Gut Microbiome and Maternal Glycemia: A Scoping Review Protocol

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ABSTRACT

Introduction

Gestational diabetes mellitus (GDM) is a metabolic disorder characterized by the onset of hyperglycemia during pregnancy and is associated with short and long-term health complications for both mother and child. Research is emerging that targeting the capacity of the gut microbiome, the collection of archaea, eukarya, and bacteria found within the gastrointestinal tract, may be an approach to prevent and manage GDM. To date, initial studies show that women with GDM may exhibit a distinct gut microbiota profile with differences in structure, metabolic function, and composition in comparison to women without GDM during pregnancy. However, an examination of the gut microbiome and maternal glycemic control is in its infancy and a comprehensive, systematic synthesis on this knowledge has yet to be performed. Our scoping review described in this protocol aims to fill that gap.

Inclusion Criteria

This scoping review inclusion criteria considers non-interventional human studies and animal studies in English that examine the relationship between gut microbiome, GDM, and maternal glycemia in pregnancy.

Method and Analysis

This scoping review will be conducted using the framework by the Johanna Briggs Institute, which is an enhancement of the original scoping review framework by Arksey & O'Malley (2005) and the updated version by Levac and colleagues (2010). The search strategy consisted of a predefined criterion to search five electronic databases (PubMed, Embase, CINAHL Plus with Full Text, CENTRAL, and ClinicalTrials.gov). The Covidence online platform will be used to navigate screening and data extraction by two independent reviewers. Data will be extracted in tabular and graphical form in addition to a narrative summary that corresponds with the objectives of this review. Any disagreement during screening and data extraction will be resolved during bi-weekly team meetings and discussion with reviewers.

BACKGROUND

Gestational diabetes mellitus (GDM) is a metabolic disorder characterized by the onset of hyperglycemia during pregnancy and is associated with short and long-term health complications for both mother and child.[1–4] For the child, these complications include increased risks for macrosomia, metabolic syndrome, lifelong obesity, type 2 diabetes (T2D), and even infant mortality.[2,3,5–9] Women diagnosed with GDM experience higher risk for obstetric complications, such as pre-eclampsia and pre-term delivery, and have a 30-70% chance of developing GDM in future pregnancies.[2,10,11] Furthermore, women who develop GDM have an increased risk for developing T2D and cardiovascular disease. [2,10,11] While the actual prevalence of GDM is uncertain due to differences in screening, the current global estimates range from 8-14% within all pregnancies.[6–8,10,11] Thus, targeting ways to prevent and manage GDM is a clinical and public health priority that will improve the cardiometabolic health of women and future generations.

Currently, lifestyle modifications, which includes increasing physical activity and medical nutrition therapy (MNT) is the first line intervention to reduce the onset of and manage glucose levels in women with GDM. However, there is no consistent evidence or consensus on the best dietary approaches for preventing and managing GDM that would benefit maternal health and the developing fetus.[9,12,13] Furthermore, lifestyle modifications are effective for only half of women diagnosed with GDM, and pharmacotherapy, such as insulin and metformin, have varying safety profiles.[12,14] Therefore, there is a need for innovative and safe non-pharmacological approaches for preventing and managing GDM.

Leveraging the capacity of the gut microbiota, the collection of archaea, eukarya, and bacteria found within the gastrointestinal tract may be an approach to prevent and manage GDM.[15] Evidence shows that the gut microbiota can play a significant role in maintaining human health by producing metabolites and nutrients that regulate energy metabolism, intestinal permeability and immune responses.[12] More specifically, the gut microbiota is intrinsically involved in glucose homeostasis, for both pregnant and non-pregnant individuals and may play a role in the development and persistence of GDM.[16] Furthermore, the gut microbiota's structure, composition, and metabolic activity are influenced by environment, physical activity, antibiotics, pregnancy hormones, diet, and dietary supplements; lending a salient target for preventing and managing GDM.[16–18]

To date, initial studies show that women with GDM may exhibit a distinct gut microbiota profile, similar to adults with T2D, with differences in structure, composition and metabolic function in comparison to non-GDM women.[19–22] Moreover, a few human and animal studies have emerged examining the dynamic nature of the maternal gut microbiome across pregnancy to understand its role in glycemia.[19–25] Yet, an examination of the gut microbiome and maternal glycemic control is in its infancy and a comprehensive, systematic synthesis on this knowledge has yet to be performed.

The objective of this scoping review is to understand relationships between the maternal gut microbiome and maternal glycemic outcomes during pregnancy. Our primary question is how does gut microbial structure, composition and metabolic activity change relate to maternal glycemia and the onset of GDM. Two recently published narrative reviews examined the relationship of diet, probiotics, gut microbiome, and maternal glycemia, however, the approach was not comprehensive failing to include animal studies.[12,26] Our review will comprehensively examine existing literature as it relates to the gut microbiome and maternal glycemia in humans and animal models through an established systematic scoping process.

METHODS

Framework and Tools

Our proposed review will be conducted using the framework by the Johanna Briggs Institute (JBI), which is an enhancement of the original scoping review framework by Arksey & O'Malley (2005) and the updated version by Levac and colleagues (2010).[27–29] We will use the Preferred Reporting Items for Systematic Reviews and Meta-Analyses-Extension for Scoping Reviews (PRISMA-SCR) checklist. Also, we will use Covidence (Melbourne, Australia), an online platform that helps streamline the systematic process for writing review articles.

Search Strategy

We conducted an exploratory search (in MEDLINE) to inform the authors on this topic, identify published scoping studies which rendered no evidence in this area, and published a commentary.[30] Following this, we consulted with an information scientist from the University of Illinois at Chicago Library of Health Sciences to discuss the aims of the project, construct the search strategy, identify the relevant databases, and to finalize the search terms used in this study.

The search strategy was designed around two conceptual domains: (1) the gut microbiome and (2) gestational diabetes mellitus. The full search strategy for the PubMed database is included in **Table 1**. The following databases were used to search for articles that meet the eligibility criteria:

- PubMed (new platform; includes MEDLINE)
- Embase
- CINAHL Plus with Full Text
- CENTRAL
- ClinicalTrials.gov

Searches were initially conducted between June 29 and July 15, 2020. No restrictions were placed on date or language of search results. In addition, we will perform a backwards search, which requires hand searching all included studies' reference list along with searching any relevant review papers identified in the search process. Also, we will conduct a forward citation search, which will identify articles that cited the

included studies. The backwards and forward searches will identify additional relevant articles that were not captured in the initial database search.

Study Eligibility

Studies are eligible for this review if they examined a relationship between gut microbiome, maternal glycemic outcomes including a diagnosis of gestational diabetes among pregnant women and animals. Studies were included based on a pre-defined PCC (Population or Participants, Concept, Context) criterion.

Population

This scoping review will consider all studies that focus on pregnant women or pregnant animals.

Concept

This review will examine the relationships between the gut microbiome and maternal glycemia. Glycemic outcomes include but are not limited to glucose intolerance (ex. high levels of Hba1c, insulin, and/or glucose), insulin resistance, and low insulin sensitivity. Only sequencing gut microbial analysis will be included (ex. 16S RNA) as it relates to structural, composition, and functional activities of the microbiome.

Context

There were no restrictions on the context of included studies outside of focusing on studies that examined pregnant women and animal models.

Inclusion and Exclusion Criteria

This scoping review encourages identifying a breadth of knowledge for data analysis and to develop the discussion section when publishing our findings. However, since starting the title and abstract screening a larger number of articles thus far have been included for full-text review. To narrow the focus to a reasonable number of articles for data analysis two rounds of criteria has been developed. Our initial search for title and abstract screening for example, included all study types and grey literature such as clinical trial registries and conference proceedings. For full-text screening, further inclusion and exclusion criteria will be used. The following is our inclusion and exclusion criteria for each round.

- Round 1: Inclusion Criteria
    - All study types (including experimental, observational, and mixed methods)
    - Grey literature (including clinical trial registries)
    - English only articles with no limitations on publication year
- Round 1: Exclusion Criteria:
    - Review articles, conference proceedings, and commentaries
    - Studies that only examined women or animals outside of the gestational period
- Round 2: Inclusion Criteria

    - Observational (cohort studies [prospective and retrospective], cross sectional studies, and case control studies)
- Round 2: Exclusion Criteria:
    - Human experimental research design, including interventions/treatment studies
    - Abstracts, editorials, research protocols/design papers

Relevant studies that fell within the first round of the inclusion criteria and review articles will be used to inform the introduction and discussion sections of this review.

Data Screening

We will use the Covidence online platform to support data screening. Title and abstract screening will be conducted by two reviewers independently to identify potential papers that meet the first round of inclusion criteria. Disputes between reviewers on specific articles will be settled at least bi-weekly with the research team by a consensus. Articles included for the full-text screening will be evaluated using the first and second round of inclusion/exclusion criteria. These articles will be downloaded in full and the full text will be reviewed by two reviewers independently to confirm they meet the inclusion criteria. If the articles do not meet the inclusion criteria based on the first and second rounds, they will be pushed back to title and abstract screening and re-voted on by two independent reviewers. If the articles meet the inclusion criteria but do not fit the aims of this review or was lacking important information, the articles will be excluded and reasons for exclusion will be noted. Disputes between reviewers will be discussed with the research team and resolved by a consensus.

Data Extraction

Data will be extracted from each included study by two reviewers independently using a predefined, yet flexible, data extraction form shown in **Table 2** and **Table 3**. This form will be developed by the research team prior to data extraction and include standard information, such as study details and characteristics, and further detailed information that corresponds with the research questions of this study. Furthermore, based on the Levac et al., the form will go through an initial validation process where two reviewers independently extract data from four random studies. Next, a consultation with the team will review the form to ensure consistency, accuracy, and all the necessary information is captured. During this consultation, any amendments to this form will be agreed upon by the team for a finalized version of the form. The extraction process will be continued by the same two reviewers independently with weekly consultations for any inconsistencies or disagreements which will be discussed and resolved with the two reviewers.

Data Analysis

As a scoping review, this study will provide a descriptive synthesis of the main findings from the included studies to determine the current evidence on the relationship between GDM and maternal glycemia with the gut microbiome. Therefore, we will identify the

major concepts, methodologies and assessments used, and gaps in research, if any, to address where further research is needed in observational human and animal studies related to this topic.

DISCUSSION

To our knowledge this is the first scoping review to examine and assimilate the current evidence on the relationships between maternal gut microbiome and maternal glycemia including the diagnosis of GDM. This is an emerging research area with great potential for improving the overall health status of mothers and their children. Furthermore, this review will provide timely insight on the extent of research on this topic to inform future investigations.

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COMPETING INTEREST

The authors report that they have no competing interests.

REFERENCES

1. Metzger BE, Lowe LP, Dyer AR, et al. Hyperglycemia and Adverse Pregnancy Outcomes. *N Engl J Med*. 2008;358(19):1991-2002. doi:10.1056/nejmoa0707943

2. Damm P, Houshmand-Oeregaard A, Kelstrup L, Lauenborg J, Mathiesen ER, Clausen TD. Gestational diabetes mellitus and long-term consequences for mother and offspring: a view from Denmark. *Diabetologia*. 2016;59(7):1396-1399. doi:10.1007/s00125-016-3985-5

3. Reece EA, Leguizamón G, Wiznitzer A. Gestational diabetes: the need for a common ground. *Lancet*. 2009;373(9677):1789-1797. doi:10.1016/S0140-6736(09)60515-8

4. Catalano PM. The impact of gestational diabetes and maternal obesity on the mother and her offspring. *J Dev Orig Health Dis*. 2010;1(4):208-215. doi:10.1017/S2040174410000115

5. American Diabetes Assocation. Diagnosis and Classification of Diabetes Mellitus: Definition and description of diabetes mellitus. *Diabetes Care*. 2014;37 (Supple(January):S81-S90. doi:10.2337/dc14-S081

6. Centers for Disease Control and Prevention. Diabetes. Gestational. Published 2019. https://www.cdc.gov/diabetes/basics/gestational.html

7. Guariguata L, Linnenkamp U, Beagley J, Whiting DR, Cho NH. Global estimates of the prevalence of hyperglycaemia in pregnancy. *Diabetes Res Clin Pract*. 2014;103(2):176-185. doi:10.1016/j.diabres.2013.11.003

8. Lee KW, Ching SM, Ramachandran V, et al. Prevalence and risk factors of gestational diabetes mellitus in Asia: A systematic review and meta-analysis. *BMC Pregnancy Childbirth*. 2018;18(1):1-20. doi:10.1186/s12884-018-2131-4

9. Han S, Middleton P, Shepherd E, Van Ryswyk E, Crowther CA. Different types of dietary advice for women with gestational diabetes mellitus-update of the cochrane review. *Cochrane Database Syst Rev*. 2017;(2):CD009275. doi:10.1002/14651858.CD009275.pub3.www.cochranelibrary.com

10. Kanguru L, Bezawada N, Hussein J, Bell J. The burden of diabetes mellitus during pregnancy in low- and middle-income countries: A systematic review. *Glob Health Action*. 2014;7(1). doi:10.3402/gha.v7.23987

11. IDF Diabetes Atlas 9th Edition. Accessed January 26, 2021. https://www.diabetesatlas.org/data/en/

12. Ponzo V, Fedele D, Goitre I, et al. Diet-gut microbiota interactions and gestational diabetes mellitus (GDM). *Nutrients*. 2019;11(2). doi:10.3390/nu11020330

13. American Diabetes Assocation. Management of Diabetes in Pregnancy. Sec. 13. In Standards. *Diabetes Care*. 2017;40(January):114-119. doi:10.2337/dc17-S016

14. Chen P, Wang S, Ji J, et al. Risk Factors and Management of Gestational

Diabetes. *Cell Biochem Biophys*. 2014;71(2):689-694. doi:10.1007/s12013-014-0248-2

15. Thursby E, Juge N. Introduction to the human gut microbiota. *Biochem J*. 2017;474(11):1823-1836. doi:10.1042/BCJ20160510

16. Gomez-Arango LF, Barrett HL, McIntyre HD, et al. Connections between the gut microbiome and metabolic hormones in early pregnancy in overweight and obese women. *Diabetes*. 2016;65(8):2214-2223. doi:10.2337/db16-0278

17. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559-563. doi:10.1038/nature12820

18. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell*. 2016;165(6):1332-1345. doi:10.1016/j.cell.2016.05.041

19. Wang J, Zheng J, Shi W, et al. Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. *Gut*. 2018;67(9):1614-1625. doi:10.1136/gutjnl-2018-315988

20. Kuang YS, Lu JH, Li SH, et al. Connections between the human gut microbiome and gestational diabetes mellitus. *Gigascience*. 2017;6(8). doi:10.1093/gigascience/gix058

21. Liu H, Pan LL, Lv S, et al. Alterations of gut microbiota and blood lipidome in gestational diabetes mellitus with hyperlipidemia. *Front Physiol*. 2019;10(AUG). doi:10.3389/fphys.2019.01015

22. Hasan S, Aho V, Pereira P, et al. Gut microbiome in gestational diabetes: a cross-sectional study of mothers and offspring 5 years postpartum. *Acta Obstet Gynecol Scand*. 2018;97(1):38-46. doi:10.1111/aogs.13252

23. Fuller M, Priyadarshini M, Gibbons SM, et al. The short-chain fatty acid receptor, FFA2, contributes to gestational glucose homeostasis. *Am J Physiol - Endocrinol Metab*. 2015;309(10):840-851. doi:10.1152/ajpendo.00171.2015

24. Connor KL, Chehoud C, Altrichter A, Chan L, Desantis TZ, Lye SJ. Maternal metabolic, immune, and microbial systems in late pregnancy vary with malnutrition in mice. *Biol Reprod*. 2018;98(4):579-592. doi:10.1093/biolre/ioy002

25. Yao Z, Long Y, Ye • Juan, Li P, Jiang Y, Chen Y. 16S rRNA Gene-Based Analysis Reveals the Effects of Gestational Diabetes on the Gut Microbiota of Mice During Pregnancy. *Indian J Microbiol*. 2088;60. doi:10.1007/s12088-020-00862-x

26. Hasain Z, Mokhtar NM, Kamaruddin NA, et al. Gut Microbiota and Gestational Diabetes Mellitus: A Review of Host-Gut Microbiota Interactions and Their Therapeutic Potential. *Front Cell Infect Microbiol*. 2020;10:188. doi:10.3389/fcimb.2020.00188

27. Peters MDJ, Marnie C, Tricco AC, et al. Updated methodological guidance for the

conduct of scoping reviews. *JBI Evid Synth*. 2020;18(10):2119-2126. doi:10.11124/JBIES-20-00167

28. Arksey H, O'Malley L. Scoping studies: Towards a methodological framework. *Int J Soc Res Methodol Theory Pract*. 2005;8(1):19-32. doi:10.1080/1364557032000119616

29. Levac D, Colquhoun H, O'Brien KK. Scoping studies: Advancing the methodology. *Implement Sci*. 2010;5(1):69. doi:10.1186/1748-5908-5-69

30. Man B, Koenig MD, Bernabe BP, Nagelli U, Tussing-Humphreys L. The Role of the Gut Microbiota in the Prevention and Management of Gestational Diabetes Mellitus: Are We There Yet? *J Perinat Neonatal Nurs*. 2020;34(3):195-198. doi:10.1097/JPN.0000000000000497

Table 1: Example Search Strategy of PubMed (new platform) Database

Search Number	Search Keywords
#1	"Gastrointestinal Microbiome"[mesh]
#2	"Gastrointestinal Tract"[mesh] OR "Feces"[mesh]
#3	gut[tiab] OR "gastrointestinal"[tiab] OR "intestinal"[tiab] OR "intestine"[tiab] OR "intestines"[tiab] OR "GI"[tiab] OR "fecal"[tiab] OR "faecal"[tiab] OR "colon"[tiab] OR "rectum"[tiab] OR "rectal"[tiab] OR "colorectal"[tiab] OR "feces"[tiab] OR "faeces"[tiab] OR "fecal"[tiab] OR "faecal"[tiab] OR "stool"[tiab] OR "cecum"[tiab] OR "caecum"[tiab] OR "bowel"[tiab] OR "bowels"[tiab] OR "stomach"[tiab] OR "gastric"[tiab]
#4	#2 OR #3
#5	"Microbiota"[mesh] OR "microbiology"[subheading] OR "dysbiosis"[mesh] OR "probiotics"[mesh] OR "prebiotics"[mesh] OR "synbiotics"[mesh] OR "Bacteria"[mesh]
#6	"microbiome"[tiab] OR "microbiomes"[tiab] OR "microbiota"[tiab] OR "flora"[tiab] OR "bacterium"[tiab] OR "bacteria"[tiab] OR "mycobiome"[tiab] OR "microbial"[tiab] OR "microbe"[tiab] OR "microbes"[tiab] OR "dysbiosis"[tiab] OR "symbiont"[tiab] OR "symbionts"[tiab] OR "bacterial"[tiab] OR "prebiotic"[tiab] OR "prebiotics"[tiab] OR "probiotic"[tiab] OR "probiotics"[tiab] OR "synbiotic"[tiab] OR "synbiotics"[tiab] OR "microbiology"[tiab] OR "colonization"[tiab] OR "colonisation"[tiab]
#7	#5 OR #6
#8	"Metagenome"[mesh] OR "Metagenomics"[mesh] OR "Sequence Analysis"[mesh] OR "Proteome"[mesh] OR "Proteomics"[mesh] OR "Metabolome"[mesh]
#9	"DNA sequencing"[tiab] OR "exact sequence variants"[tiab] OR "operational taxonomic units"[tiab] OR "operational taxonomic unit"[tiab] OR "operational taxonomy units"[tiab] OR "operational taxonomy unit"[tiab] OR "amplicon sequence"[tiab] OR "amplicon sequencing"[tiab] OR "shotgun"[tiab] OR "metagenomic"[tiab] OR "metagenomics"[tiab] OR "metagenome"[tiab] OR "metagenomes"[tiab] OR "metabolomic"[tiab] OR "metabolomics"[tiab] OR "metabolome"[tiab] OR "metabolomes"[tiab] OR "proteomic"[tiab] OR "proteomics"[tiab]
#10	#8 OR #9
#11	#7 OR #10
#12	#4 AND #11
#13	#1 OR #12

#14	"Diabetes, Gestational"[mesh]
#15	"GDM"[tiab]
#16	#14 OR #15
#17	"Pregnancy"[mesh] OR "Pregnancy complications"[mesh] OR "Postpartum Period"[mesh] OR "Mothers"[mesh]
#18	"pregnant"[tiab] OR "pregnancy"[tiab] OR "gestational"[tiab] OR "first trimester"[tiab] OR "second trimester"[tiab] OR "third trimester"[tiab] OR "prenatal"[tiab] OR "perinatal"[tiab] OR "postnatal"[tiab] OR "obstetric"[tiab] OR "maternal"[tiab] OR "gestation"[tiab] OR "postpartum"[tiab] OR "mother"[tiab] OR "mothers"[tiab]
#19	#17 OR #18
#20	"blood glucose"[mesh] OR "insulin resistance"[mesh] OR "Insulin"[mesh] OR "Glucose Tolerance Test"[mesh] OR "Glucose Intolerance"[mesh] OR "Hyperglycemia"[mesh] OR "Prediabetic State"[mesh] OR "Diabetes Mellitus"[mh:noexp] OR "Diabetes Mellitus, Type 2"[mesh] OR "Glycated Hemoglobin A"[mesh] OR "Hypoglycemic Agents"[mesh]
#21	"diabetes"[tiab] OR "diabetic"[tiab] OR "diabetics"[tiab] OR "insulin"[tiab] OR "glucose"[tiab] OR "metabolic"[tiab] OR "metabolism"[tiab] OR "prediabetes"[tiab] OR "prediabetic"[tiab] OR "hbA1c"[tiab] OR "hb A1c"[tiab] OR "A1c"[tiab] OR "metabolomic"[tiab] OR "hyperglycemia"[tiab] OR "hyperglycaemia"[tiab] OR "hyperglycemic"[tiab] OR "hyperglycaemic"[tiab] OR "glycemic"[tiab] OR "glycaemic"[tiab]
#22	#20 OR #21
#23	#19 AND #22
#24	#16 OR #23
#25	#13 AND #24

Table 2: Preliminary Data Extraction Instrument for Animal Studies

Author, Country, Year (Reference)	
Animal Models	
Comparison and Study Duration	
Type of collection sample	
Method of gut microbiota evaluation	
Major findings	

Table 3: Preliminary Data Extraction Instrument for Human Observational Studies

Author, Country, Year (Reference)	
Participant Characteristics	
Study Type and Duration	
GDM criteria	
Type of sample/ collection site	
Method of gut microbiota evaluation	
Major findings	