UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS - ENDOCRINOLOGIA

REPERCUSSÕES MATERNO-FETAIS DA DEFICIÊNCIA DE VITAMINA D EM MULHERES COM DIABETES GESTACIONAL

TESE DE DOUTORADO

LETÍCIA SCHWERZ WEINERT

Porto Alegre, Julho de 2013

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, da Universidade Federal do Rio Grande do Sul (UFRGS) como requisito parcial para obtenção do título de Doutora em Endocrinologia.

Porto Alegre, Julho de 2013

CIP - Catalogação na Publicação

Weinert, Letícia Schwerz
Repercussões materno-fetais da deficiência de vitamina D em mulheres com diabetes gestacional / Letícia Schwerz Weinert. -- 2013.
71 f.

Orientadora: Sandra Pinho Silveiro.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Porto Alegre, BR-RS, 2013.

1. Deficiência de vitamina D. 2. Diabetes gestacional. 3. Pequeno para idade gestacional. 4. Doença hipertensiva da gestação. 5. Desfechos adversos materno-fetais. I. Silveiro, Sandra Pinho, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

Agradeço à minha amiga e orientadora, Profª Drª Sandra Pinho Silveiro, pelo incentivo à pesquisa e pela orientação iniciados há 11 anos. Desde a iniciação científica, quando acadêmica de medicina da UFRGS, tenho o prazer de receber seus ensinamentos em endocrinologia e em pesquisa. O otimismo frente às adversidades e o apoio em momentos de dificuldades também foram marcas constantes. Agradeço veementemente pelo incentivo de todos estes anos, o tempo disponibilizado para meu crescimento intelectual, a dedicação aos meus trabalhos e à confiança na minha capacidade.

Agradeço às amigas e colaboradoras Drª Angela Jacob Reichelt e Profª Drª Maria Lúcia Rocha Oppermann pela oportunidade de trabalho no ambulatório de Diabetes e Gestação, pelo convívio nestes últimos anos, pelo incentivo fornecido ao meu doutorado, e pelos ensinamentos diários na área do Diabetes Gestacional.

Agradeço à Profª Joíza Lins Camargo e à colega Roberta Boff pelo essencial apoio e suporte na dosagem sérica da vitamina D.

Agradeço à equipe do Laboratório do Serviço de Endocrinologia, em especial à Profª Daisy Crispim Moreira e à colega Tais Silveira Assman, pelo apoio no armazenamento de material biológico.

Agradeço aos alunos de iniciação científica Leonardo Rauber Schmitt, Bárbara Marina Simionato e Aline Siebeneichler, pelo trabalho árduo para que esta pesquisa fosse concretizada.

Agradeço às gestantes participantes pela colaboração com a pesquisa.

Agradeço aos meus colegas de residência médica pelo incentivo em pesquisa e análise crítica da evidência.

Agradeço também à Universidade Federal do Rio Grande do Sul, ao Hospital de Clínicas de Porto Alegre e ao Serviço de Endocrinologia, pela minha formação profissional, e ao Programa de Pós-Graduação em Ciências Médicas-Endocrinologia por proporcionar a realização deste doutorado.

Agradeço ao meu esposo, Prof Eduardo Gehling Bertoldi, que esteve ao meu lado diariamente na caminhada deste doutorado, com palavras e gestos de amor, apoio e confiança; e aos meus pais Gastão Henrique Weinert e Maria da Glória Schwerz Weinert pelo incentivo e suporte na busca do conhecimento e pelo exemplo de trabalho e honestidade.

SUMÁRIO

ABREVIATURAS E SIGLAS

25(OH)D	25-Hidroxivitamina D ou *25-Hydroxyvitamin D*
ADA	*American Diabetes Association*
BMI	*Body Mass Index*
DG	Diabetes Gestacional
DM	*Diabetes Mellitus*
GD	*Gestational Diabetes*
HDP	*Hypertensive Disorders of Pregnancy*
HOMA	*Homeostasis Model Assessment*
ICU	*Intensive Care Unit*
IG	Idade Gestacional
IMC	Índice de Massa Corporal
IOM	*Institute of Medicine*
LGA	*Large for gestational Age*
OGTT	*Oral Glucose Tolerance Test*
OR	*Odds Ratio*
PIG	Pequeno para Idade Gestacional
PTH	Paratormônio
RAS	*Renin Angiotensin System*
RN	Recém-nascido
RR	*Relative Risk*
SGA	*Small for Gestational Age*
TTOG	Teste de tolerância oral à glicose
UAE	*Urinary Albumin Excretion*
UTI	Unidade de Tratamento Intensivo

VDBP *Vitamin D Binding Protein*

Repercussões materno-fetais da deficiência de vitamina D e do diabetes gestacional

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Suporte financeiro: Fundo de Incentivo à Pesquisa e Eventos – Hospital de Clínicas de Porto Alegre

Conflito de interesse: Nenhum conflito de interesse a declarar

Resumo

O estudo das funções extra-esqueléticas da vitamina D vem ampliando-se nos últimos anos. Na gestação, a preocupação com os níveis de vitamina D maternos ocorre pela necessidade desta vitamina para a formação do esqueleto fetal e pela associação da hipovitaminose D com desfechos adversos materno-fetais. Para o recém-nascido (RN), as complicações incluem o baixo peso ao nascer, o comprometimento do crescimento longitudinal e as infecções respiratórias. Para a gestante, a deficiência de vitamina D vem sendo associada à alteração na homeostase glicêmica e ao aumento da incidência de diabetes gestacional (DG) , à pré-eclâmpsia e à vaginose bacteriana. Entretanto, a evidência científica atual ainda é controversa e não há definição estabelecida sobre o real benefício da suplementação da vitamina D na gestação.

O diabetes gestacional, por sua vez, também está associado a desfechos adversos para a gestante e para a prole. Para o feto, há aumento da incidência de prematuridade, macrossomia, distócia de ombro e hipoglicemia neonatal; enquanto para a mãe, há associação com aumento da taxa de cesariana, pré-eclâmpsia e diabetes pós-gestacional.

Desta forma, os desfechos adversos da hipovitaminose D e do DG presentes de forma simultânea na gestação podem ser aditivos. Este artigo propõe-se à revisão das repercussões da deficiência da vitamina D e do DG na gestação, para a mãe e para o RN, e discute a potencial repercussão da associação de ambas situações já que a hipovitaminose D pode estar relacionada com aumento da ocorrência de DG.

Palavras-chave: deficiência de vitamina D, hipovitaminose D, diabetes gestacional, gestação

Abstract

Extra-skeletal functions of vitamin D have been studied in the last years. During pregnancy, the concern with vitamin D levels is justified by its importance for the fetal skeleton development and by the association of hypovitaminosis D with adverse maternal and fetal outcomes. For the newborn, adverse outcomes include low birth weight, impaired longitudinal growth and respiratory infections. For the women, vitamin D deficiency has been associated with glucose homeostasis impairment and increased incidence of gestational diabetes (GD), preeclampsia and bacterial vaginosis. However, the available scientific data is still controversial and the real benefit of vitamin D supplementation during pregnancy is not defined.

Hyperglycemia during pregnancy is also associated with increased rates of perinatal adverse outcomes. For the fetus and the newborn, GD is associated with an increased incidence of prematurity, macrosomia, shoulder dystocia and neonatal hypoglycemia; for the mother, there are increased rates of cesarean delivery, preeclampsia and type 2 diabetes.

Therefore, adverse outcomes of hypovitaminosis D and GD present simultaneously during pregnancy could be additive. This manuscript aims to review the impact of vitamin D deficiency and of GD for the women and the newborn, and to discuss the potential association between these two clinical situations since hypovitaminosis D may increase the risk for GD.

Keywords: vitamin D deficiency, hypovitaminosis D, gestational diabetes, pregnancy

Vitamina D: Fisiologia e Função Biológica

A vitamina D, ou calciferol, é atualmente considerada um pró-hormônio essencial para o corpo humano. Compreende um grupo de seco-esteróis lipossolúveis, sendo a vitamina D2 (ergocalciferol) e a vitamina D3 (colecalciferol) suas principais representantes. A vitamina D3 é sintetizada na pele, a partir do 7-desidrocolesterol, após exposição à radiação ultravioleta B (UVB), sob influência de diversos fatores como estação do ano, cor da pele, latitude, altitude, uso de protetor solar, idade e extensão da pele exposta[1,2]. A exposição solar, desta forma, é a principal fonte de vitamina D, embora alguns alimentos também sejam fonte de vitamina D3[3]. Ambas as formas de vitamina D2 e D3 são fabricadas comercialmente e encontradas em suplementos alimentares. Entretanto, todas estas formas de vitamina D são consideradas biologicamente inativas até as reações enzimáticas de hidroxilação. A primeira ocorre no fígado, mediada pela 25-hidroxilase, e sintetiza a 25-hidroxivitamina D (25(OH)D), forma circulante mais abundante de vitamina D; a segunda reação ocorre no rim, mediada pela 1α-hidroxilase, e produz a 1,25-dihidroxivitamina D (calcitriol), hormônio biologicamente ativo. A síntese renal de calcitriol é regulada positivamente pelo paratormônio (PTH) e negativamente pelo *fibroblast-like growth fator-23*. Os níveis séricos de cálcio e fósforo também influenciam esta reação[2].

A ação clássica da vitamina D é a regulação da homeostase do cálcio e do fósforo, e a manutenção da saúde óssea. Entretanto, a maioria dos tecidos do corpo possuem receptores para a forma ativa da vitamina D e muitos destes contêm a enzima para a conversão de 25-hidroxivitamina D em 1,25-dihidroxivitamina D para consumo local[4,5]. Assim, diversas funções extra-esqueléticas vêm sendo atribuídas à vitamina D, como a regulação do sistema imune, da proliferação e diferenciação celular, e da secreção hormonal, incluindo o estímulo para a secreção de insulina[4,5].

Devido à importância da vitamina D, o *Institute of Medicine* (IOM) publicou recentemente[6] as recomendações sobre a ingestão diária recomendada de cálcio e vitamina D, baseando-se na evidência vigente de indicadores de saúde óssea, doenças crônicas e desfechos em saúde para a população norte-americana. Devido à preocupação com a prevenção do câncer de pele, a recomendação proposta pelo IOM é considerar mínima a exposição solar e assumir que toda a vitamina D advém da ingestão. Durante a gestação e lactação, a ingestão diária recomendada é de 600 UI, considerando-se a necessidade do feto e a produção materna de leite. As recomendações também consideram relevante a medida sérica da 25-hidroxivitamina D, já que este biomarcador reflete a síntese endógena e a suplementação de vitamina D.

Deficiência de Vitamina D

O IOM recomenda que o ponto de corte para o diagnóstico da deficiência de vitamina D é abaixo de 20 ng/mL (50 nmol/L)[6] (A conversão de ng/mL para nmol/L dá-se através da multiplicação pelo valor 2,5; a unidade ng/mL será adotada neste artigo). Na literatura atual, incluindo a última recomendação da *Endocrine Society,* encontramos também valores para a definição da insuficiência de vitamina D, com valores de 20 a 30 ng/mL[2,3], embora o IOM ressalte que pontos de corte mais elevados do que o apropriado podem resultar em aumento artificial da prevalência da hipovitaminose D. Não há evidência atual demonstrando benefício no rastreamento populacional para a deficiência de vitamina D, e a recomendação vigente é a realização da medida da 25-hidroxivitamina D em indivíduos em risco[3]. Desta forma, o rastreamento é indicado para indivíduos com raquitismo, osteomalácia, osteopenia ou osteoporose, doença renal crônica, insuficiência hepática, má-absorção, obesos, gestantes e lactantes, e usuários de anticonvulsivantes, antirretrovirais e glicocorticoide[3].

A deficiência de vitamina D vem sendo reconhecida atualmente como uma epidemia mundial, incluindo crianças, adultos e idosos[2,7]. No Brasil, a alta prevalência da hipovitaminose D assemelha-se à mundial; entretanto, percebe-se diferença regional devido à ampla extensão geográfica. Em Belo Horizonte, 132 pacientes em acompanhamento ambulatorial foram avaliados, sendo que 0,8% foram considerados com deficiência e 42,4% com insuficiência de vitamina D, com pontos de corte de 14 e 32 ng/mL, respectivamente[8]. Em mulheres na pós-menopausa, 43,7% apresentam nível sérico de vitamina D abaixo de 20 ng/mL em Recife[9], e 27,1% no Rio de Janeiro, embora, neste último estudo, as participantes já tivessem diagnóstico prévio de osteopenia ou osteoporose[10]. Em São Paulo, estudo com idosos apresentou valores de vitamina D abaixo de 20 ng/mL em 71,2% dos participantes institucionalizados e 43,8% dos ambulatoriais[11], enquanto estudo com participantes saudáveis demonstrou prevalência de 77,4% de hipovitaminose D definida por valor inferior a 30 ng/mL[12]. Em Porto Alegre, no Rio Grande do Sul, latitude 30º sul, estudo em idosos institucionalizados, durante a primavera, demonstrou alta prevalência de hipovitaminose D já que 85,7% dos participantes apresentaram valores inferiores a 20 ng/mL[13]. Também nesta cidade, 89 médicos residentes com idade média de 26 anos foram avaliados e 57,4% apresentaram vitamina D abaixo de 20 ng/mL[14].

A principal causa de deficiência de vitamina D é a baixa exposição ao sol. O uso de protetor solar, o envelhecimento, a maior pigmentação da pele, e o inverno (em especial em locais abaixo e acima da latitude 33°) são condições associadas com

redução da síntese de vitamina D[2,15]. A má absorção intestinal, o aumento do catabolismo da vitamina D (por exemplo, por uso de anticonvulsivante e terapia antirretroviral), insuficiência renal, síndrome nefrótica e insuficiência hepática também são fatores associados à deficiência de vitamina D[2,16]. A obesidade, por sua vez, é relacionada com a hipovitaminose D devido à redução da biodisponibilidade desta vitamina, tanto de origem dietética ou cutânea, pela sua deposição no tecido adiposo[17].

As consequências da hipovitaminose D vêm sendo bastante estudadas. Há redução intestinal da absorção de cálcio e fósforo, e elevação do nível de PTH[8,9,14], o que resulta em redução da densidade óssea[18]. Em adultos, esta situação pode resultar em osteomalácia, osteopenia, osteoporose e aumento do risco de fraturas ósseas; e em crianças, em raquitismo[2]. Além do dano ósseo, tem sido demonstrado associação da deficiência de vitamina D com outras comorbidades[2,5] como neoplasias[19], doenças autoimune – incluindo diabetes mellitus tipo 1 (DM1)[20], infecções[21], transtorno afetivo[22], resistência insulínica[23] e diabetes mellitus tipo 2 (DM2)[24-26], doença cardiovascular[27] e morte[28].

Vitamina D e Gestação

Importância e prevalência da deficiência de vitamina D

Durante a gestação, há necessidade de mobilização de cálcio materno para o desenvolvimento do esqueleto fetal. Desta forma, ocorrem diversas adaptações fisiológicas, como o aumento materno da mobilização óssea e da absorção intestinal de cálcio, ao menos em parte mediado pela 1,25 dihidroxivitamina D. O nível de calcitriol total aumenta no primeiro trimestre, e o de calcitriol livre, no terceiro trimestre, e retornam ao normal no puerpério e lactação. A síntese renal parece ser a mais importante, estimulada pela prolactina e lactogênio placentário, embora a placenta, a decídua e os rins do feto também expressem 1α-hidroxilase[29]. É importante ressaltar que a vitamina D circula quase inteiramente ligada a proteínas séricas e, na gestação, a proteína ligadora de vitamina D encontra-se aumentada. Entretanto, apesar deste aumento da proteína ligadora, os níveis do calcitriol livre elevam-se na gestação[30]. Desta forma, mulheres com deficiência de vitamina D podem encontrar dificuldades em manter o metabolismo mineral ideal durante a gestação sem comprometer a sua massa óssea ou a do feto.

Esta preocupação vem tomando maiores proporções nos últimos anos, já que a prevalência de hipovitaminose D na gestação é bastante elevada e as consequências para a saúde materno-fetal vêm mostrando-se graves. Diversos autores[15] e entidades

médicas como o *National Institute for Health and Clinical Excellence*[31] recomendam mais estudos sobre a deficiência de vitamina D na gestação.

Em Londres, estudo recente em gestantes de diversas etnias[32], a deficiência (definida por valores < 10 ng/mL) e a insuficiência de vitamina D (entre 10 e 30 ng/mL) atingiram prevalência de 36% e 45%, respectivamente, ou seja, apenas 18% das mulheres apresentaram níveis adequados desta vitamina. Mulheres com cor da pele escura apresentaram quadro ainda mais preocupante, já que apenas 8% possuíam níveis considerados adequados. A obesidade e os meses de inverno também foram associados a maior prevalência de hipovitaminose. Outra avaliação britânica, apenas com mulheres caucasianas, demonstrou que aproximadamente metade delas apresentava valor < 20 ng/mL[33]. Em Detroit, 71,7% das gestantes apresentaram diagnóstico de deficiência (< 20 ng/mL) e 20,8% de insuficiência de vitamina D (entre 20 e 30 ng/mL)[16], na Bélgica, 44,6% e 29,5%, respectivamente[34], e, em Montreal, 39% foram consideradas deficientes[35]. No projeto perinatal colaborativo, que engloba 12 centros norte-americanos, mais de 2 mil gestações foram avaliadas com dosagem de 25-hidroxivitamina D, sendo que 55,9% das gestantes apresentaram valores abaixo de 20 ng/mL[36]. Em nosso meio, não há avaliação publicada da prevalência da deficiência de vitamina em gestantes, porém o estudo de Premaor et al, o qual encontrou 57,4% dos participantes com valores de vitamina D abaixo de 20 ng/mL, avaliou indivíduos em idade fértil (média de 26 anos), sendo 49,3% do sexo feminino[14].

Repercussões para o feto

Como descrito acima, o feto depende do suprimento materno de 25-hidroxivitamina D e cálcio e, portanto, a alta taxa de deficiência de vitamina D na gestante também repercute na prole (Tabela 1) [15,37]. A concentração plasmática de vitamina D no recém-nascido (RN) é cerca de 60 a 70% do nível materno[38], mas outros estudos reportam valores ainda mais baixos[39]. Em estudo canadense, a deficiência de vitamina D em RNs de mães que repunham vitamina D durante a gestação alcançou 46% de prevalência (definida por vitamina D< 11 ng/mL no cordão), com variação de acordo com a estação o ano e a cor da pele[40], enquanto em estudo irlandês apenas 8% dos RN apresentavam vitamina D acima de 20 ng/mL[37].

A incidência de RN pequeno para idade gestacional (PIG) está relacionada ao nível de vitamina D em estudos observacionais. Em coorte prospectiva, a vitamina D no segundo trimestre da gestação abaixo de 10 ng/mL foi associada a risco três vezes maior de RN PIG, embora não se tenha encontrado associação contínua significativa entre vitamina D e peso ao nascer para idade gestacional[41]. Em outra coorte multiétnica, com mais de 3 mil gestações avaliadas, a deficiência da vitamina D,

avaliada com 13 semanas de idade gestacional, foi associada com RN de menor peso, e maior risco de ocorrência de PIG[42]. Em estudo caso-controle americano, com população residente em latitude 40ºN, a razão de chances para RN PIG foi de 7,5 em gestações com nível de 25-hidroxivitamina D < 15 ng/mL avaliada antes de 22 semanas de idade gestacional, e de 2,1 em gestações com nível >30 ng/mL, em comparação com gestações com nível entre 15 e 30 ng/mL, mostrando uma relação em "U" em gestantes brancas. Neste estudo, não houve tal associação em gestantes negras[43]. O consumo de leite durante a gestação, fonte de vitamina D (leite com suplemento de vitamina D), cálcio e proteínas, também foi associado ao peso ao nascer, sendo que cada um micrograma adicional de vitamina D na dieta resultou em aumento de 11 g no peso do RN, independentemente de outros fatores avaliados, embora a vitamina D sérica não tenha sido medida[44]. Em gestação complicada por pré-eclâmpsia grave, a vitamina D também foi associada com o percentil de crescimento ao nascer e incidência de RN PIG[45]. No início de 2013, em análise de mais de 2 mil nascimentos norte-americanos, a 25-hidroxivitamina D > 15 ng/mL antes de 26 semanas de idade gestacional foi associada a maior peso ao nascer e circunferência craniana, e metade do risco de RN PIG[36]. Por outro lado, outros estudos observacionais não demonstraram associação entre deficiência de vitamina D e peso ou comprimento ao nascer [33,46]. Meta-análise de estudos observacionais publicada em 2013 reforça a associação entre deficiência de vitamina D e risco para RN PIG[47].

É sabido que o baixo peso ao nascer é associado com aumento do risco de doença cardiovascular e DM2 no adulto[48]. Entretanto, apesar da provável associação de hipovitaminose D durante a gestação e a restrição de crescimento intrauterino, suas consequências na prole, em longo prazo, não foram avaliadas adequadamente. Aos 9 anos de idade, apenas 30% dos filhos de mães avaliadas para hipovitaminose D durante a gestação retornaram para avaliação, na coorte britânica de maior tempo de acompanhamento até este momento[33]. Do ponto de vista cardiovascular, foram avaliadas a pressão arterial, a estrutura cardíaca através de ecocardiograma e a espessura da íntima da carótida e estas não foram associadas ao nível sérico de vitamina D na gestação. Também não houve diferença antropométrica significativa[33].

O crescimento longitudinal e a mineralização óssea na prole também podem ser comprometidos pela deficiência de vitamina D na gestação[37,46,49,50]. Durante o período fetal, a vitamina D parece estar associada com o crescimento femoral[37,49], e, ao nascer, os neonatos de mulheres com vitamina D mais baixa apresentaram menor comprimento de ossos longos[46]. Já a massa óssea, avaliada por densitometria após o nascimento, é menor em RN com deficiência de vitamina D[50]. Aos 9 anos de idade, a menor massa óssea corporal total e de coluna lombar das crianças também foi

associada a concentrações mais baixas de vitamina D materna durante o final da gestação[51].

A associação da deficiência de vitamina D com prematuridade ainda é um ponto controverso. Embora um estudo japonês tenha reportado valores mais baixos de vitamina D em mulheres com parto prematuro[52], outros estudos longitudinais não corroboram esta associação[53,54]. De forma semelhante, a idade gestacional ao nascer é semelhante entre os grupos com e sem deficiência de vitamina D em algumas coortes[55], enquanto outras apresentam menor idade gestacional no grupo deficiente[42,46]. Entretanto, esta diferença de idade gestacional pode ser muito pequena quanto 0,2 semanas[42] ou perder significância estatística após ajustes[46].

As doenças pulmonares na prole também vêm sendo estudadas, em especial as complicações infecciosas e a sibilância. O risco de infecção do trato respiratório inferior pelo vírus sincicial respiratório, causa frequente de bronquiolite e pneumonia em crianças com menos de um ano de idade, é associado à deficiência de vitamina D no cordão umbilical em RN saudáveis e a termo acompanhados por um ano[56]. Camargo et al realizou seguimento de 922 RN por 3 meses, e, de forma semelhante, encontrou associação inversa entre o nível de vitamina D e o risco de infecções respiratórias em geral[57]. Também o risco de sibilância recorrente parece ser menor em crianças cujas mães ingeriram maior quantidade de vitamina D durante a gestação[58] e quando o nível de vitamina D no cordão foi mais elevado[57]. Entretanto, a associação com asma ainda permanece incerta, já que uma coorte de crianças avaliadas com 9 anos demonstrou aumento do risco (RC de 5,4; IC95%1,09-26,65) quando a vitamina D materna foi > 30 ng/mL[33], enquanto outro estudo não encontrou relação com o risco de asma aos 5 anos[57].

O risco de outras manifestações atópicas, como o eczema[33] e a alergia alimentar[59], podem apresentar associação positiva com a vitamina D materna. Por outro lado, 231 RN avaliados no primeiro ano de vida apresentaram maior risco de eczema quando a vitamina D no cordão foi < 20 ng/mL[60]. Assim, a relação entre vitamina D e a resposta imunológica ainda permanece contraditória e pouco explorada.

Evidências contraditórias ainda envolvem o risco de DM1 na prole. Estudo caso-controle aninhado em coorte recente, encontrou um risco duas vezes maior para o desenvolvimento de DM1 na prole de mães com vitamina D na gestação no quartil inferior[61]. Por outro lado, estudo finlandês não encontrou diferença nos níveis de vitamina D materna cujos filhos desenvolveram ou não DM1[62].

Em coorte espanhola, a vitamina D materna foi relacionada positivamente com os escores mental e psicomotor da prole avaliados em torno dos 14 meses de idade[63].

Revisão sistemática relata associação entre o mês de nascimento e o risco de desenvolvimento de esclerose múltipla, especialmente em área de baixa exposição à luz solar, e o que poderia sugerir uma possível influência da vitamina D durante a gestação[64]. A ingestão de leite e vitamina D materna também pode estar associada à redução do risco da prole em desenvolver esclerose múltipla[65]. Entretanto, estudo caso-controle não encontrou associação entre a vitamina D materna durante a gestação e o risco de esclerose na prole[66]. Mais estudos são necessários para estabelecer a relação entre a vitamina D na gestação e o desenvolvimento neuropsicomotor e as doenças neurológicas na prole.

Repercussões para a gestante

Uma das complicações maternas mais relacionadas com a deficiência da vitamina D durante a gestação é a pré-eclâmpsia (Tabela 1). Muitos estudos observacionais demonstram aumento do risco para pré-eclâmpsia em mulheres com valores mais baixos de vitamina D[35,67-69]. Entretanto, outros estudos não comprovam esta associação[54,70-72], especialmente em subgrupos de risco mais elevado[70,72]. Meta-análise de estudos observacionais publicada em 2013 aponta para uma relação entre a hipovitaminose D e a incidência de pré-eclâmpsia, porém análise estratificada para estudos com ajustes estatísticos para possíveis fatores de confusão tornam esta associação não significativa[47].

A deficiência de vitamina D também vem sendo consistentemente relacionada à maior incidência de vaginose bacteriana durante a gestação[73,74], e esta associação foi recentemente confirmada por revisão sistemática[47].

O aumento da taxa de cesariana em mulheres com hipovitaminose D é reportado por alguns autores[75,76], porém outros estudos apontam para uma independência deste desfecho em relação à vitamina D materna[54].

Na coorte de Amsterdam, a deficiência de vitamina D materna avaliada no início da gestação foi associada com níveis mais altos de sintomas depressivos em questionário aplicado com 16 semanas de idade gestacional[77]. Entretanto, uma relação causal não pode ser estabelecida, já que a medida da vitamina D e a aplicação do questionário foram realizadas temporalmente muito próximas[77]. Em africanas americanas, a vitamina D sérica no início da gestação também apresentou relação inversa com escala de depressão aplicada no segundo trimestre[78].

O papel da vitamina D no desenvolvimento do DM2 e do DG também vem sendo estudado e a deficiência desta vitamina parece estar associada com a alteração da homeostase glicêmica na gestação[47,79], que será discutida abaixo.

Tabela 1. Possíveis repercussões materno-fetais da deficiência de vitamina D na gestação*

Repercussões Fetais	Repercussões Maternas
Evidências consistentes	
RN pequeno para IG	Diabetes Gestacional
Desenvolvimento e mineralização óssea	Vaginose bacteriana
Infecção respiratória	
Sibilância	
Evidências iniciais ou contraditórias	
DM 1	Pré-eclâmpsia
Prematuridade	Aumento da taxa de cesariana
Esclerose múltipla	Sintomas depressivos
Piora do desenvolvimento neuropsicomotor	

RN: Recém-nascido; IG: idade Gestacional; DM: Diabetes Mellitus
*O estudo das consequências da deficiência de vitamina D para a gestante e para o feto ainda está em andamento

Suplementação de vitamina D na gestação

Os ensaios clínicos sobre reposição de vitamina D durante a gestação também são heterogêneos e os resultados sobre a redução de desfechos materno-fetais ainda são incipientes. Em 1980, a reposição de 1000 unidades ao dia de ergocalciferol em mulheres asiáticas, durante o terceiro trimestre, resultou em redução não significativa da taxa de RN PIG de 29 para 15% e os RNs do grupo controle apresentaram fontanelas maiores, sugerindo prejuízo da ossificação [39]. Não houve diferença na idade gestacional ao nascer entre os grupos[39]. No ensaio clínico de Hollis et al[80], 350 mulheres foram randomizadas para receber 400, 2000 ou 4000 unidades de vitamina D ao dia e foram seguiram no estudo até o parto. A dose de 4000 unidades foi a mais efetiva em atingir níveis considerados suficientes de vitamina D para a gestante. Entretanto, não houve diferença na idade gestacional ao nascer, peso do RN ou necessidade de internação em unidade de tratamento intensivo neonatal[80]. Análise publicada em 2012 pela *Cochrane Collaboration* descreve menor incidência de RN com peso abaixo de 2500 g nas gestações com suplementação de vitamina D, porém com significância limítrofe (risco relativo 0,48; 95% IC 0,23-1,01)[81]. Entretanto, não foi detectada redução da taxa de pré-eclâmpsia, síndrome nefrítica, natimorto ou morte neonatal[81]. Novos ensaios clínicos foram publicados em 2013. Em um deles, a reposição de vitamina D nas doses de 2000 e 4000 unidades versus 400 unidades ao dia novamente não resultou em diferença na antropometria fetal ou idade gestacional ao nascer, porém o objetivo principal do estudo era a avaliação do nível sérico de 25(OH)D na mãe e no cordão umbilical[82]. Já no estudo de Wagner et al, onde 257 gestantes foram randomizadas para receber 2000 ou 4000 unidades de vitamina D por dia, foi encontrada associação entre o percentil de peso neonatal e a dose de vitamina

D ingerida. Além disso, este estudo sugere associação negativa entre o valor da 25(OH)D final e parto prematuro e infecção[83]. Em análise combinada de dois ensaios clínicos recentes, com reposição de vitamina D de 400, 2000 e 4000 unidades ao dia, a concentração final de 25-hidroxivitamina D foi associada à redução de desfechos adversos combinados –DG, doença hipertensiva da gestação, infecção, vaginose bacteriana e parto prematuro[84]. É importante ressaltar que nos estudos citados acima, as doses elevadas de vitamina D foram consideradas seguras durante a gestação, e mais eficazes em atingir níveis adequados de 25(OH)D[80,82,83]. Desta forma, os autores consideram as recomendações tradicionais de suplementação de vitamina D na gestação insuficientes[83]. Outros dois estudos utilizaram dose semanal de vitamina D de 35000[85] e 50000 unidades/semana[86], as quais se mostraram capazes de elevar o nível de vitamina D materna e no cordão ou no RN, sem efeitos adversos. Desta forma, estudos adicionais são ainda necessários para definir a dose ideal de suplementação de vitamina D na gestação e, principalmente, seu real benefício na redução da incidência de desfechos adversos para a mãe e o neonato.

Diabetes Gestacional

O DG é definido pela hiperglicemia detectada pela primeira vez na gestação, excluindo-se o diabetes não diagnosticado anteriormente. Sua prevalência é crescente, em parte pelo aumento da prevalência da obesidade entre mulheres jovens e também pela mudança recente do critério diagnóstico[87,88].

O diagnóstico do DG diverge entre as diferentes entidades internacionais. A *International Association of Diabetes and Pregancy Study Groups*[88] propôs, em 2010, modificação do critério diagnostico após análise dos dados do estudo HAPO[89]. Esta proposta inclui o rastreamento para diabetes pré-gestacional na primeira consulta pré-natal através da glicemia de jejum. Neste momento, pode-se diagnosticar diabetes (glicemia ≥ 126 mg/dL) ou DG (92 - 125 mg/dL). Nas gestantes normoglicêmicas (< 92 mg/dL), realiza-se o teste de tolerância oral a glicose 75 g (TTOG 75 g) entre 24 e 28 semanas de idade gestacional, e um ponto alterado é suficiente para o diagnóstico de DG (glicemia de jejum ≥ 92 mg/dL, 1h ≥180 mg/dL ou 2h ≥ 153 mg/dL). Este rastreamento é universal, ou seja, deve ser realizado em todas as mulheres, independentemente do risco. A *American Diabetes Association*[87] endossou este novo critério diagnóstico enquanto o *American College of Obstetricians and Gynecologists*[90] mantém sua proposta diagnóstica antiga e a Organização Mundial de Saúde ainda não publicou seu posicionamento.

O aumento do risco de desfechos adversos materno-fetais na gestação com diabetes é bem definido, e este risco possui associação contínua com os níveis de

glicose materna (Tabela 2)[89]. Para a gestante, o DG associa-se ao aumento de complicações como pré-eclâmpsia, elevação das taxas de cesariana e de permanência do diabetes após o parto. Para o feto, macrossomia fetal, distócia de ombro, prematuridade e hipoglicemia neonatal estão associados à hiperglicemia materna[89,91]. Em longo prazo, o DG também acarreta consequências para a prole. Crianças e adolescentes expostos ao DG materno apresentam maior índice de massa corporal, gordura subcutânea, circunferência abdominal[92], resistência insulínica, maior risco de DM2/intolerância aos carboidratos[93,94], e maior prevalência dos componentes da síndrome metabólica[95]. Além disso, a obesidade materna, comorbidade frequentemente associada ao DG, também está associada à obesidade e à síndrome metabólica na prole[95].

O tratamento da hiperglicemia no DG reduz a morbidade perinatal[91,96,97], porém outros fatores de risco para desfechos adversos materno-fetais potencialmente modificáveis devem ser identificados e manejados adequadamente.

Tabela 2. Desfechos materno-fetais associados ao diabetes gestacional

Desfechos Fetais	**Desfechos Maternos**
Em curto prazo	
Macrossomia e RN grande para IG	Pré-eclâmpsia
Distócia de ombro	Cesariana
Hipoglicemia neonatal	
Hiperinsulinismo fetal	
Prematuridade	
Internação em UTI neonatal	
Em longo prazo	
Aumento do IMC e circunferência abdominal	Diabetes Mellitus tipo 2
Aumento de gordura corporal	
Resistência insulínica	
Intolerância aos carboidratos e DM 2	
Síndrome Metabólica	

RN: Recém-nascido; IG: Idade Gestacional; UTI: Unidade de Tratamento Intensivo; IMC: Índice de Massa Corporal; DM: Diabetes Mellitus

Diabetes Gestacional e deficiência de vitamina D

Os fatores de risco para o DG são obesidade, história prévia de DG ou filho macrossômico, síndrome dos ovários policísticos e história familiar de DM2[98]. Entretanto, mais recentemente, o papel da deficiência de vitamina D no desenvolvimento do DM2 e do DG vem sendo estudado. O risco de DM2 é mais elevado em pacientes com hipovitaminose D[24-26] e os mecanismos envolvidos são a resistência insulínica e a disfunção da célula beta[23,99,100]. Polimorfismos dos genes do

receptor da vitamina D e de sua proteína ligadora, função imune, inflamação, nível sérico de cálcio e PTH, e a obesidade também parecem estar envolvidos na relação entre a vitamina D e a resistência insulínica[99]. De forma semelhante, a deficiência de vitamina D está associada com a alteração da homeostase glicêmica na gestação e DG[79]. Quando avaliada no segundo trimestre da gestação, a hipovitaminose D está associada ao aumento da ocorrência de DG, e esta associação parece ser independente da idade, raça ou peso materno[55,101,102]. Em coorte de gestantes com vitamina D avaliada antes de 16 semanas de idade gestacional, o aumento da vitamina D foi associado à redução do risco de hiperglicemia materna avaliada entre 24 e 28 semanas de idade gestacional apenas em mulheres tabagistas[103]. Esta associação entre hipovitaminose D e aumento do risco de DG foi recentemente confirmada em meta-análise[47]. Assim, a vitamina D pode ser um fator de risco para DG potencialmente modificável.

A prevalência da hipovitamose D é bastante elevada em mulheres com DG, sendo 84% no Canadá (definida por vitamina D < 29,4 ng/mL)[101], e 83,3% no Iran (<20 ng/mL)[104]. No Rio Grande do Sul, local de alta prevalência de hipovitaminose D, dados ainda não publicados apontam para deficiência de vitamina D (<20 ng/mL) em 53,3% e insuficiência (20 a 30 ng/mL) em 33,2% das gestantes[105].

A potencial contribuição da deficiência de vitamina D para a elevada taxa de complicações materno-fetais na gestação com DG ainda não foi estudada e merece atenção das entidades médicas. De forma semelhante, a suplementação de vitamina D nesta população ainda não foi avaliada e não há ensaio clínico específico para este grupo de mulheres de alto risco.

Conclusão

A deficiência de vitamina D é uma situação de alta prevalência mundial, na população geral e em gestantes. As consequências desta hipovitaminose em longo prazo ainda não estão completamente esclarecidas e, na gestação, os estudos apontam para aumento da incidência de desfechos adversos maternos e fetais. Entretanto, os resultados dos estudos observacionais na gestação ainda apresentam resultados contraditórios, em parte por população em estudo heterogênea, amostra pequena, diferentes grupos controle, diferentes métodos de aferir a vitamina D, diferentes pontos de corte para o diagnóstico de sua deficiência e controle incompleto para fatores de confusão.

Apesar da prevalência alarmante e das potenciais consequências da deficiência de vitamina D na gestação, sua avaliação e suplementação na gestação ainda não são realizadas rotineiramente no Brasil. Por outro lado, a maioria dos

especialistas e entidades internacionais concordam sobre a necessidade de rastreamento da deficiência de vitamina D e de suplementação durante a gestação, embora ainda não exista concordância sobre a dose ideal e sobre o real benefício para os desfechos materno-fetais. Mais estudos são necessários para esclarecer a associação entre a deficiência de vitamina D e desfechos adversos materno-fetais, em especial em populações de alto risco como mulheres com diabetes e hipertensão, e para definir a melhor maneira de realizar a suplementação desta vitamina durante a gestação.

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Prevalence of vitamin D deficiency and maternal outcomes in a cohort of women with gestational diabetes

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Funding: Fundo de Incentivo à Pesquisa e Eventos, Hospital de Clínicas de Porto Alegre; Universidade Federal do Rio Grande do Sul, CNPq

Conflict of interest: None

Abstract

Introduction: Vitamin D deficiency is highly prevalent in pregnant women and has been associated with elevated risk of adverse maternal and fetal outcomes. As hypovitaminosis D is reported to increase the incidence of gestational diabetes (GD), the association of both conditions may amplify the risk of related complications.

Objective: To evaluate the maternal consequences of vitamin D deficiency in pregnancies complicated by GD.

Methods: A cohort of 184 pregnant women with GD referred to specialized prenatal monitoring was followed throughout pregnancy. Women were classified according to the presence of vitamin D deficiency (defined as < 20ng/mL) and adverse maternal outcomes were evaluated. Associations with outcomes were analyzed with Poisson regression with robust standard errors and multiple linear regression.

Results: 98 women (53.3%) were diagnosed with vitamin D deficiency. Dark skin tone and overweight or obesity were more frequent in vitamin D deficient women (P=0.048 and P=0.037, respectively). There was no difference between groups in the incidence of hypertensive disorders of pregnancy, cesarean delivery, postpartum diabetes, insulin resistance, requirement of drug treatment for GD and infection. However, in white women, serum vitamin D presented a significantly negative correlation with the systolic blood pressure at beginning (P=0.002) and end (P=0.018) of the third trimester and vitamin D significantly affected systolic blood pressure after adjustment for confounders.

Conclusions: In this cohort of pregnant women with GD, vitamin D deficiency did not affect the incidence of hypertensive disorders of pregnancy or any other adverse outcome. However, in white women, vitamin D was an independent predictor of systolic blood pressure during pregnancy.

Keywords: vitamin D, vitamin D deficiency, hypovitaminosis D, gestational diabetes, gestational hypertension, preeclampsia

Introduction

The prevalence of vitamin D deficiency is very high in pregnant women of several populations[1-3] and it has been reported to increase the risk of adverse maternal and fetal outcomes, such as gestational diabetes (GD)[4-6].

Low maternal vitamin D levels have been associated with complications for the mother[6,7], such as preeclampsia[8-11], bacterial vaginosis[12], periodontal disease[13], shorter duration of gestation[14], high rate of cesarean section[15], depressive symptoms in pregnancy[16] and GD[4-6], although some data are controversial. Likewise, GD is associated with increased rates of adverse maternal outcomes such as primary cesarean section, delivery before 37 weeks and preeclampsia[17]. However, the consequences of vitamin D deficiency in women with GD have not been evaluated.

Preeclampsia is the leading cause of maternal morbidity and mortality and its complete pathogenesis is not entirely elucidated[18]. Traditional risk factors are nulliparity or multiple pregnancy, previous preeclampsia, age of 40 years or older, obesity, family history, hypertension, renal disease, diabetes and antiphospholipid antibodies[18]. Some case-control and longitudinal studies have suggested that maternal vitamin D deficiency is an independent risk factor for preeclampsia[8-11], but other authors disagree[19-22]. This issue remains controversial, especially regarding the subgroup of high-risk women.

Therefore, the objective of this study was to evaluate the maternal consequences of vitamin D deficiency, mainly hypertensive disorders of pregnancy (HDP), in women with GD.

Methods

Study population and research design

The study was conducted at Hospital de Clínicas de Porto Alegre, Brazil, by the diabetes and pregnancy specialized multidisciplinary team of assistance and research. Pregnant women with hyperglycemia are systematically referred to high risk prenatal monitoring. All pregnant women with a diagnosis of GD evaluated between November 2009 and May 2012 were invited to participate and the cohort of women included in the study was prospectively followed until the puerperium.

Socio-demographic characteristics, medical history and pregnancy data were evaluated. Physical examination was performed in all prenatal visits. Fasting maternal

blood was sampled at the third trimester, since the diagnosis of GD is usually made after the 24th week of gestation. The serum was prepared by centrifugation and stored at -80°C until delivery of all pregnancies. At this moment, serum 25-hydroxyvitamin D (25(OH)D) was measured and women were classified according to the presence of vitamin D deficiency (risk group) or not (control). Season of maternal blood collection was categorized as winter (June 21 – September 22), spring (September 23 – December 20), summer (December 21 – March 20), and autumn (March 21 – June 20). Blood was sampled at all seasons. Porto Alegre city is at latitude 30°S.

Until the end of 2010, the diagnosis of GDs at our institution followed the recommendations of the 2nd Meeting of The Diabetes and Pregnancy Task Force[23]: after a positive screening (fasting plasma glucose ≥ 85 mg/dL), a 75 g oral glucose tolerance test (75 g OGTT) was performed and GD was diagnosed if fasting plasma glucose ≥ 110 mg/dL or 2h plasma glucose ≥ 140 mg/dL. After the publication of the International Association of Diabetes and Pregnancy Study Groups[24] proposal for the diagnosis of GD and its adoption by the American Diabetes Association (ADA), we also included in the study women who had fasting plasma glucose ≥ 92 mg/dL or 1h plasma glucose ≥ 180 mg/dL or 2h plasma glucose ≥ 153 mg/dL after the 75 g OGTT.

All participants provided informed written consent and the study was approved by the Ethical Committee of our hospital.

Covariates

Several variables were evaluated at the maternal interview: maternal age, self-reported skin color (white or dark skin tone), marital status, work outside home, socioeconomic status (Brazilian classification[25]), years of study, parity, self-reported prepregnancy weight, active smoking at registration, chronic diseases, regular use of medicines and supplements, previous history of a child with macrosomia, of GD, and of gestational hypertension or preeclampsia/eclampsia. Height was measured at first prenatal visit and body mass index (BMI) was calculated as pregestacional weight/height² (kg/m²). Blood pressure was measured at all prenatal visits according to World Health Organization recommendations[26]. Mean blood pressure was calculated as (systolic blood pressure + diastolic blood pressure x 2) / 3. Data about maternal weight gain, delivery route and medical emergencies were extracted from medical records. HbA1c, urinary albumin excretion (UAE) in 24 hours, lipid profile, fasting insulin and C-peptide were measured at the third trimester in addition to the routine laboratory prenatal evaluation. HbA1c was also measured at the end of the follow-up.

Insulin and C-peptide were measured only in women who were not in treatment with insulin at the moment of blood sampling.

Outcomes

The primary outcome was the occurrence of gestational hypertension (defined as systolic blood pressure ≥ 140 or diastolic ≥ 90 mmHg on at least two occasions, after 20 weeks of gestation[26]), preeclampsia or preeclampsia superimposed to chronic hypertension (defined as proteinuria of 300 mg/L or more in two random urine specimens in patients with gestational hypertension or in patients with chronic hypertension, respectively[26]), and eclampsia (defined as tonic-clonic seizures in the hypertensive women). HDP was a composite outcome defined as gestational hypertension, preeclampsia, preeclampsia superimposed on chronic hypertension and eclampsia.

Secondary outcomes were gestational age at delivery, pregnancy weight gain, HbA1c, requirement of drug treatment for GD (insulin or oral medication), insulin resistance, rate of cesarean delivery, of postpartum prediabetes (impaired fasting glucose or impaired glucose tolerance) or diabetes, and of infection (urinary tract, pulmonary, and puerperal infections). Homeostasis model assessment (HOMA-IR) >4.31 was considered as insulin resistance (the population specific 75th percentile of HOMA-IR).

Laboratory

Glucose, cholesterol and triglyceride were measured by enzymatic colorimetric assays, UAE by imunoturbidimetry (Advia 1800, Siemens Healthcare, Erlangen, Germany), HbA1c by high performance liquid chromatography (Variant II, BioRad Laboratories, Hercules, CA), and fasting insulin and C-peptide by chemiluminescence (Advia Centaur XP, Siemens Healthcare, Erlangen, Germany). Insulin resistance was calculated by HOMA-IR with the equation (glucose in mg/dL x 0,0555 x insulin / 22.5)[27].

Serum 25(OH)D was measured by the chemiluminescent immunoassay (DiaSorin LIAISON®, MN, USA). This assay measures between 4.0 and 150 ng/mL and the inter-assay precision had a coefficient of variation of < 4% and < 6% for 15 and 50 ng/mL values, respectively. Vitamin D deficiency was defined as 25(OH)D below 20 ng/mL, in agreement with current recommendations of the Institute of Medicine[28] and the Endocrine Society[29]. Levels between 20 and 30 ng/mL were considered insufficient[29].

Statistical analysis

Descriptive statistics were used to present clinical and demographic variables in each vitamin D deficiency category (below or above 20 ng/mL). Number, percentage, means ± standard deviation, median and interquartile range were used. Analysis was also performed between ethnical subgroups.

Differences in the distribution of categorical variables were analyzed with chi-square test. Continuous variables were evaluated with T-test when parametric and with Mann-Whitney test when non-parametric. A general mixed model (Poisson regression with robust standard errors[30]) was used to study the association of HDP with serum vitamin D. Confounding factors were considered not to influence the main result and were removed from the model if their inclusion did not change relative risk (RR) in at least 5%. Correlations between blood pressure and serum vitamin D were evaluated with Pearson´s test and multiple linear regression was used to study the predictors of blood pressure levels. Models for adjustment took into consideration potential biological confounders as maternal glucose at OGTT and HbA1c, maternal BMI, maternal age, blood pressure, ethnicity, previous HDP, nulliparity, medication requirement for diabetes treatment and season.

Statistical analysis was performed in SPSS version 20.0. Statistical significance was considered when $P<0.05$.

Results

A hundred and eighty-four women agreed to participate and had blood sampled for storage. Clinical and socio-demographic data of the whole group of pregnant women and according to vitamin D status are shown in Table 1. Mean 25(OH)D was 19.7 ± 8.6 ng/mL for the whole group and 98 women (53.3%) had deficiency of vitamin D (Figure 1). As expected, women with vitamin D deficiency were more frequently of dark skin tone, had higher prevalence of overweight or obesity and had blood sampled in sunny seasons less frequently. Among the 48 women with dark skin tone, 24 were self-reported as black and 24 as mixed. Most women (74.5%) were married, 66.3% work outside home and 66.7% had eight or more years of study, without difference between groups. No woman was regularly taking vitamin D supplement.

Table 1. Clinical and laboratory characteristics of the whole group and according to vitamin D status

	All (N=184)	25(OH)D ≥ 20 ng/mL (N=86)	25(OH)D < 20 ng/mL (N=98)	*P*
25(OH)D (ng/mL)	19.7 ± 8.6	27.4 ± 4.8	12.9 ± 4.4	<0.001
Season* (n,%)				
Summer	58 (31.5)	47 (54.7)	11 (11.2)	<0.001
Autumn	57 (31.0)	25 (29.1)	32 (32.7)	
Winter	25 (13.6)	3 (3.5)	22 (22.4)	
Spring	44 (23.9)	11 (12.8)	33 (33.7)	
Age (years)	32.2 ± 6.0	32.0 ± 6.3	32.3 ± 5.8	0.744
White (n,%)	136 (73.9)	69 (80.2)	67 (68.4)	0.048
Socioeconomic class A or B (n,%)	45 (31.9)	17 (28.3)	28 (34.6)	0.432
Parity (n)	2 (2-4)	2 (2-4)	3 (2-4)	0.505
Nulliparity (n,%)	38 (20.7)	18 (20.9)	20 (20.4)	0.930
Previous HDP (n,%)	40 (21.7)	20 (23.3)	20 (20.4)	0.640
Chronic Hypertension (n,%)	21 (11.4)	7 (8.1)	14 (14.3)	0.191
Smoking (n,%)	21 (11.4)	9 (10.5)	12 (12.2)	0.705
Prepregnancy weight (kg)	74.0 ± 17.3	73.0 ± 17.8	74.9 ± 16.9	0.452
BMI (kg/m²)	29.7 ± 6.7	29.1 ± 6.6	30.2 ± 6.9	0.290
Overweight or obesity (n,%)	128 (69.9)	53 (62.4)	75 (76.5)	0.037
75 g OGTT (mg/dL)				
Fasting glucose	100 ± 27	97 ± 26	102 ± 27	0.215
1h glucose	177 ± 28	177 ± 32	178 ± 27	0.924
2h glucose	162 ± 26	165 ± 25	160 ± 27	0.269
First blood pressure (mmHg) ¥				
Systolic	115 ± 11	115 ± 12	116 ± 11	0.389
Diastolic	72 ± 10	72 ± 10	72 ±10	0.848
Last blood pressure (mmHg)				
Systolic	119 ± 16	118 ± 13	120 ± 19	0.595
Diastolic	77 ± 11	76 ± 11	78 ± 11	0.455
Lipids (mg/dL)				
Total cholesterol	228 ± 49	228 ± 48	228 ± 49	0.939
LDL	128 ± 44	130 ± 44	127 ± 45	0.750
HDL	58 ± 14	57 ± 14	59 ± 14	0.357
Triglyceride	208 ± 69	209 ± 67	208 ± 71	0.970
C-Peptide (ng/mL)	2.1 ± 0.9	2.1 ± 1.0	2.0 ± 0.8	0.396
Creatinine (mg/dL)	0.48 ± 0.10	0.49 ± 0.12	0.47 ± 0.10	0.304
UAE (mg/24h)	5.1 (0-11.7)	4.2 (0-11.7)	5.3 (0-11.4)	0.848

Data expressed as number (%), mean±sd, median (interquartile range)
25(OH)D: 25-hydroxyvitamin D; HDP: Hypertensive disorders of pregnancy; BMI: Body mass index; OGTT: Oral glucose tolerance test; UAE: Urinary albumin excretion
*Season of blood sample; ¥Blood pressure at beginning of the third trimester

Maternal outcomes are presented in Table 2. There was no significant difference between groups in the incidence of hypertensive disorders and of any secondary outcome. Eclampsia has happened in just one patient whose serum 25(OH)D was 32.1 ng/mL and there was no maternal death. The only significant clinical

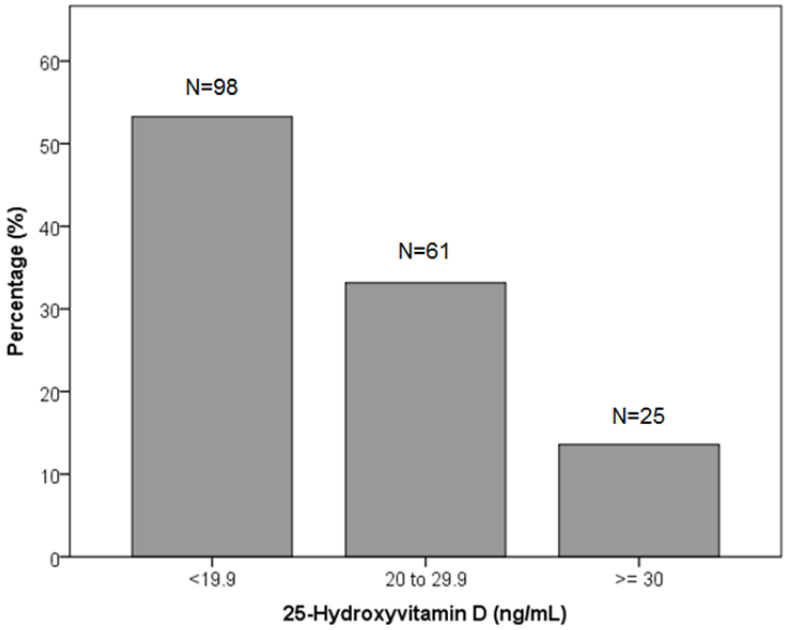

Figure 1. Prevalence of insufficiency and deficiency of vitamin D

predictor of HDP was the blood pressure at beginning (P<0.001 for both systolic and diastolic) and end of the third trimester (P=0.001 for both systolic and diastolic), after adjustment for previous HDP, season, ethnicity and HbA1c.

Table 2. Maternal outcomes and vitamin D deficiency

	25(OH)D ≥ 20 ng/mL (N=86)	25(OH)D < 20 ng/mL (N=98)	*P*
Gestational age at delivery (weeks)	38.4 (38.0-38.9)	38.6 (37.5-39)	0.552
Pregnancy weight gain (kg)	10.06 ± 7.14	9.57 ± 7.69	0.660
First HbA1c (%)	5.6 (5.1-6.0)	5.6 (5.3-6.1)	0.756
Last HbA1c (%)	5.8 (5.2-6.3)	5.9 (5.4-6.5)	0.214
Drug treatment for GD (n,%)	49 (57.0)	61 (62.2)	0.467
HOMA-IR	3.05 (2.10-4.74)	2.93 (2.20-4.30)	0.615
HOMA-IR≥4.31 (n,%)	16 (27.6)	16 (23.2)	0.570
Gestational hypertension (n,%)	14 (16.3)	12 (12.2)	0.433
Preeclampsia* or eclampsia (n,%)	10 (11.6)	6 (6.1)	0.186
HDP (n,%)	15 (17.4)	15 (15.3)	0.696
Caesarian (n,%)	48 (56.5)	53 (54.1)	0.746
Postpartum hyperglycemia[¥] (n,%)	12 (29.3)	9 (20.5)	0.346
Diabetes	4 (9.8)	1 (2.3)	0.143
Infection (n,%)	19 (22.4)	26 (26.5)	0.513

Data expressed as number (%), mean±sd, median (interquartile range)25(OH)D: 25-hydroxyvitamin D; GD: Gestational diabetes; HOMA: Homeostasis model assessment; HDP: Hypertensive disorders of pregnancy (composite outcome)

* Preeclampsia and preeclampsia superimposed to chronic hypertension; [¥] Diabetes and prediabetes

Serum 25(OH)D had a significant negative correlation with the systolic blood pressure at beginning of the third trimester (P=0.049, r= -0.146). However, multiple linear regression demonstrated no significance (P=0.077) for serum vitamin D after adjustment for BMI and previous HDP. Other blood pressure measurements had no significant correlation with serum vitamin D.

Analyzing data according to ethnic group, we found no difference between white and dark skin tone women in the incidence of gestational hypertension (12.5% vs 18.8%, P=0.285, respectively), of preeclampsia/eclampsia (8.1% vs 10.4%, P=0.632), and of the composite outcome of HDP (15.4% vs 18.8%, P=0.594). There were also no differences in clinical or laboratorial characteristics, except for the UAE which was higher in women with dark skin tone (3.9 mg/24h (0-9.7 mg/24h) vs 7.8 mg/24h (3.5-15.2 mg/24h), P=0.005). Blood pressure at all moments during prenatal monitoring was similar between white and dark skin tone women as well. On the other hand, in white women, serum 25(OH)D had significant negative correlation with the systolic and mean blood pressure at beginning and end of the third trimester (Figure 2). Serum 25(OH)D significantly affected blood pressure after adjustment for confounders in multiple linear regression (Table 3). In women with dark skin tone, vitamin D did not influence blood pressure in multiple linear regression.

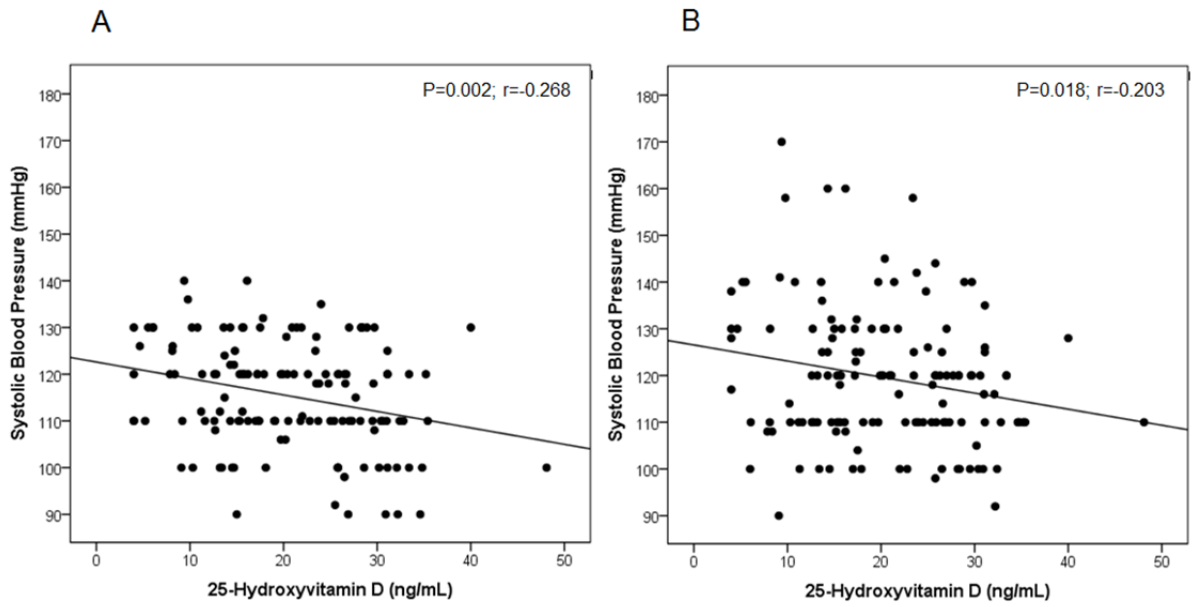

Figure 2. Correlation between 25-hydroxyvitamin D and systolic blood pressure at beginning (A) and end (B) of the third trimester in white women

Table 3. Association between serum 25(OH)D and maternal blood pressure in white women

	Pearson correlation		Multiple linear regression	
	r	P	B coefficient	P
First blood pressure (mmHg)*				
Systolic	-0.268	0.002	-0.255[¥]	0.011
Diastolic	-0.133	0.123	-0.071[¥]	0.429
Mean	-0.195	0.023	-0.132[¥]	0.118
Last Blood pressure (mmHg)				
Systolic	-0.203	0.018	-0.401[£]	0.004
Diastolic	-0.163	0.059	-0.177[€]	0.092
Mean	-0.193	0.024	-0.275[£]	0.013

* Blood pressure at beginning of third trimester
[¥]Adjusted for BMI, previous HDP and HbA1c; [£]Adjusted for BMI, previous HDP and season; [€]Adjusted for BMI and previous HDP

Only 85 (46.2%) women returned for postpartum diabetes screening. There was no difference between groups of vitamin D levels in the incidence of postpartum diabetes or prediabetes (Table 2).

Discussion

Hypovitaminosis D had a high prevalence in our cohort of pregnant women with GD and just 13.6% of them had levels considered to be sufficient. This finding, however, was not unexpected, since similar prevalence has been reported in other populations of pregnant women[1-3], and, furthermore, GD[4-6] and obesity[31] are medical conditions associated with lower levels of vitamin D. Moreover, our city is located at a latitude of low sun exposure during many months throughout the year [32], no woman was receiving vitamin D supplements and serum 25(OH)D measurement is not a routine in Brazilian prenatal care. One more point was that our sampling for vitamin D measurement was performed in the third trimester of pregnancy, and it has been reported that its levels decrease from the first to the third trimester, independently of the season[22]. On the other hand, vitamin D and its metabolites circulate almost entirely bound to albumin and to vitamin D binding protein (VDBP), and mean VDBP are reported to increase in pregnancy [33]. Therefore, these “elevated” serum levels of total vitamin D tended to underestimate the real prevalence of vitamin D deficiency in our cohort.

No association was demonstrated between vitamin D deficiency and the development of gestational hypertension or preeclampsia in our study population. In contrast, previous case-control and longitudinal studies had demonstrated that maternal vitamin D deficiency is a risk factor for preeclampsia in non-diabetic

pregnancies[8-11]. However, data are still controversial[19-22]. A recent meta-analysis of observational studies showed an association between preeclampsia and vitamin D insufficiency, but stratified analysis by adjustment of critical confounders as well as by study design led to a more conservative and non-significant association[6]. Together these suggest that the association may vary with study quality. Moreover, the visual inspection of funnel plot raised the possibility of publication bias, although it was not statistically significant[6]. In high-risk women, the association of preeclampsia and vitamin D is still less studied and it seems that there is no difference in the rates of preeclampsia according to vitamin D status[11,21]. In the study of Wey et al[11], the association between hypovitaminosis D and preeclampsia was stronger for nulliparous women without other risk factors and it was not significant in the subgroup of high-risk participants. In the single and small case-control study with type 1 diabetes women, serum vitamin D levels in the first, second or third trimesters of pregnancy were not significantly associated with development of preeclampsia[19]. In a cohort of women with clinical or biochemical risk factors for preeclampsia, there was also no difference in the rates of preeclampsia or gestational hypertension according to vitamin D concentration[21]. In this study, 12.7% of participants had a diagnosis of preeclampsia, a higher rate than ours (8.7% for the whole group). But both these rates of preeclampsia are higher than that reported by studies performed in low-risk women described above, such as the study of Bodnar et al, with a rate of 4.9%[9], and higher than the traditional reported incidence of preeclampsia of 2-8%[18]. Therefore, considering our population of diabetic women, 70% with overweight or obesity, 22% with previous HDP and 11.4% with chronic hypertension, our finding of absence of association between vitamin D deficiency and gestational hypertension or preeclampsia is an additional evidence that vitamin D deficiency is not associated with HDP in high-risk women.

We did not measure VDBP and free vitamin D, but the association of free 25(OH)D level, calculated by measurement of total 25(OH)D and VDBP, with hypertensive disorders was previously studied by Powe et al [20]. One more time, first trimester free 25(OH)D and VDPB levels were not different in women with preeclampsia and controls.

No studies of vitamin D supplementation alone had been published at the time of the Cochrane review[34] and a single study [35] of vitamin D and calcium combined supplementation reported no benefit in decreasing rates of preeclampsia although blood pressure levels were significantly lower in the supplemented group at 32 and 36 weeks. This finding is in agreement with our results. A combined analysis of two recent

trials of vitamin D supplementation with 400, 2000 and 4000 IU/day did not find significantly reduction of HDP but there was a trend of lower rates with increasing dose[36]. Analysis by final maternal 25(OH)D concentration also did not find decreasing rates of HDP in women with levels ≥ 32 ng/mL, although there was a difference in the rates of combined maternal comorbidities - HDP, infection, bacterial vaginosis, preterm birth and GD[36].

In general, studies about association between preeclampsia and vitamin D do not show data about blood pressure levels. One exception is the nested case-control study of Powe et al which did not find association of total 25(OH)D and blood pressure in the first trimester. Similar results are reported for the analysis restricted to white women[20]. In Pakistani parturients, maternal 25(OH)D was inversely correlated with mean blood pressure at the time of admission to the labor suite [37]. Therefore, association between blood pressure in pregnancy and vitamin D are scarce, but available data, including our results, suggest that 25(OH)D levels may influence blood pressure at the end of pregnancy, but not at the first trimester. More studies are necessary to clarify the association of vitamin D and blood pressure levels in pregnancy. One of the possible mechanisms for the relationship between them is that 1.25 hydroxyvitamin D has been considered a negative endocrine regulator of the renin-angiotensin system (RAS)[38]. It is known that during pregnancy occurs an overexpression of many components of the RAS, such as an increase in renin, in angiotensinogen liver and angiotensin II[39].

Many disparities exist in rates of adverse outcomes between white and black women, such as preeclampsia, preterm delivery, intrauterine or neonatal death and GD[40], and vitamin D has been considered to be one of the potential causative variables. Moreover, there are few data about black pregnant women although vitamin D deficiency is more common in women with darker skin pigmentation[40]. Our data could contribute for the understanding of the differences between ethnic groups, regarding hypertensive disorders, since we found that vitamin D is a predictor of blood pressure levels only in white women. The RAS activation in blacks appears to differ from whites since some studies suggest a greater activation of renal RAS in blacks and also a blunted suppression of intrarenal RAS activity with a high-sodium diet[41]. Therefore it could explain the different association of vitamin D and blood pressure between ethnicities during pregnancy. Moreover, women with dark skin tone in our cohort presented higher UAE, what is in agreement with the reported excessive rate of target-organ injury in blacks[41] as well with the activation of RAS.

The association of other adverse maternal outcomes with vitamin D deficiency is still controversial. Higher cesarean delivery rates have been reported in women with hypovitaminosis D by some authors[15] but it is not an universal finding[6,22]. Cesarean delivery rates were much lower in previous studies than in ours (17%[15] and 22.5%[22], vs 56% in our cohort), probably because they did not evaluated diabetic women. Lower gestational age at birth has also been associated with lower levels of vitamin D[14], while some authors suggest an inverse relationship [37]. Most importantly, there was no evidence of an increase in the incidence of preterm birth in women with hypovitaminosis D [21,22,42], except in one study of Japanese pregnancies[43]. Vitamin D deficiency is consistently associated with an increase in bacterial vaginosis rates among different studies [6,12,44], but we did not evaluate this complication. Since hyperglycemia is associated with an increase in the rates of cesarean section and preterm birth[17], our findings that vitamin D deficiency was not a risk factor for cesarean and lower gestational age at birth in women with GD are very important.

As far we know, this is the first study that analyzed the pregnancy outcomes of vitamin D deficiency in women with GD. We did not find higher insulin resistance, drug treatment rates, postpartum type 2 diabetes or worst glucose control in women with vitamin D deficiency. Maghbooli et al[45] found a correlation of 25(OH)D levels with HOMA index and a higher rate of insulin resistance in pregnant women with vitamin D deficiency, but only 7% of women had a GD diagnosis. Clifton-Bligh et al[46] also described a negative correlation of serum vitamin D with fasting glucose, insulin and HOMA, but the associations with insulin and HOMA were no longer significant after adjusting for confounders. Once more, a minority of women had GD. A recent trial showed that supplementation with 50 000 IU/day of vitamin D every two weeks improved insulin resistance in comparison with 200 IU/day in pregnant women with less than 12 weeks of pregnancy[47]. The only data available in women with GD described that lower vitamin D levels were independently associated with higher HbA1c. However, this was a retrospective study and there was no data regarding hard endpoints[48]. In non-pregnant adults, vitamin D deficiency has been associated with increased risk of type 2 diabetes[49] and insulin resistance[50]. The influence of vitamin D levels in glucose metabolism and decreased tolerance should be further evaluated in pregnant women with diagnosed hyperglycemia. The women of our cohort are currently participating of an ongoing follow up in order to evaluate the association of vitamin D deficiency during pregnancy with the incidence of type 2 diabetes in the future.

Our study has important methodological strengths. First, it evaluated serum vitamin D in a longitudinal design and blood samples were collected before the development of pregnancy outcomes. Second, we studied a homogenous high-risk population of pregnant women with GD that had not been evaluated until this moment. Third, vitamin D levels were measured by the accurate DiaSorin LIAISON assay. Fourth, our sample includes 48 women with dark skin pigmentation, enabling analysis in ethnical subgroups.

The limitations of the study were: First, serum VDBP was not measured and, therefore, our serum total 25(OH)D may overestimate the available vitamin D and underestimate our prevalence of vitamin D deficiency. Second, we considered both the Brazilian and the ADA recommendations for GD diagnosis, since there was no consensus among international associations and the best cut point is still not defined. However, analysis of data after exclusion of women whose diagnosis was made solely by the ADA criteria did not change the results.

In conclusion, the prevalence of hypovitaminosis D in pregnant women with GD is high. Vitamin D deficiency was not associated with increased rates of HDP, postpartum diabetes or other maternal adverse outcomes in women with GD. However, serum vitamin D level was an independent predictor of systolic blood pressure levels in white women.

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Vitamin D deficiency increases the risk of adverse neonatal outcomes in gestational diabetes

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Funding: Fundo de Incentivo à Pesquisa e Eventos, Hospital de Clínicas de Porto Alegre; Universidade Federal do Rio Grande do Sul, CNPq

Conflict of interest: None

Abstract

Introduction: Gestational diabetes (GD) and vitamin D deficiency have been associated with increased risk of adverse perinatal outcomes but the consequences of both conditions simultaneously present in pregnancy have not yet been evaluated.

Objective: To study the influence of vitamin D deficiency in neonatal outcomes of pregnancies with GD.

Methods: 184 pregnant women with GD referred to specialized prenatal monitoring were included in this cohort and had blood sampled for 25-hydroxyvitamin D measurement. Vitamin D was measured by chemiluminescence and deficiency was defined as < 20 ng/mL. Participants were followed until puerperium and adverse neonatal outcomes were evaluated. Associations with outcomes were analyzed with Poisson regression with robust standard errors.

Results: Newborns of women with vitamin D deficiency had higher incidences of intensive care unit hospitalization (ICU) (32 vs 19%, P=0.048), of hypoglycemia (any, 17.3 vs 7.1%, P=0.039; requiring ICU, 15.3 vs 3.6%, P=0.008), and were small for gestational age (SGA) more frequently (17.3 vs 5.9%, P=0.017). After adjustment, relative risk (RR) for hypoglycemia requiring ICU was 3.63 (95%CI 1.09-12.11) and for SGA was 4.32 (95%CI 1.75-10.66). The RR for ICU hospitalization was no longer statistically significant. The incidence of prematurity, jaundice and shoulder dystocia were higher in the group of vitamin D deficient women, but there was no significant difference.

Conclusions: In this cohort of pregnant women with GD, vitamin D deficiency was associated with an important increase in the incidence of adverse neonatal outcomes such as SGA newborns and neonatal hypoglycemia.

Keywords: vitamin D, vitamin D deficiency, gestational diabetes, small for gestational age

Introduction

Several physiologic adaptations in calcium and vitamin D metabolism are necessary during pregnancy in order to provide sufficient nutrients to the fetus and the neonate[1]. Fetuses depend entirely on the mother as a source of vitamin D and plasma levels of 25-hydroxyvitamin D (25(OH)D) in the newborn average 60 to 70% of maternal level[2]. However, pregnant women have an unacceptably high prevalence of vitamin D deficiency and insufficiency[2-4] and it has been reported to increase the risk of adverse maternal and neonatal outcomes.

The birth weight is one the most studied neonatal outcomes in general pregnancies. Studies have demonstrated that lower maternal levels of 25(OH)D result in reduction of birth weight and that vitamin D deficiency is associated with a higher risk of neonates small for gestational age (SGA)[5-9]. Reduced intrauterine long bone growth may also occur[10-12], and poor skeletal mineralization and lower bone mineral content has been detected in the newborns[13] and in the 9-year-old children[14] of vitamin D deficient mothers. Other offspring problems which have been associated with low vitamin D status in pregnancy are respiratory tract infections[15], type 1 diabetes[16], lower mental and psychomotor scores[17] and higher insulin resistance[18].

Similarly to hypovitaminosis D, gestational diabetes (GD) is associated with adverse perinatal outcomes, such as macrosomia, neonatal hypoglycemia and shoulder dystocia[19]. Moreover, long-term evaluation of the offspring of women with GD reveals an increased rate of adiposity[20], type 2 diabetes and pre-diabetes[21].

Therefore, our objective was to investigate the neonatal complications of vitamin D deficiency in the presence of GD.

Methods

Study population and research design

The diabetes and pregnancy specialized multidisciplinary team of assistance and research of Hospital de Clínicas de Porto Alegre, Brazil, assists pregnant women with hyperglycemia referred to high risk prenatal monitoring. Between November 2009 and May 2012, we invited all women with a diagnosis of GD to participate in the study. Women answered a questionnaire about socio-demographic variables, medical history and pregnancy data; physical examination was performed at all prenatal visits. Blood was sampled after 8 hours fasting in the third trimester of pregnancy since the

diagnosis of GD is usually made after the 24th week of gestation; serum was prepared by centrifugation and was stored at -80°C until analysis. This cohort was prospectively followed until the puerperium period. At this moment, 25(OH)D was measured and women were classified according to the presence of vitamin D deficiency (risk group) or not (control). All participants provided informed written consent and the study was approved by the Ethical Committee of our hospital.

Blood was sampled at all seasons. Season was categorized by winter (June 21 – September 22), spring (September 23 – December 20), summer (December 21 – March 20), and autumn (March 21 – June 20). Porto Alegre city is located at latitude 30°S.

The diagnosis of GD at our institution, until the end of 2010, followed the recommendations of the 2nd Meeting of The Diabetes and Pregnancy Task Force[22]: a 75 g oral glucose tolerance test (75 g OGTT) was performed if the screening was positive (fasting plasma glucose ≥ 85 mg/dL) and gestational diabetes was defined by the cut point of fasting plasma glucose ≥ 110 mg/dL or 2h plasma glucose ≥ 140 mg/dL. After the publication of the diagnostic proposal of the International Association of Diabetes and Pregnancy Study Groups[23] and its adoption by the American Diabetes Association (ADA), we also included in the study women who had fasting plasma glucose ≥ 92 mg/dL or 1h plasma glucose ≥ 180 mg/dL or 2h plasma glucose ≥ 153 mg/dL after the 75 g OGTT.

Covariates

Several clinical variables were evaluated at the maternal interview: maternal age, self-reported skin color (white or dark skin tone), socioeconomic status (Brazilian classification[24]), years of study, parity, self-reported prepregnancy weight, active smoking at registration, chronic diseases, regular use of medicines and supplements, parity, previous history of a child with macrosomia, of GD, and of gestational hypertension or preeclampsia/eclampsia. Height was measured at first prenatal visit and body mass index (BMI) was calculated as pregestacional weight/height² (kg/m²). Routine prenatal laboratory tests and HbA1c were evaluated. Data about delivery route, medical emergencies, neonatal intensive care unit (ICU) hospitalization, neonatal weight immediately after birth, sex and health status were extracted from medical records.

Outcomes

We evaluated the following neonatal outcomes: birth weight, incidence of neonate SGA or large for gestational age (LGA) (< percentile 10 and > percentile 90 for sex and gestational age, respectively[25]), of neonatal hypoglycemia (glucose < 45 mg/dL) with or without ICU hospitalization, of prematurity (gestational age <37 weeks), of ICU hospitalization of any cause, of jaundice, of shoulder dystocia, and of death (fetal or neonatal).

Gestational age was based on an ultrasound early in pregnancy. Adequacy of birth weight for gestational age was based in the traditional curve of Alexander et al[25], but we also analyzed the results for the recently published Brazilian charts[26].

Laboratory

Glucose was measured by the colorimetric method (Advia 1800, Siemens Healthcare, Erlangen, Germany) and HbA1c by high performance liquid chromatography (Variant II, BioRad Laboratories, Hercules, CA). Serum 25(OH)D was measured by the chemiluminescent immunoassay (DiaSorin LIAISON®, MN, USA). This assay measures between 4.0 and 150 ng/mL, and inter-assay precision had a coefficient of variation of < 4% and < 6% for 15 and 50 ng/mL values, respectively. Vitamin D deficiency was defined as 25(OH)D below 20 ng/mL, in agreement with current recommendations of the Institute of Medicine[27] and the Endocrine Society[28]. Levels between 20 and 30 ng/mL were considered insufficient[28].

Statistical analysis

Descriptive statistics were used to present the data about clinical and demographic variables of mother and neonate in each vitamin D category (below or above 20 ng/mL). Number, percentage, means ± standard deviation, median and interquartile range were used.

Comparison between risk and control groups was performed. Categorical variables were analyzed with chi-square test, and continuous variables were evaluated with t-test when parametric and with Mann-Whitney test when non-parametric. A general mixed model (Poisson regression with robust standard errors) was used to study the association of SGA, neonatal hypoglycemia, and ICU hospitalization with serum vitamin D. Models for adjustment took into consideration potential biological confounders as maternal glucose at OGTT, HbA1c, maternal BMI, skin pigmentation, birth weight, gestational age, smoking, nulliparity, hypertensive disorders of pregnancy (gestational hypertension and preeclampsia), medication requirement for diabetes

treatment, maternal age, low socioeconomic status, years of study and season. Confounding factors were considered not to influence the main result and were removed from the model if their inclusion did not change relative risk (RR) in at least 5%. Correlations were evaluated with Pearson´s or Spearman´s test for parametric and non-parametric variables, respectively. Analysis was also performed between ethnical subgroups.

Statistical analysis was performed in SPSS version 20.0 and statistical significance was considered when $P<0.05$.

Results

A hundred and eighty-four pregnant women with GD were included in the study, had blood sampled for storage and were followed until postpartum. Maternal clinical and laboratorial characteristics are shown in Table 1. Mean 25(OH)D was 19.7 ± 8.6 ng/mL for the whole group and 98 women (53.3%) had deficiency of vitamin D. No woman was regularly taking vitamin D supplement.

Table 2 and Figure 1 show neonatal adverse outcomes according to vitamin D status. There was just one fetal death with 33 weeks of pregnancy, in a women who developed gestational hypertension and preeclampsia; her serum 25(OH)D was 16.2 ng/mL. Three neonatal infections were diagnosed as pneumonia, meningitis and neonatal septicemia; all infections occurred in the group of vitamin D deficiency. ICU hospitalization was necessary for variable neonatal disorders such as jaundice with phototherapy, severe hypoglycemia, grunting, infection, tachypnea of the newborn, prematurity and other less frequent reasons. Table 3 shows the neonatal outcomes after adjustment for significant confounders. Only SGA newborns and neonatal hypoglycemia requiring ICU hospitalization remained associated with lower vitamin D levels.

The incidence of SGA newborns was higher in the group of vitamin D deficient women (Table 2 and Figure 1). The analysis of SGA newborns with the charts of Alexander et al presented an RR for vitamin D deficiency of 4.32 (95%CI 1.75-10.66) after adjustments (Table 3), while the analysis with the Brazilian chart showed a similar significant RR of 4.55 (95%CI 1.30-16.04). When analysis was performed in order to compare the groups of newborns who were SGA or not, we detected a lower 25(OH)D level in the group SGA (Figure 2). However, serum 25(OH)D did not present a statistically significant linear correlation with birth weight ($P=0.177$).

Table 1. Clinical and laboratorial characteristics of the 184 women with gestational diabetes

	All (N=184)	25(OH)D ≥ 20 ng/mL (N=86)	25(OH)D < 20 ng/mL (N=98)	*P*
25(OH)D (ng/mL)	19.7 ± 8.6	27.4 ± 4.8	12.9 ± 4.4	<0.001
Season* (n,%)				
Summer	58 (31.5)	47 (54.7)	11 (11.2)	<0.001
Autumn	57 (31.0)	25 (29.1)	32 (32.7)	
Winter	25 (13.6)	3 (3.5)	22 (22.4)	
Spring	44 (23.9)	11 (12.8)	33 (33.7)	
Age (years)	32.2 ± 6.0	32.0 ± 6.3	32.3 ± 5.8	0.744
White (n,%)	136 (73.9)	69 (80.2)	67 (68.4)	0.048
Eight years of study or more (n,%)	122 (66.3)	58 (67.4)	64 (66.0)	0.834
Socioeconomic class A or B (n,%)	45 (24.5)	17 (28.3)	28 (34.6)	0.432
Parity (n)	3 (2-4)	2 (2-4)	3 (2-4)	0.505
Previous HDP (n,%)	40 (21.7)	20 (23.3)	20 (20.4)	0.640
Hypertension	34 (18.5)	20 (23.3)	14 (14.3)	0.130
Preeclampsia or eclampsia	31 (16.9)	15 (17.5)	16 (16.4)	0.592
Chronic Hypertension (n,%)	17 (9.2)	5 (5.8)	12 (12.2)	0.130
Smoking (n,%)	21 (11.4)	9 (10.5)	12 (12.2)	0.705
BMI (kg/m²)	29.7 ± 6.7	29.1 ± 6.6	30.2 ± 6.9	0.290
75 g OGTT (mg/dL)				
Fasting glucose	100.4 ± 27.1	97.8 ± 26.1	102.8 ± 27.8	0.215
1h glucose	177.8 ± 28.9	177.0 ± 32.5	178.2 ± 27.7	0.924
2h glucose	162.9 ± 26.6	165.4 ± 25.5	160.8 ± 27.5	0.269
HbA1c (%)[¥]	5.6 (5.2-6.0)	5.6 (5.1-6.0)	5.6 (5.3-6.1)	0.756

Data expressed as number (%), mean±sd, median (interquartile range)
25(OH)D: 25-hydroxyvitamin D; HDP: Hypertensive disorders of pregnancy; BMI: Body mass index; OGTT: Oral glucose tolerance test;
*Season of blood sampling; [¥]HbA1c at beginning of the third trimester

Prematurity rates was not statistically different between the group with or without vitamin D deficiency (Table 2) and serum 25(OH)D did not correlated with gestational age at delivery (P=0.695). On the other hand, the analysis of newborns with or without premature delivery showed a trend for lower serum vitamin D in preterm births (20.2±8.5 vs 16.9±8.4 ng/ml, P=0.059).

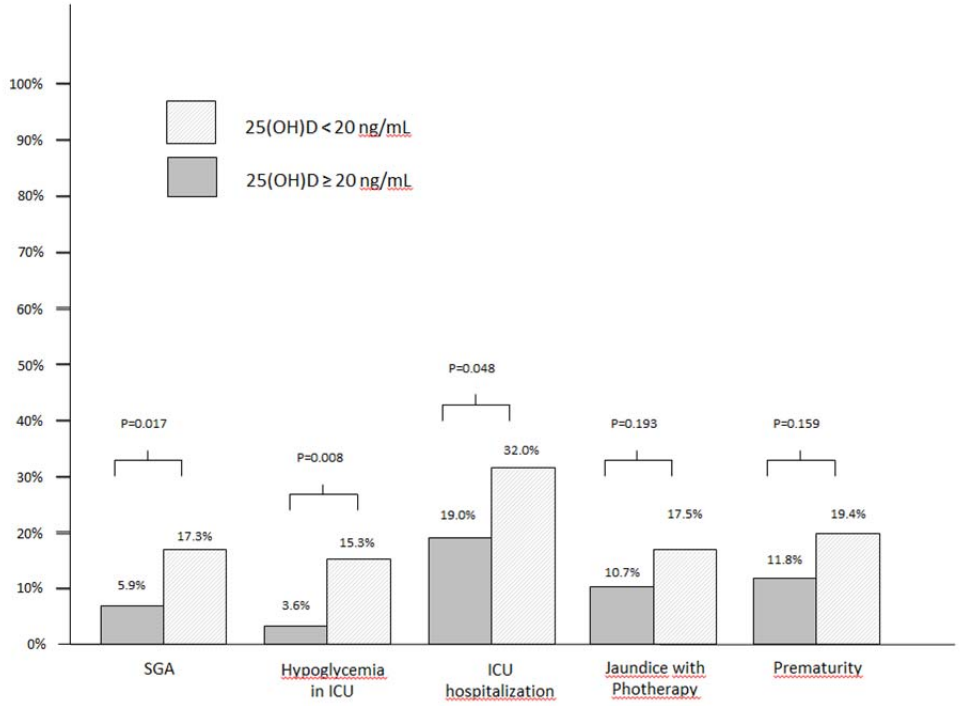

Figure 1. Adverse neonatal outcomes and maternal vitamin D

Table 2. Neonatal outcomes according to maternal vitamin D status

	25(OH)D ≥ 20 ng/mL (N=86)	25(OH)D < 20 ng/mL (N=98)	*P*
Gestational age (weeks)	38.4 (38.0-38.9)	38.6 (37.6-39)	0.552
Prematurity (n,%)	10 (11.8)	19 (19.4)	0.159
Birth Weight (g)	3260 ± 535	3179 ± 586	0.334
SGA (n,%)	5 (5.9)	17 (17.3)	0.017
LGA (n,%)	8 (9.4)	8 (8.2)	0.766
Neonatal hypoglycemia (n,%)			
Any hypoglycemia	6 (7.1)	17 (17.3)	0.039
Requiring ICU	3 (3.6)	15 (15.3)	0.008
Jaundice (n,%)			
Any jaundice	15 (17.9)	24 (24.7)	0.261
With phototherapy	9 (10.7)	17 (17.5)	0.193
Shoulder dystocia (n,%)	3 (3.6)	7 (7.1)	0.292
Neonatal infection (n,%)	0	3 (3.1)	-
ICU hospitalization* (n,%)	16 (19)	31 (32)	0.048
Death (n,%)	0	1 (1)	-

Data expressed as number (%), mean±sd, median (interquartile range)
25(OH)D: 25-hydroxyvitamin D; SGA: Small for gestational age; LGA: Large for gestational age; ICU: Intensive care unit
*ICU hospitalization of any cause

Table 3. Results of Poisson regression analysis for neonatal outcomes

	RR, crude	95% CI	RR, adjusted	95% CI
SGA* (n,%)	2.95	1.14-7.66	4.32	1.75-10.66
Neonatal hypoglycemia[£] (n,%)				
Any hypoglycemia	2.43	1.00-5.88	2.40	0.90-6.42
Requiring ICU	4.29	1.29-14.30	3.63	1.09-12.11
ICU hospitalization[¥] (n,%)	1.68	0.99-2.85	1.31	0.70-2.42

25(OH)D: 25-hydroxyvitamin D; SGA: small for gestational age; ICU: intensive care unit
*Adjusted for maternal glucose at OGTT, maternal body mass index, smoking and season;
[£]Adjusted for maternal glucose at OGTT, birth weight, gestational age and season;
[¥]Adjusted for maternal glucose at OGTT, maternal body mass index, birth weight, gestational age, and season;

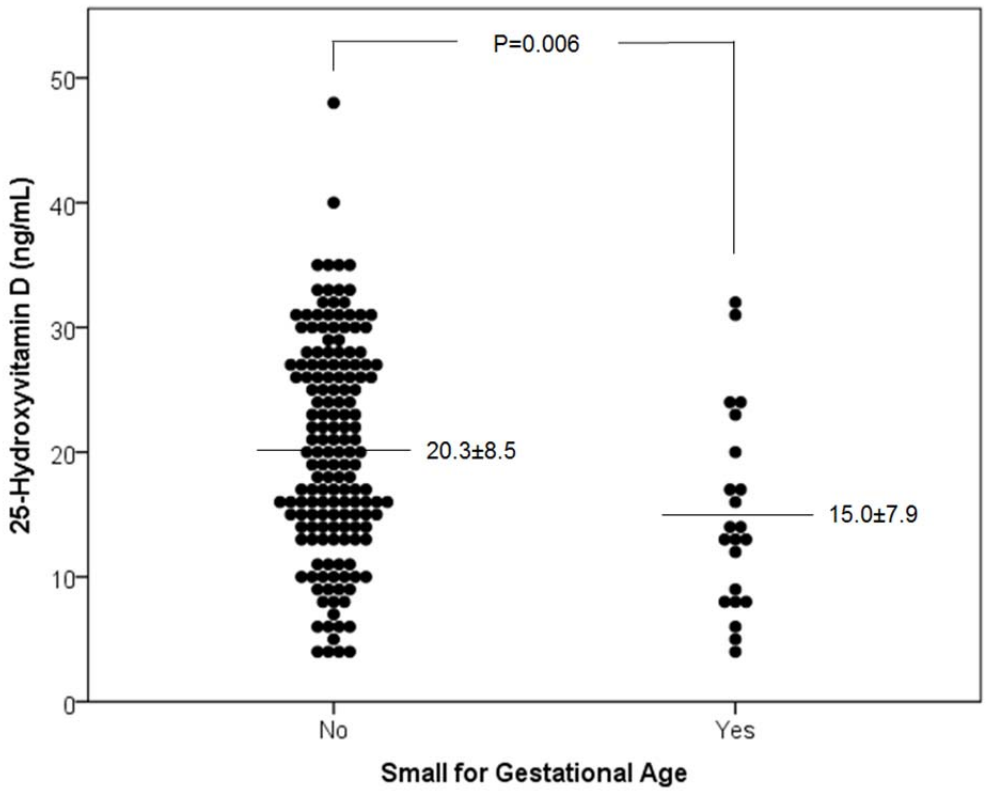

Figure 2. Serum 25-hydroxyvitamin D in newborns according to birthweight adequacy

Analysis of data considering skin pigmentation differences showed an incidence of SGA of 9.6% in white and 18.8% in dark skin tone women (P=0.095). In white women, the vitamin D deficient group had a higher incidence of SGA than women with 25(OH)D ≥ 20 ng/mL (16.4% vs 2.9%, P=0.008). The RR for SGA in white women with 25(OH)D < 20 ng/mL was 6.04 (CI95% 1.90-19.23) after adjustment for maternal

glucose at OGTT, maternal body mass index, smoking and season. However, in women with dark skin tone, there was no difference in the rates of SGA according to vitamin D status (19.4% in the deficient group vs 17.6% in the group with 25(OH)D ≥ 20 ng/mL, P=0.885).

Discussion

Vitamin D deficiency was associated with increased rates of neonatal hypoglycemia requiring ICU hospitalization and of SGA newborns in our cohort of pregnant women with GD. Many previous studies have reported an increased risk of newborns SGA in women with deficiency of vitamin D. This finding is consistent around different populations and variable trimester of 25(OH)D measurement. A large multi-ethnic cohort in The Netherlands evaluated 3730 relatively healthy women with measurement of vitamin D in early pregnancy and found that deficient vitamin D levels resulted in an odds ratio (OR) of 2.4 (CI95% 1.9-3.2) for SGA[8]. Bodnar et al evaluated nulliparous pregnant women in a nested case-control study and showed a U-shaped relation between serum 25(OH)D and risk of SGA in white women. The OR for SGA in women with 25(OH)D < 15 ng/mL in comparison to 15-30 ng/mL was 7.5[5]. In an U.S. cohort, maternal vitamin D deficiency in the first trimester was also associated with SGA incidence, and birth weight increased with rising levels of 25(OH)D[7]. In one more cohort, with second trimester plasma 25(OH)D measurement, lower levels of vitamin D were associated with higher odds of SGA[6]. A recent meta-analysis on this issue reinforced the association[9]. In agreement with these previous data in general pregnancies, we found an important increase in the incidence of SGA neonates in pregnancies with GD. Therefore, as far as we know, we are the first to show this association in a population of diabetic women whose offspring is at higher risk of being LGA. Moreover, few studies have measured vitamin D at the third trimester, when the fetal growth velocity is greater.

Some studies have found an association between serum vitamin D and birth weigth[7,29] although this relationship could be nonlinear[7]. We did not find a continuous relationship but we understand that birth weight is not a pathologic outcome. Excessive or restricted fetal growth should be stronger representatives of abnormal intrauterine development.

Since vitamin D deficiency is more prevalent in women with dark skin tone, it could be one of the possible mechanisms to explain ethnical differences in neonatal outcomes. However, available data are not conclusive. In our cohort, vitamin D

influenced SGA incidence in white women, but it did not change the risk in non-white women. Previous studies also did not find any relation between serum 25(OH)D and SGA risk in black women[5] or described a limited role of vitamin D in the explanation of multi-ethnic differences[8]. In contrast, Burris et al demonstrated a higher incidence of SGA in black women and they raised the possibility of vitamin D contribution for racial disparities[6].

The biological mechanism of vitamin D influence on fetal growth is still not fully understood. One of the plausible explanations is that maternal vitamin D deficiency may result in abnormal maternal calcium metabolism and lower calcium levels in cord blood have association with lower birth length[30]. Other possible speculated mechanism is that vitamin D may regulate glucose metabolism[31], what affects fetal mass. Moreover, human placenta may have a role in the influence of vitamin D on fetal growth since vitamin D receptors and enzymes to convert 25(OH)D in 1.25 dihydroxyvitamin D have been identified in the placenta tissue, and calcitriol may regulate human chorionic gonadotropin and sex steroid secretion [32,33].

It is also important to highlight that intrauterine growth restriction is a relevant neonatal outcome since it results in increased fetal and perinatal morbidity and mortality [34-36]. Moreover, fetal origins of adult disease theory assumes that SGA infants are at increased risk for adult coronary heart disease and related disorders[37]. A large cohort reported an increased risk of ischemic heart disease mediated by poor fetal growth[38] and another British cohort have found lower birth weight to be associated with reduced kidney function over 60 years later[39]. Therefore, fetal growth restriction is associated with an increased rate of complications early or late in the life, and more longitudinal studies are need to completely understand the long-term consequences of SGA neonates.

Transient asymptomatic hypoglycemia may be part of the transition to extrauterine environment, but severe or persistent neonatal hypoglycemia could be associated with ICU hospitalization, higher cost and neurologic damage. This is the first study to show the association of vitamin D deficiency and increased rates of neonatal hypoglycemia. The association is significant for any hypoglycemia but, more importantly, since the blood glucose levels to define neonatal hypoglycemia have been discussed [40], we also show an association of vitamin D deficiency with severe hypoglycemia requiring ICU hospitalization. This is a relevant outcome in an at risk population because they are newborns of women with GD. More studies are necessary to reinforce this association.

We also showed the novel data about higher rate of neonatal ICU hospitalization in the group of newborns from mothers with 25(OH)D < 20 ng/mL. However, after regression analysis, vitamin D does not appear to be an independent predictor of hospitalization. ICU hospitalization increases the risk of hospital acquired morbidity, impose a period of separation from parents and increase costs[41]. Therefore, modifiable risk factors for ICU requirement deserve attention.

Although the incidence of prematurity was higher in the pregnancies with vitamin D deficiency and the serum vitamin D was lower in the group with preterm newborns, there was no statistically significant difference in our study. The association of gestation length and vitamin D is controversial. Some studies did not find a lower gestation age at birth in pregnancies complicated by vitamin D deficiency[6,42] while other data reported an association[8,11]. However, the significant difference may be rather small as 0.2 weeks in the study of Leffelaar et al[8]. The association with prematurity is also not uniform. A Japanese study found lower 25(OH)D levels after the 30th week of pregnancy in mothers with premature delivery[43], while other studies with vitamin D measurement earlier in pregnancy did not[44-46]. Therefore, we believe that the association of vitamin D and prematurity deserves further investigation and that the trimester of pregnancy for vitamin D measurement should be taken into account.

Based on observational studies findings, vitamin D supplementation has been suggested during pregnancy. Two trials from eighties reported a lower incidence of SGA neonates[47] and a better birthweight[48] in mothers who performed vitamin D supplementation . A Cochrane evaluation[49] found a lower rate of babies with birthweight below 2500 grams in women who received supplements, although statistical significance was borderline. There was no data about prematurity, death or admission to neonatal ICU. The trial of Hollis et al did not find any difference in gestational length, birth weight or ICU hospitalization among groups of supplementation of vitamin D with 400, 2000 or 4000 IU/day. There was no report about the incidence of SGA newborns [50]. Other recent trial also did not find difference in birth weight according to vitamin D supplementation in Arab women and they had a low incidence of SGA. Authors argue that the sample size was too small to evaluate the effect on growth[51]. Another placebo-controlled trial of 35000 IU/week also did not find any difference in birth weight, gestational age at birth, stillbirth or other adverse events, but only 160 women were randomized[52]. A combined analysis of two trials did not demonstrate differences in neonatal birth weight and prematurity rates in women who supplemented 2000 or 4000 IU/day of vitamin D vs 400 IU/day[53]. Therefore,

although observational data is alarming, there is no evidence of the real benefit of vitamin D supplementation during pregnancy and larger studies are necessary.

This cohort study adds novel data about vitamin D deficiency in a high-risk group of women with GD. This population has a high incidence of vitamin D deficiency and it is associated with increased rates of adverse neonatal outcomes. Other strengths of our study is the prospective design, the expert laboratory team to measure serum 25(OH)D and the inclusion of diverse skin pigmentation women. Moreover, we used both a standard international chart for birthweight analysis and the recently built national chart. Relative risk for SGA was very similar between the charts, what reinforce our findings. However, this study has also some limitations. First, we have not assessed calcium intake or serum level. It is relevant since calcium levels in cord blood have been reported to be associated with birth length[30]. Second, serum vitamin D binding protein was not measured and therefore we were not able to estimate free 25(OH)D, but we understand that it is not a routine in clinical practice and does not invalidate the results. Third, we used both the Brazilian and the American Diabetes Association recommendations for GD diagnosis, since there was no current consensus, what may render the study population somewhat heterogeneous. However, analysis of data after exclusion of women whose diagnosis was made solely by the ADA criteria did not change the results.

Our perspective is to follow the cohort of newborns during childhood and adolescence in order to evaluate the long-term consequences of the exposure to both GD and vitamin D deficiency during pregnancy. If the phenomenon of development plasticity[37] is real, our cohort of newborns may have an increase in the incidence of metabolic and osseous adverse outcomes.

In conclusion, vitamin D deficiency was associated with an increased incidence of adverse neonatal outcomes, such as neonatal hypoglycemia requiring ICU and newborns SGA, in our cohort of high-risk pregnant women with GD. We believe it is imperative to confirm our findings in other independent large cohorts. Vitamin D is a modifiable risk factor in pregnancy with important public health implications and we believe that national authorities should implement routine vitamin D measurement during pregnancy. Additional research on the underlying biological mechanisms of vitamin D action in pregnancy, on the long-term consequences to the offspring, and on the real benefit of vitamin D supplementation is essential.

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Conclusão

O estudo das funções extra-esqueléticas da vitamina D vem ampliando-se nos últimos anos. Na gestação, a preocupação com os níveis de vitamina D maternos ocorre pela necessidade desta vitamina para a formação do esqueleto fetal e pela associação da hipovitaminose D com desfechos adversos materno-fetais. Para o recém-nascido (RN), as complicações incluem o baixo peso ao nascer, o comprometimento do crescimento longitudinal e as infecções respiratórias. Para a gestante, a deficiência de vitamina D vem sendo associada à alteração na homeostase glicêmica e ao aumento da incidência de diabetes gestacional (DG) , à pré-eclâmpsia e à vaginose bacteriana. Entretanto, a evidência científica atual ainda é controversa e não há definição estabelecida sobre o real benefício da suplementação da vitamina D na gestação. O diabetes gestacional, por sua vez, também está associado a desfechos adversos para a gestante e para a prole. Desta forma, os desfechos adversos da hipovitaminose D e do DG presentes de forma simultânea na gestação podem ser aditivos.

O objetivo desta tese foi avaliar a deficiência de vitamina D em mulheres com DG e analisar sua associação com desfechos adversos maternos e fetais/neonatais. Uma coorte de 184 mulheres com DG foi avaliada e classificada conforme a presença de deficiência de vitamina D (grupo de risco – 25-hidroxivitamina D < 20 ng/mL; grupo controle – 25-hidroxivitamina D ≥ 20 ng/mL).

A prevalência de deficiência de vitamina D foi de 53,3%. Para a gestante, não houve aumento da incidência, no grupo com deficiência de vitamina D, de desfechos adversos como hipertensão gestacional, pré-eclâmpsia/eclâmpsia, cesariana, diabetes pós-parto, infecção, resistência insulínica ou necessidade de tratamento medicamentoso para o DG. Por outro lado, em gestantes com cor da pele branca, o nível sérico de vitamina D apresentou correlação significativa e negativa com os níveis de pressão arterial sistólica, e foi preditor independente deste desfecho, no terceiro trimestre da gestação.

Para o RN, a deficiência de vitamina D materna foi associada com maior taxa de hipoglicemia neonatal com necessidade de hospitalização em unidade de tratamento intensivo (UTI) neonatal e de RN pequeno para idade gestacional. A necessidade de hospitalização em UTI por qualquer causa também foi mais frequente no grupo de RN filhos de gestantes com deficiência de vitamina D, porém o risco relativo desta associação perdeu significância estatística após ajuste para

confundidores. As incidências de prematuridade, macrossomia, icterícia e distócia de ombro não foram diferentes entre os grupos.

Desta forma, concluímos que a deficiência de vitamina D em gestantes diagnosticadas com DG está associada ao aumento da incidência de desfechos adversos neonatais e correlaciona-se de forma inversa com os níveis de pressão arterial sistólica em mulheres brancas.