at the time of enrolment in those who replied “yes” accounted for 0.2% (Table 1) and thus it was unlikely we have many participants who already had diabetes prior to their first pregnancy and thus mis-classified as GDM.

Data collection began in 1997 and patient records would date to before then and the definition of gestational diabetes has since changed. Before 1997 there was no World Health Organisation (WHO) definition of GDM and different hospitals utilised different cut-off points for blood glucose, therefore acquisition of GDM status would still not be as accurate through hospital records.

It is recognised that risk factors for GDM exist that do warrant screening during pregnancy such as: BMI >30 kg/m^2, previous GDM, family history of GDM, and an ethnicity with high prevalence of diabetes mellitus [21]. GDM is sometimes classified into the two categories true GDM and pre-existing diabetes based on the International Association of Diabetes and Pregnancy Study Groups consensus [22]. True GDM is diagnosed in pregnancy and resolves after pregnancy, however pre-existing diabetes tends to be present before and after as well as requiring insulin therapy. Knowing that classifications and differing cut-offs for GDM exist, these are liable to affect numbers in the literature throughout the years and makes the studying the effects of GDM difficult.

We were unable to control for use of bone protection during the follow up and as such did not adjust in our analyses. However, inability to control for such co-variate is likely to produce attenuation in effect size for the relationship and thus observed effects are likely an underestimation.

Comparison with literature

To the best of our knowledge, the only other study looking at the association between BMD and GDM came from work by Kee et al. [6] in which they measured BMD of women with gestational diabetes during pregnancy at 20 and 36 weeks gestation without further follow up after pregnancy. The authors concluded that the decrease in BMD they observed during pregnancy was due to physiological changes and that factors such as nutrition may have a role. The differing results of Kee et al. with our study could be explained by the differences in follow up and our ability to control for dietary covariates and supplements in the statistical model.

To date no studies have been conducted on GDM and fracture risk, however it is well established that both type 1 and 2 diabetes mellitus are associated with an increased fracture risk [13].

Interpretation of findings

Diet is linked to both BMI and GDM and could be the driving force behind the strong association. BMD loss after GDM exists in this sample in those who have an increased BMI and remains unclear for the rest. An increased hip fracture risk remained after adjustment for covariates and no other studies exist to confirm the result. The exact mechanism for hip fracture in GDM is unclear and a new direction in research on this field could address this with

the use of a prospective study and DEXA for measuring BMD. Another mechanism explaining this finding could be patients developing type 2 DM later in life, and therefore retinopathy and neuropathy precipitating the fracture. This would explain the strong association of increased BMI with BUA/VOS in both the univariate and multivariate analysis.

Despite the strong link between development of type 2 diabetes mellitus in later life for GDM, the GDM group were less likely to have prevalent diabetes (Table 1). It could be that there remains the possibility of a pre-diabetic state in participants or a diagnosis given after baseline given the strong evidence of association between GDM and type 2 diabetes mellitus.

Clinical and research implications

Effect of GDM on BMD remains an under researched area and its increasing risk to fractures and falls may warrant further follow up. Follow up would include typical advice to reduce risk of type 2 DM and cardiovascular risk as well as a DEXA scan after menopause to identify those at high risk. Lifestyle advice in terms of appropriate diet and increased physical activity should maintain or increase BMD and mitigate the risks of fractures. Future research studies investigating fracture risk in those with history GDM would require larger cohorts.

Conclusion

This study aimed to investigate the link between GDM and BMD, fractures, and falls in the EPIC cohort. It was found that a history of GDM increased risk of hip fracture and falls. There is currently no literature investigating the association between GDM and long-term fracture risk and our study provides better understanding of this relationship. Further research is required to gain deeper insight regarding the relationship between GDM, BMD and fractures.

Contributors

Annes Ahmeidat performed literature review, analysed the data, and prepared the draft manuscript.

Sohinee Bhattacharya conceived the study and supervised drafting of the manuscript.

Robert N Luben performed data linkage.

Kay-Tee Khaw is one of the PIs of the EPIC-Norfolk study.

Phyo K Myint conceived the study and supervised drafting of the manuscript.

All authors contributed to the writing of the paper. PKM is the guarantor.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

The EPIC-Norfolk study was approved by the Norwich Research Ethics Committee.

Ethical Statement for Solid State Ionics

Hereby, I **Phyo K Myint**, consciously assure that for the manuscript **“Long term effects of gestational diabetes on bone mineral density and fracture risk: European Prospective Investigations into Cancer (EPIC)-Norfolk prospective population-based study”** the following is fulfilled:

1) This material is the authors' own original work, which has not been previously published elsewhere.

2) The paper is not currently being considered for publication elsewhere.

3) The paper reflects the authors' own research and analysis in a truthful and complete manner.

4) The paper properly credits the meaningful contributions of co-authors and co-researchers.

5) The results are appropriately placed in the context of prior and existing research.

6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.

7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences.

To verify originality, your article may be checked by the originality detection software iThenticate. See also http://www.elsevier.com/editors/plagdetect.

I agree with the above statements and declare that this submission follows the policies of Solid State Ionics as outlined in the Guide for Authors and in the Ethical Statement.

Provenance and peer review

This article was not commissioned and was externally peer reviewed.

Research data (data sharing and collaboration)

There are no linked research data sets for this paper. Data will be made available on request to the steering committee of EPIC-Norfolk.

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References

[1] Farrar D, Simmonds M, Griffin S, Duarte A, Lawlor DA, Sculpher M, Fairley L, Golder S, Tuffnell D, Bland M, Dunne F, Whitelaw D, Wright J, Sheldon TA. The identification and treatment of women with hyperglycaemia in pregnancy: An analysis of individual participant data, systematic reviews, meta-analyses and an economic evaluation. *Health Technol Assess (Rockv)*. 2016;20(86):47–54. DOI:10.3310/hta20860[PubMed:27917777]

[2] Kim SY, England L, Wilson HG, Bish C, Satten GA, Dietz P. Percentage of Gestational Diabetes Mellitus Attributable to Overweight and Obesity. *Am J Public Heal*. 2010;100(6):1047–52. DOI:10.2105/AJPH.2009.172890[PubMed:20395581]

[3] Makgoba M, Steer PJ. An analysis of the interrelationship between maternal age , body mass index and racial origin in the development of gestational diabetes mellitus. *BJOG*. 2012;119(3):276–82. DOI:10.1111/j.1471-0528.2011.03156.x[PubMed:22044452]

[4] Narayan B, Nelson-Piercy C. Medical problems in pregnancy. *Clin Med*. 2017;17(3):251–7. DOI:10.7861/clinmedicine.17-3-251[PubMed:28572227]

[5] Chander S, Vinnamala S, Jenkins D. Gestational Diabetes and Future Risk of Diabetes. 2013;5(2):92–6. DOI:10.4021/jocmr1201w[PubMed:23519363]

[6] To WWK, Wong MWN. Bone mineral density changes in gestational diabetic pregnancies – a longitudinal study using quantitative ultrasound measurements of the os calcis. *Gynecol Endocrinol*. 2008;24(9):519–25. DOI:10.1080/09513590802288184[PubMed:18958773]

[7] Salari P, Abdollahi M. The influence of pregnancy and lactation on maternal bone health: a systematic review. *J Fam Reprod Heal*. 2014;8(4):135–48. [PubMed:25530765]

[8] Siddiqi S, Borse A, Pervez A, Anjum S. A study of bone turnover markers in gestational diabetes mellitus. *Indian J Endocrinol Metab*. 2017;21(1):38–44. DOI:28217496

[9] Khaw K, Reeve J, Luben R, Bingham S, Welch A, Wareham N, Oakes S, Day N. Prediction of total and hip fracture risk in men and women by quantitative ultrasound of the calcaneus : EPIC-Norfolk prospective population study. *Lancet*. 2004;363(9404):197–202. DOI:10.1016/S0140-6736(03)15325-1[PubMed:14738792]

[10] Frost ML, Blake GM, Fogelman I. Quantitative Ultrasound and Bone Mineral Density Are Equally Strongly Associated with Risk Factors for Osteoporosis. *J Bone Min Res*. 2009;16(2):406–16. DOI:10.1359/jbmr.2001.16.2.406[PubMed:11204441]

[11] U.S. Dept. of HHS. Bone health and osteoporosis: a report of the Surgeon General. *US Heal Hum Serv* [Internet]. 2004;437. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20945569DOI:10.2165/00002018-200932030-00004[PubMed:20945569]

[12] Elvey MH, Pugh H, Schaller G, Dhotar G, Patel B, Oddy MJ. Failure in the application of fragility fracture prevention guidelines. *Ann R Coll Surg Engl*. 2014;96(5):381–5. DOI:10.1308/003588414X13946184901164[PubMed:24992424]

[13] Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes - A meta-analysis. *Osteoporos Int*. 2007;18(4):427–44. DOI:10.1007/s00198-006-0253-4

[14] Day N, Oakes S, Luben R, Khaw K, Binghama S, Welch A, Wareham N. EPIC-Norfolk : cohort study design and characteristics of the cohort. European Prospective Investigation of Cancer. *Br J Cancer*. 1999;80(1):95–103. [PubMed:10466767]

[15] Connelley R, Gayle V, Lambert PS. A Review of occupation-based social classifications for social survey research. *Methodol Innov*. 2016;9(1):1–14. DOI:doi.org/10.1177/2059799116638003

[16] Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, Day NE. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Heal Nutr*. 2003;6(4):407–13. DOI:10.1079/PHN2002439[PubMed:12795830]

[17] Julian C, Lentjes MAH, Huybrechts I, Luben R, Wareham N, Moreno LA, Khaw K. Fracture Risk in Relation to Serum 25- Hydroxyvitamin D and Physical Activity : Results from the EPIC-Norfolk Cohort Study. *PLoS One*. 2016;11(10):1–16. DOI:10.1371/journal.pone.0164160[PubMed:27749911]

[18] Sinha S, Myint PK, Luben RN, Khaw KT. Accuracy of death certification and hospital record linkage for identification of incident stroke. *BMC Med Res Methodol*. 2008;10(8):74. DOI:10.1186/1471-2288-8-74[PubMed:19000303]

[19] High cholesterol: Overview. *Inst Qual Effic Heal Care*. 2017;

[20] NICE. National Institute for Health and Care Excellence (2015): Diabetes in pregnancy: management from preconception to the postnatal period (NG3). *NICE Guidel [NG3]*. 2015;63(2008):42.

[21] Antenatal care for uncomplicated pregnancies: clinical guideline [CG62] [Internet]. 2019 [cited 2019 Dec 2]. Available from: https://www.nice.org.uk/guidance/cg62

[22] Mirghani Dirar A, Doupis J. Gestational diabetes from A to Z. *World J Diabetes*. 2017;8(12):489–511. DOI:10.4239/wjd.v8.i12.489[PubMed:29290922]

Table 1. Baseline characteristics of 10,526 women in EPIC-NORFOLK aged 40-78 at first health check (1993-1997) according to diabetes in pregnancy status

	GDM n = 183	No GDM n = 10,343	p-value
Age, years	56.7 (8.8)	58.7 (9.2)	**0.004**
BMI, Kg/m^2	26.4 (4.1)	26.1 (4.3)	0.251
Smoker			0.592
Current smoker	21 (11.5)	1151 (11.1)	
Former smoker	52 (28.4)	3302 (31.9)	
Never smoked	110 (60.1)	5890 (56.9)	
Alcohol, g	5.0 (8.0)	5.7 (8.5)	0.331
Level of activity			0.436
Inactive	50 (27.3)	2857 (27.6)	
Moderately inactive	55 (30.1)	3395 (32.8)	
Moderately active	50 (27.3)	2409 (23.3)	
Active	28 (15.3)	1682 (16.3)	
Area deprivation index	-2.2 (2.0)	-2.1 (2.1)	0.317
Social class			0.181
Professional	18 (9.8)	691 (6.7)	
Manager	65 (35.5)	3603 (34.8)	
Skilled non-manual	36 (19.7)	1994 (19.3)	
Skilled manual	38 (20.8)	2244 (21.7)	
Semi-skilled	24 (13.1)	1401 (13.5)	
Non-skilled	2 (1.1)	410 (4.0)	
Education			0.170
None	69 (37.7)	4340 (42.0)	
O-level	27 (14.8)	1232 (11.9)	
A-level	69 (37.7)	3722 (36.0)	
Degree	18 (9.8)	1049 (10.1)	

Dietary vitamin D, µg	3.4 (1.9)	3.5 (1.9)	0.730
Dietary calcium, mg	969.0 (272.4)	991.6 (288.6)	0.292
Vitamin D supplement			0.391
Yes	41 (22.4)	2530 (24.5)	
No	142 (77.6)	7813 (75.5)	
Calcium supplement			0.391
Yes	6 (3.3)	527 (5.1)	
No	177 (96.7)	9816 (94.9)	
Phosphorus, mg	1443.1 (343.2)	1456.2 (362.2)	0.626
Protein, g	81.0 (19.5)	81.5 (20.8)	0.770
Total Cholesterol, mmol/L	6.2 (1.1)	6.3 (1.2)	0.172
Self-reported stroke			0.437
Yes	3 (1.6)	95 (0.9)	
No	180 (98.4)	10247 (99.1)	
Prevalent diabetes			**<0.001**
Yes	30 (16.4)	231 (2.2)	
No	153 (83.6)	10112 (97.8)	
Systolic BP, mmHg	131.3 (18.9)	133.6 (18.7)	0.101
Diastolic BP, mmHg	80.1 (11.3)	80.8 (11.0)	0.402
ACE inhibitors			0.640
Yes	6 (3.3)	281 (2.7)	
No	177 (96.7)	10062 (97.3)	
Diuretics			0.074
Yes	9 (4.9)	840 (8.1)	
No	174 (95.1)	9503 (91.9)	
Calcium channel blockers			0.365

Yes	6 (3.3)	467 (4.5)	
No	177 (96.7)	9876 (95.5)	
Beta blockers			1.000
Yes	11 (6.0)	630 (6.1)	
No	172 (94.0)	9713 (93.9)	
Statins			1.000
Yes	1 (0.5)	67 (0.6)	
No	182 (99.5)	10276 (99.4)	
HT			0.631
Current	41 (22.4)	2188(21.2)	
Former	24 (13.1)	1197 (11.6)	
Never	118 (64.5)	6958 (67.3)	
Menopausal status			**0.039**
Premenopausal	44 (24.0)	1790 (17.3)	
Early perimenopause	10 (5.5)	588 (5.7)	
Late perimenopause	31 (16.9)	1953 (18.9)	
Post-menopausal	98 (53.6)	6012 (58.1)	
Trips, falls, stumbles			0.304
Yes	27 (14.8)	1549 (15.0)	
No	156 (85.2)	8794 (85.0)	
Hip fractures			**<0.001**
Yes	19 (10.4)	563 (5.4)	
No	164 (89.6)	9780 (94.6)	
All fractures			**0.014**
Yes	28 (15.3)	1238 (12.0)	
No	155 (84.7)	9105 (88.0)	

Data presented as mean (standard deviation) for continuous and number (percentage) for categorical.BMI: body mass index, BP: blood pressure, ACE: angiotensin converting enzyme, HT: hormone therapy

Table 2. Linear regression for GDM with BMD outcomes

	B	SE	p-value
BUA (dB/MHz) n = 7478			
Unadjusted	-2.23	1.56	0.153
Model A	-0.37	1.40	0.793
Model B	-0.438	1.39	0.752
VOS (m/s) n = 7478			
Unadjusted	-9.61	3.81	0.012
Model A	-5.41	3.48	0.12
Model B	-5.81	3.45	0.092

Model A: diabetes during pregnancy, age at baseline, BMI, smoking status, activity level, area deprivation index, self-reported stroke, diabetes mellitus prevalence, vitamin D nutrition, calcium nutrition, diuretics for > 3months, calcium and vitamin D supplement, social class and education, statin and total blood cholesterol, HT, menopausal status.

Model B: significant variables from univariate analysis

Table 3. Cox regression for GDM with fracture outcomes

	Hazard ratio (95%CI)	p-value
Trips, falls, stumbles n = 1576		
Unadjusted	1.22 (0.83-1.79)	0.304
Model A	1.17 (0.79-1.74)	0.427
All fractures n = 1266		
Unadjusted	1.60 (1.10-2.33)	0.014
Model A	1.60 (1.09-2.35)	0.017
Hip fractures n = 582		
Unadjusted	2.47 (1.57-3.91)	<0.001
Model A	2.46 (1.54-3.92)	<0.001

Model A: diabetes during pregnancy, age at baseline, BMI, smoking status, activity level, area deprivation index, self-reported stroke, diabetes mellitus prevalence, vitamin D nutrition, calcium nutrition, diuretics for > 3months, calcium and vitamin D supplement, social class and education, statin and total blood cholesterol, HT, menopausal status.