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Lactogenic hormones in relation to maternal metabolic health in pregnancy and postpartum: protocol for a systematic review

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ABSTRACT

Introduction: Maternal metabolic disease states (such as gestational and pre-gestational diabetes, and maternal obesity) are reaching epidemic proportions worldwide and are associated with adverse maternal and fetal outcomes. Despite this, their aetiology remains incompletely understood. Lactogenic hormones, namely human placental lactogen and prolactin, play often overlooked roles in maternal metabolism and glucose homeostasis during pregnancy and (in the case of prolactin) postpartum, and have clinical potential from a diagnostic and therapeutic perspective. This manuscript presents a protocol for a systematic review which will synthesise the available scientific evidence linking these two hormones to maternal and fetal metabolic conditions/ outcomes.

Methods and analysis: Medline (via OVID), CINAHL and EMBASE will be systematically searched for original observational and interventional research articles linking human placental lactogen and/ or prolactin levels (in pregnancy and/ or up to 12 months postpartum) to key maternal metabolic conditions/ outcomes (including pre-existing and gestational diabetes, markers of glucose/ insulin metabolism, postpartum glucose status, weight change obesity and polycystic ovary syndrome). Relevant fetal outcomes (birthweight and placental mass, macrosomia and growth restriction) will also be included. Two reviewers will assess articles for eligibility according to pre-specified selection criteria, followed by full text review, quality appraisal and data extraction. Where possible, meta-analysis will be performed, otherwise a narrative synthesis of findings will be presented.

Ethics and dissemination: Formal ethical approval is not required as no primary data will be collected. The results will be published in a peer-reviewed journal and presented at conference meetings, and will be used to inform future research directions.

PROSPERO registration details receipt 262771, CRD registration number pending

ARTICLE SUMMARY

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Novel and relevant research area linking lactation hormones to maternal metabolic health, with particular relevance to pregnancies affected by obesity and/ or diabetes
- Protocol is for the first systematic review in this area
- Employs rigorous, standardised methodology; and will involve an exhaustive literature search and quality appraisal
- Limitations include the anticipated heterogeneity in study designs, most of which will likely be observational in nature and hence unable to establish causality.

INTRODUCTION

Pregnancy entails profound maternal physiological and metabolic adaptations to accommodate the needs of the growing fetus, and to prepare for lactation. An increase in insulin resistance of 50-60% between pre-pregnancy and the late third trimester is a physiologic change in every pregnancy (regardless of glucose tolerance), and is essential to prioritise the delivery of glucose across the placenta for fetal development [1]. This is paralleled - in a normal pregnancy - by adaptive changes in the islets of the maternal endocrine pancreas to allow increasing insulin synthesis and secretion, including an increased beta-cell mass. Overall, this results in maintenance of maternal glucose homeostasis [1].

Gestational diabetes mellitus (GDM) may develop when there is failure to balance insulin secretion with the composite of pre-pregnancy and pregnancy-induced insulin resistance, and is an increasingly prevalent condition (affecting between 2 and 38% of pregnant women worldwide) [2]. GDM is associated with multiple adverse maternal and fetal outcomes, including macrosomia, pre-eclampsia and gestational hypertension, polyhydramnios, stillbirth, and neonatal hypoglycaemia; as well as an increased lifetime risk of obesity and dysglycaemia in the offspring [3]. In women with pre-existing diabetes mellitus (type 1 or type 2), superimposed pregnancy-induced insulin resistance exacerbates established pre-gestational insulin resistance and/ or deficiency, with similar potential complications.

Lactogenic hormones, chiefly human placental lactogen (hPL) and prolactin (PRL), are well-recognised for their roles in the antenatal preparation of the breast for lactation, and – in the case of PRL – in establishing and maintaining lactation after delivery. However, these hormones also have central roles in maternal metabolism: during gestation, both contribute to insulin resistance but are also likely to act as stimuli for the adaptation of maternal pancreatic islet function. Postpartum, the hormonal control of lactation (primarily mediated by PRL) may

fundamentally alter carbohydrate and lipid metabolism and adipocyte biology, guarding lactating postpartum women against progression to type 2 diabetes [4].

Human placental lactogen is a peptide hormone produced by the placenta. It is detectable as early as 6 weeks' gestation and increases across gestation, peaking at around 30 weeks. The secretion rate of hPL near term is about 1g/ day (a rate considerably greater than that of any other protein hormone) [5] and the peak concentration of hPL is at least 25-fold that of PRL [4]. hPL binds with high affinity to the PRL receptor, and is increasingly recognised as playing a major role in the modulation of maternal metabolism to meet the energy requirements of the growing fetus [6]. It is also involved in lactogenesis I (secretory initiation), supporting alveolar and ductal growth in the breast in preparation for milk production [5].

As one of the major 'diabetogenic' hormones of pregnancy (alongside placental growth hormone, progesterone, cortisol, and PRL), hPL increases maternal insulin resistance and reduces maternal glucose utilisation, elevating maternal blood glucose levels (supporting transplacental glucose transfer and adequate fetal nutrition) [4]. However, this appears to be matched by upregulation of insulin secretory capacity. In rodent models, placental lactogens significantly increase glucose-induced insulin secretion, beta-cell proliferation and survival in isolated pancreatic islets [7-9]. In humans, in vitro evidence using human islet cell tissue suggests that hPL also acts (likely via the PRL receptor) on the endocrine pancreas to promote maternal beta-cell function, enhancing insulin synthesis and glucose-stimulated insulin secretion [9]. The net effect of this is – in a healthy pregnancy – maintenance of maternal normoglycaemia.

Human placental lactogen also increases lipolysis and release of free fatty acids (FFAs). With maternal fasting, hPL release increases the availability of FFAs to the mother for use as fuel; sparing glucose and amino acids for placental transport and fetal nutrition [10]. hPL is also likely to play a role in inducing and maintaining the state of physiological hyperleptinaemia

but relative leptin-resistance seen in pregnancy, which provides maternal appetite stimulus even with increasing adipose deposition [4]. Human placental lactogen (and PRL) also seem to increase appetite and food intake via other mechanisms, with widespread distribution of PRL receptors in the hypothalamus and induction of hyperphagia after intracerebroventricular administration suggesting a central mode of action [11].

Being placentally-derived, hPL is also positively correlated with birthweight and placental mass; with potential clinical application in the antenatal prediction of macrosomia and/ or fetal growth restriction in both metabolically-normal and abnormal pregnancies [12].

Prolactin is a peptide hormone produced by lactotrophs in the anterior pituitary gland, and has close structural homology to hPL. Basal serum PRL increases progressively during normal pregnancy, with peak values in late gestation approximately 10-fold higher than pre-conception [4]. Whilst best known for its lactogenic effect on the female mammary gland, PRL also alters insulin sensitivity and lipid metabolism. PRL induces insulin resistance outside of pregnancy (as demonstrated in non-pregnant prolactinoma patients with pathological PRL elevation) [13]; and, like hPL, is likely to contribute to the insulin resistant state of pregnancy, ensuring the availability of glucose for the fetal-placental compartment. However, the physiological contribution of PRL to glucose tolerance in pregnancy and postpartum is thought to differ from other states of relative or absolute hyperprolactinaemia [4]. In vitro evidence suggests that PRL (like hPL) can directly enhance insulin secretion from human islets, although the latter hormone may have the dominant effect during human pregnancy due to its higher concentrations [9]. It is worth noting that rodent evidence for the effect of PRL on maternal beta-cell function during pregnancy is striking: knockout mice specifically lacking PRL receptors on pancreatic beta-cells have normal glucose tolerance outside of pregnancy, but become progressively glucose intolerant with gestation due to corresponding failure of beta-cell proliferation – essentially, developing GDM [14, 15].

Postpartum, physiological hyperprolactinaemia is the key endocrine change responsible for the initiation and maintenance of lactation. Prolactin concentrations during lactation are intermediate between those in the non-pregnant state and those in late pregnancy, and the pulsatile nature of secretion (lost during pregnancy) is restored. PRL surges occur following nursing, and peaks are higher in women who exclusively breastfeed their infants than in those who supplement with formula or only feed formula. In women who do not breastfeed, PRL falls to non-pregnant concentrations within 3 weeks postpartum [4].

Lactation – under the chief control of PRL – is a unique metabolic state associated with an elevation of plasma FFAs, and with the mobilisation of lipids from diet and adipose stores to the breast for milk production. Observational evidence suggests that lactation is associated with maternal metabolic benefits, with consistent findings of lower rates of persistent postpartum dysglycaemia and progression to type 2 diabetes in women who breastfeed compared with those who do not (both in the general population [16] and following GDM pregnancy [17]). As such, PRL may link effective and sustained lactogenesis to improved maternal metabolic status postpartum. Whether this is primarily mediated by improved insulin secretory capacity or reduced insulin resistance remains unclear, as there are putative biological mechanisms for both [4, 18, 19]. Regardless, lactation may present a particular window of opportunity for women with postpartum insulin resistance (relevant to many women following a GDM pregnancy) to significantly improve long-term health outcomes by improving insulin secretion and/or sensitivity. Indeed, some authors have argued that lactation (quite apart from its other benefits to mother and offspring) may be seen as a therapeutic intervention in this patient cohort, analogous to the prescription of an insulin-sensitising medication [4].

It is also increasingly apparent that the relationship between impaired glucose/ insulin metabolism and poor lactation outcomes may be bidirectional. Whilst lactation outcomes are not the focus of this review, women with obesity and/ or diabetes are at increased risk of lactogenesis delay and persistent poor milk supply [20, 21], reasons for which may include a

suboptimal PRL response to infant suckling [22] and impaired insulin-receptor dynamics at the level of the lactocyte [23]. Authors linking PRL to glucose dynamics during lactation have suggested that "good beta-cell plasticity" in metabolically-healthy women may exert a permissive effect on lactation, allowing PRL to play its primary evolutionary role [18]. As such, the women who stand to benefit most from the metabolic benefits of sustained lactation may face the most barriers to achieving it. A more complete understanding of lactogenic hormone action, and how it is altered in metabolically-abnormal pregnancies, is essential to promote and support lactation in this population.

Narrative reviews (which constitute the majority of the existing work in this area, and have produced many of the current mechanistic hypotheses) are often incomplete or reach subjective conclusions. Systematic reviews focused on key physiological questions are under-utilised in contemporary endocrine literature, and provide an opportunity to move toward extensive synthesis with objective, evidence-based conclusions. This review aims to systematically examine the relationship between hPL and PRL and maternal metabolism in pregnancy and postpartum, particularly in relation to common gestational metabolic conditions; as well as the association between hPL and PRL and key fetal outcomes. It also aims to provide mechanistic insights and to examine the clinical implications of these findings, from both a diagnostic and therapeutic perspective.

SYSTEMATIC REVIEW QUESTION

In pregnant women (participants) what is the relationship between hPL/ PRL levels (exposures) and

(a) maternal gestational metabolic status/ outcomes?

(b) relevant fetal outcomes?

(c) maternal metabolic outcomes up to 12 months postpartum?

METHODS/ DESIGN

Rigorous international gold-standard methodology will be adopted in this review, which will conform to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [24]. This review has been submitted for registration in the international prospective register of systematic reviews (PROSPERO), receipt code 262771 (CRD number pending)**.** We used the PRISMA-P checklist when writing this protocol paper [25]. Any future amendments to this protocol will be reported on PROSPERO and published with the results of the review.

ELIGIBILITY CRITERIA

Selection criteria using a modified version of the Participant, Exposure, Comparison, Outcome and Study Type (PECOT) framework [26] (table 1), established a priori, will be used to determine the eligibility of articles to include in this review. There will be no date or language limits for eligibility. It should be noted that the review aims to elucidate the *relationship* between maternal serum hPL/PRL levels and metabolic/ fetal conditions/ outcomes, without assuming causality or directionality. The designation of hPL and PRL levels as 'exposure' and the listed outcomes as 'outcomes' is somewhat arbitrary and may not apply to all studies: some may work in the opposite direction. For example, studies that enrol women with pre-existing diabetes or GDM (relevant metabolic exposure) and look at PRL and hPL levels across gestation (outcome) would warrant inclusion. It is acknowledged by the reviewers that the relationship between lactogenic hormones and maternal metabolism is likely bidirectional; and the inclusion criteria will reflect this.

Table 1: Modified PECOT framework for study inclusion/ exclusion

	Participants (P)	Exposure (E)	Comparison (C)	Outcomes (O)	Study types (T)
Inclusion criteria	Pregnant women Women up to 12 months postpartum (regardless of lactation status)	Endogenous maternal serum hPL* (recorded at least once during pregnancy) **OR** Endogenous maternal serum PRL (recorded at least once during pregnancy and/ or up to 12 months postpartum)	Studies with any /multiple control group/s or no control group will be included	**Maternal:** Glucose status during pregnancy and up to 12 months postpartum (pre-existing diabetes [any type] or GDM) Metabolic indices related to maternal glucose/ lipid metabolism (e.g. glucose measurements, insulin secretion, sensitivity/ resistance, beta-cell function) during pregnancy or postpartum Obesity, gestational weight gain Postpartum weight change Polycystic ovary syndrome Lipid profile **Infant:** Birthweight (absolute / centiles, macrosomia) Growth restriction Placental mass	Longitudinal cohort Case control Cross-sectional studies Randomised controlled trials Clinical observational human trials (eg. infusion/ clamp studies) Systematic reviews (to be examined for eligible articles)
Exclusion criteria	Non-pregnant populations Males Pathological / iatrogenic elevation of PRL (e.g. prolactinoma, medication-induced hyperprolactinaemia) or hPL (e.g. molar pregnancy)	hPL/PRL levels in other fluids (e.g. amniotic fluid), in fetus or infant, or in cord blood hPL/PRL administered exogenously Trials examining an intervention/ procedure (e.g. amniocentesis, induction of labour, drug treatment) with hPL/PRL as outcome Trials focused on ART and ART outcomes Trials examining 'lactation' as exposure (in relation to metabolic outcomes) without PRL measured	None	Outcomes unrelated to named key maternal metabolic or infant outcomes, (e.g. placental function / perfusion / blood flow without mention of weight or FGR [e.g. Doppler indices alone]) Fetal structural abnormalities/ congenital malformations Miscarriage / pregnancy loss Diabetic retinopathy Lactation outcomes / parameters (milk transfer, milk production, infant weight change during breastfeeding)	Animal studies In vitro/ tissue culture studies Narrative reviews Commentaries/ letters Case reports Conference abstracts Expert opinion Protocol papers

*alternative name, *human chorionic somatomammotropin*, also included in search (and studies eligible for inclusion)
Abbreviations: hPL, human placental lactogen; PRL, prolactin; ART, assisted reproductive technologies; GDM, gestational diabetes mellitus; FGR, fetal growth restriction.

SEARCH STRATEGY

A systematic search strategy using relevant search terms, in accordance with the selection criteria (Table 1) has been developed (see *supplementary material 1*), in consultation with expert subject librarians. A combination of keywords and database-specific subject headings will be used. The following electronic databases will be searched:

- MEDLINE via OVID
- MEDLINE ePub ahead of print, in-process, in-data review and other non-indexed citations via OVID
- CINAHL plus
- EMBASE

Bibliographies of relevant studies identified by the search strategy, and relevant reviews/ meta-analyses, will also be manually searched for identification of additional eligible studies.

INCLUSION OF STUDIES

References will be screened and managed using EndNote x9 and Covidence software. Two reviewers will scan the titles, abstracts and keywords of every record retrieved by the search strategy, assessing eligibility according to the inclusion and exclusion criteria in Table 1 (and in consultation with a third reviewer where required). A pilot test of the selection criteria will be conducted on 20-30 article titles and abstracts in order to refine and clarify the criteria prior to the formal commencement of screening.

If initial information suggests that an article meets the selection criteria for eligibility, the full text will be retrieved for further assessment by two reviewers. Disagreement between reviewers as to whether a study meets inclusion criteria will be resolved by discussion, with referral to a third reviewer if consensus cannot be reached. Studies excluded based on full text review will

be tabulated along with reasons for their exclusion. Following PRISMA guidelines [24], a flow diagram will be created to illustrate the selection process.

QUALITY APPRAISAL OF THE EVIDENCE

Methodological quality of the included studies will be assessed by two independent reviewers using criteria established *a priori*, outlined in the Monash Centre for Health Research and Implementation (MCHRI) Evidence Synthesis Program critical appraisal template [27], see *supplementary material 2*. Individual quality items will be investigated using a descriptive component approach. Assessment will be based on criteria relating to external validity (population, setting, clarity of study objectives, inclusion and exclusion criteria, appropriateness of study design, and follow-up) and internal validity (selection, performance and detection bias, attrition, exposure and outcome measurement, reporting bias and potential confounders). Other domains for assessment will include potential conflicts of interest, study power, and appropriateness/ quality of statistical methodology. Any disagreement or uncertainty will be resolved by discussion among review authors. Using this approach, each study will be allocated a risk of bias rating.

DATA EXTRACTION

Data will be extracted from all included studies by two independent reviewers, using a specifically-developed data extraction form. Pilot testing of the form will be conducted using 3-5 studies of different formats to ensure all required data are captured, particularly given the anticipated heterogeneity in study design. Key anticipated domains for extraction are shown in Table 2. Relevant data which are not reported in published studies will be requested from corresponding authors.

Table 2. Key domains for data extraction

Study	First author Journal Country and year of publication Study design Follow-up duration
Participants	Number of participants Participant characteristics (at baseline) - Baseline (pre-pregnancy) metabolic conditions, if present - Mean age - Parity - Ethnicity - Singleton/ multiple pregnancy - Gestation at enrolment/ recruitment - Mean BMI - Delivery mode - Breastfeeding status If control group present, control characteristics (at baseline) - Mean age - Parity - Ethnicity - Singleton/ multiple pregnancy - Gestation at enrolment/ recruitment - Mean BMI - Delivery mode - Breastfeeding status
Exposure* (lactogenic hormone)	Hormone measured (hPL/ PRL/both) Number of timepoints Time points (pregnancy), with concentration and units of hormone at each time point Time points (postpartum), with concentration and units of hormone at each time point
Key maternal metabolic outcome(s)* of interest *Glucose status in pregnancy (pre-existing diabetes mellitus of any type OR gestational diabetes)* *Postpartum glucose status* *Continuous metabolic indices related to maternal glucose/ lipid metabolism* *e.g. measures of* *-fasting glucose* *-1h and 2h OGTT glucose* *-insulin secretion* *-insulin sensitivity* *-insulin resistance* *-beta-cell function* *(during pregnancy or postpartum)* *Gestational weight gain* *Obesity* *Postpartum weight change* *Polycystic ovary syndrome* *Lipid profile (total cholesterol, HDL and LDL cholesterol, triglycerides)*	Key maternal "outcomes" assessed (from list)* For diabetes in pregnancy - Pre-existing (T1/T2DM) or gestational - Gestation at diagnosis - Method used for diagnosis (eg OGTT) - Diagnostic criteria if stated - Treatment (diet/ oral medications/ insulin); and treatment commencement timepoint For postpartum glucose status - Time point - Method used for diagnosis (eg OGTT) - Diagnostic criteria, if stated - Prevalence of persistent dysglycaemia postpartum Relationship of said outcome(s) to hPL/PRL levels (as t-test result, odds ratio, regression coefficient etc) - Unadjusted - After adjustment (with list of covariates included in model/s) Conclusions regarding the above
Key infant metabolic outcome(s)* of interest *Birthweight (absolute/ centiles)*	Key infant outcomes assessed (from list)

Macrosomia *Growth restriction* *Placental mass*	Relationship of said outcome to hPL/PRL levels (as t-test result, odds ratio, regression coefficient etc) - Unadjusted - After adjustment (with list of covariates included in model/s) Conclusions regarding the above

*Due to the likely bidirectional nature of the lactogenic hormone/ maternal metabolism relationship, some studies will consider hPL/PRL as 'exposure' and a metabolic parameter (e.g. postpartum glucose tolerance) as 'outcome'. Others may consider a metabolic parameter (e.g maternal pre-gestational DM) as 'exposure' with hPL/PRL levels during pregnancy, in comparison to healthy controls, as 'outcome'. The extraction template will accommodate both.

Abbreviations: hPL, human placental lactogen; PRL, prolactin; BMI, body mass index; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; OGTT, oral glucose tolerance test; HDL, high density lipoprotein; LDL, low density lipoprotein

STATISTICAL ANALYSIS

Analysis for the two lactogenic hormones of interest, hPL and PRL, will be undertaken separately. Key exposure / outcome associations for each hormone will be determined based on the number of studies available. It is anticipated that hPL will be analysed primarily in relation to maternal metabolic / glycaemic status during pregnancy, and to fetal outcomes (birthweight, macrosomia, growth restriction, placental mass). For PRL, it is anticipated that key outcomes will be maternal metabolic / glycaemic status and related maternal metabolic indices (measures of insulin secretion, sensitivity and beta-cell function) both during pregnancy and postpartum. After data extraction, the reviewers will determine whether meta-analysis is appropriate (based on the number of studies for each hormone/outcome relationship and the heterogeneity of their designs and participant groups). If meta-analysis is possible, Review Manager statistical software will be used for analysis with random effects models employed to generate weighted mean differences. Statistical heterogeneity will be assessed using the I^2 test, with I^2 values > 50% indicating moderate to high heterogeneity. Sensitivity analyses will be performed where applicable to explore the effects of studies with high risk of bias on the overall results. Subgroup analyses will also be performed where possible (for example; by type of diabetes). Where a meta-analysis is not possible, a narrative synthesis of results will be performed.

Data will be presented in summary tables and in narrative format to describe the populations, exposures and key outcomes of the included studies. Forest plots and funnel plots will be used to present results from meta-analyses (where applicable) and publication bias assessments, respectively. Meta-analysis results will be reported according to PRISMA guidelines [24].

ETHICS AND DISSEMINATION

This project will collate aggregate data from published studies (or aggregate data provided by study investigators upon request), and thus ethical approval will not be required.

Findings will be disseminated via publications in peer-reviewed journals and presentations at scientific meetings. If deemed appropriate, findings will also be communicated to relevant stakeholders to guide clinical practice and public health actions in this area.

DATA AVAILABILITY STATEMENT

No data have been generated or analysed in this manuscript.

PATIENT AND PUBLIC INVOLVEMENT STATEMENT

It was not feasible or appropriate to involve patients or members of the public in the design, planning or conduct of the planned research.

DISCUSSION

The proposed review will be the first, to our knowledge, to systematically collate and synthesise the existing scientific literature linking two key lactogenic hormones, hPL and PRL, to maternal metabolic health in pregnancy and postpartum (and, by extension, to infant outcomes). Systematic reviews which evaluate biomarkers or aim to explore physiological questions are rare in the endocrine literature, and represent an under-utilised opportunity to move beyond subjective, narrative work towards inclusive, extensive reviews with the potential for objective and evidence-based conclusions.

Whilst these hormones have long been recognised for their roles in the antenatal preparation of the breast for lactation and (in the case of PRL) for the postnatal initiation and maintenance of lactation, their metabolic roles have been relatively under-appreciated. Both hormones contribute to the insulin resistance associated with the pregnant state, but also potentially have central roles in the adaptation of the maternal pancreas during gestation, stimulating beta-cell adaptation and increasing beta-cell mass and insulin secretion [1, 9]. During a normal pregnancy, this may allow compensation for pregnancy-induced insulin resistance, resulting in overall maintenance of euglycaemia.

Despite likely playing a key role in the regulation of glucose and insulin dynamics during pregnancy, the relationship between hPL levels and the pathophysiology of GDM remains unclear. Several studies have investigated possible links, with some reporting no association between maternal hPL levels and GDM status [28-30] and others reporting higher hPL in GDM subjects than controls [31-33], particularly if insulin-treated [34]. For hPL levels during pregnancies affected by pre-existing diabetes (T1DM/ T2DM), the majority of authors report serially-higher hPL throughout gestation in diabetic women compared with controls [29, 31, 35-37], although other studies in T1DM have shown lower levels in the setting of poor control [38].

Importantly however, this area of the literature is particularly dated, with many studies performed well prior to the 21st century and prior to contemporary diagnostic definitions of diabetes in pregnancy. As such, the exact type of maternal diabetes among study participants is often unclear (they are simply deemed to be 'diabetic', are defined according to the now-historical White's Classification of diabetes in pregnancy [39], or are termed 'insulin-dependent') [31, 32, 35-38]. Such studies provide valuable basic insights into the pathophysiology of the lactogen/maternal metabolism relationship, but comparison to the available better-described contemporary cohorts [28] will present challenges. Furthermore, higher hPL levels are clearly related to increased placental weight and macrosomia, and several

authors have suggested that increased levels of hPL in many diabetic pregnancies may simply reflect higher placental mass [31, 35]. This does not suggest it is aetiologically unimportant, however – it is possible that the placentomegaly seen in maternal diabetes causes higher hPL levels, stimulating maternal and fetal beta-cell expansion and increasing fetal insulin production, thus promoting glycogenesis, fat deposition and further fetal growth [6].

Acknowledging these challenges, a better understanding of the role of hPL in metabolically-abnormal pregnancies has potential clinical application. For example, accurate antenatal prediction of fetal macrosomia remains challenging, and current macrosomia prediction strategies (including physical examination and ultrasound assessment) are both resource-intensive and imprecise. There is thus a clear requirement for maternal serum biomarkers in improving antenatal macrosomia prediction, particularly in women at high risk of the outcome (such as those with pre-gestational diabetes or GDM). Whilst several candidate maternal biomarkers have been assessed for their association with birthweight or macrosomia (both in diabetic and non-diabetic pregnancies), evidence is mixed and uncertainties around clinical utility persist [40]. hPL has recently been largely overlooked as a candidate biomarker in this capacity, but previous work suggests it may have significant potential if revisited. For instance, one 1998 study measured hPL at the time of GDM screening (n=257) and found that among the subset of women with a normal glucose challenge test but whose infants ultimately weighed >4000 g (n=11), mean hPL at the time of testing had in fact been similar to the mean hPL found in women with GDM [41]. This suggests that hPL may warrant evaluation as a biomarker for macrosomia prediction, both in women with diagnosed diabetes and those without.

Unlike hPL (which, as a placentally-derived hormone, is washed from the circulation following delivery), PRL has probable influence in maternal metabolism both during pregnancy and postpartum, particularly if lactation ensues. The literature here is similarly conflicting. For example, maternal serum PRL levels during GDM pregnancy have been examined by several groups, with the majority reporting similar levels to normal pregnancies [28, 30, 42]. However,

two more recent studies have directly contradicted this, finding that higher PRL levels in the first [43] and third [44] trimester of pregnancy were associated with reduced glucose tolerance on OGTT, with both groups suggesting that PRL may be independently involved in GDM pathogenesis.

Postpartum, lactation (under the chief control of PRL) appears to confer maternal metabolic benefits, but the mechanism by which this occurs is unclear. One group found that maternal serum PRL in late pregnancy was significantly higher in women who progressed to normal glucose tolerance postpartum than in those who progressed to postpartum prediabetes/ diabetes; and that higher antepartum PRL independently predicted improved postpartum insulin secretion capacity [28]. That group suggested that these findings may reflect a postpartum extension of the beneficial effects of PRL on beta-cell mass and islet adaptation that are thought to occur during gestation. Another group, who measured PRL postpartum, presented different findings and discussion: women with higher circulating PRL in the context of lactation in their study had reduced beta-cell function and lower insulin secretion indices; but were less insulin resistant [18]. Authors have suggested that this improvement in insulin resistance may result from the mobilisation of muscle and liver lipids into breast milk under the control of PRL [4], an action that may be particularly beneficial in women who are insulin resistant at baseline (women with recent GDM are known to have increased intramyocellular lipid content, IMCL, at 4-6 months post-delivery compared with controls) [45].

There is thus a clear need for a systematic review of the literature in this field – both lactogenic hormones clearly have central roles in the regulation of maternal metabolism (both during pregnancy and postpartum, and for women with normal and abnormal pregnancies). However, to date the evidence has not, to our knowledge, been effectively synthesised.

Some limitations of the review process should be noted. Firstly, owing to the intentionally-broad scope of the review, included studies will be heterogeneous in their design, methodology

and research questions. In the analysis phase, hPL and PRL will thus be considered separately and studies will be grouped according to similar outcomes; but it is possible that marked heterogeneity will preclude meaningful conclusions and statistical meta-analysis. Secondly, some of the basic clinical work on hPL and PRL levels in normal and diabetic pregnancies is now very dated, extending back to the 1970s and 1980s. Whilst robust and worthy of inclusion, differences in experimental design and (in particular) the classification and treatment of maternal diabetes will present challenges when comparing such studies to modern cohorts. If this proves problematic, we will endeavour to conduct a subgroup analysis by publication year range or otherwise perform a narrative comparison between older and newer studies. Thirdly, as previously described, the relationship between lactogenic hormones and maternal metabolism is almost certainly bidirectional, whereby some studies examine the effects of lactogenic hormones (exposure) on metabolic conditions (outcome), whilst in others, exposure and outcome are reversed. The review is designed to capture both, but – particularly in the postpartum context – the bidirectional nature of the relationship can bias observational studies. While this cannot be directly addressed in our review methodology, it will be acknowledged in the synthesis and interpretation of the findings.

CONCLUSION

In summary, this systematic review will rigorously and systematically collate and synthesise current evidence linking the key lactogenic hormones hPL and PRL to maternal metabolic health in pregnancy and postpartum (and thus to related infant outcomes). Both hormones have key roles in the maintenance of glucose homeostasis during pregnancy, including direct actions on the beta-cells of the maternal endocrine pancreas. However, the exact roles of these hormones – particularly in metabolically abnormal pregnancies – remain unclear, and evidence is conflicting. Further, hPL may have untapped potential clinical application in the antenatal prediction of macrosomia, while the hormonal control of lactation, led by PRL, may regulate glucose and lipid metabolism and help to guard postpartum women against persistent

dysglycaemia. Through this review process, the available scientific evidence will be synthesised to clarify these relationships and inform future research and practice in the field of maternal metabolic and endocrine health.

Author contributions: KR is the project lead, conceptualised and designed the protocol, wrote the first draft of the manuscript, and will coordinate and conduct the systematic review process along with co-reviewer RG. AMM has contributed to the design of the search strategy and will provide support with evidence synthesis. AM, AMM, AJ, and HJT reviewed and edited the manuscript, and AM, AJ, and HJT will provide oversight and supervision for the systematic review process. All authors contributed substantial intellectual input to the manuscript in line with ICMJE criteria for authorship and have approved the final version for publication.

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1 (pregnan* or gestation* or post?partum or post-partum or lactat* or breastfe*).ti,ab.

2 exp pregnancy/

3 postpartum period/

4 lactation/

5 Breast Feeding/

6 1 or 2 or 3 or 4 or 5

7 prolactin.ti,ab.

8 prolactin/

9 placenta* lactogen*.ti,ab.

10 placental lactogen/

11 somato-mammotropin.ti,ab.

12 somato?mammotropin.ti,ab.

13 7 or 8 or 9 or 10 or 11 or 12

14 ((pregnan* or gestation* or matern* or post?partum or post-partum or birth or f?etal or baby or infant* or newborn* or neonat*) adj1 weight*).ti,ab.

15 (placenta* adj1 weight*).ti,ab.

16 (placenta* adj1 mass*).ti,ab.

17 (diabet* or glucose or obes* or metabolic).ti,ab.

18 polycystic ovar*.ti,ab.

19 exp diabetes mellitus/

20 glucose intolerance/

21 exp diabetes, gestational/

22 obesity, maternal/

23 birth weight/

24 fetal weight/

25 polycystic ovary syndrome/

26 pregnancy outcome/

27 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26

28 6 and 13 and 27

29 exp animals/ not humans.sh.

30 28 not 29

Embase Classic+Embase <1947 to current>

1 (pregnan* or gestation* or post?partum or post-partum or lactat* or breastfe*).ti,ab.

2 exp pregnancy/

3 lactation/

4 breast feeding/

5 1 or 2 or 3 or 4

6 prolactin.ti,ab.

7 prolactin/

8 placenta* lactogen*.ti,ab.

9 placenta lactogen/

10 somato-mammotropin.ti,ab.

11 somato?mammotropin.ti,ab.

12 6 or 7 or 8 or 9 or 10 or 11

13 ((pregnan* or gestation* or matern* or post?partum or post-partum or birth or f?etal or baby or infant* or newborn* or neonat*) adj1 weight*).ti,ab.

14 (placenta* adj1 weight*).ti,ab.

15 (placenta* adj1 mass*).ti,ab.

16 (diabet* or glucose or obes* or metabolic).ti,ab.

17 polycystic ovar*.ti,ab.

18 exp diabetes mellitus/

19 glucose intolerance/

20 pregnancy diabetes mellitus/

21 maternal obesity/

22 birth weight/

23 fetus weight/

24 ovary polycystic disease/

25 pregnancy outcome/

26 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25

27 5 and 12 and 26

28 (exp animal/ or exp invertebrate/ or nonhuman/ or animal experiment/ or animal tissue/ or animal model/ or exp plant/ or exp fungus/) not (exp human/ or human tissue/)

29 27 not 28

CINAHL PLUS

S26	S6 AND S12 AND S25
S25	S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24
S24	(MH "Pregnancy Outcomes")
S23	(MH "Polycystic Ovary Syndrome")
S22	(MH "Fetal Weight")
S21	(MH "Birth Weight")
S20	(MH "Obesity, Maternal")
S19	(MH "Diabetes Mellitus, Gestational")
S18	(MH "Glucose Intolerance")
S17	(MH "Diabetes Mellitus+")
S16	polycystic ovar*
S15	diabet* or glucose or obes* or metabolic
S14	placenta* N1 (weight* OR mass*)
S13	(pregnan* or gestation* or matern* or post?partum or postpartum or birth or f?etal or baby or infant* or newborn* or neonat*) N1 weight*
S12	S7 OR S8 OR S9 OR S10 OR S11
S11	somatomammotropin
S10	(MH "Placental Hormones")
S9	"placenta* lactogen*"
S8	(MH "Prolactin")
S7	prolactin
S6	S1 OR S2 OR S3 OR S4 OR S5
S5	(MH "Breast Feeding")
S4	(MH "Lactation")
S3	(MH "Postnatal Period+")
S2	(MH "Pregnancy+")
S1	pregnan* or gestation* or post?partum or postpartum or lactat* or breastfe*

Template for critical appraisal of a cohort study
Document evidence from the article in quotation marks.

Study ID		
Study citation		
EXTERNAL VALIDITY – IS THIS STUDY AND ITS RESULTS GENERALIZABLE TO MY SYSTEMATIC REVIEW QUESTION?		
Patient/population/ participants	Describe whether they were gender specific, had a particular condition or the general population, age and any other relevant characteristics (e.g. BMI)	
Control population (if appropriate)	Describe whether they were gender specific, had a particular condition or the general population, age and any other relevant characteristics (e.g. BMI)	
N	Where possible, list the number of participants that were: • Screened • Enrolled • Allocated/randomised • Assessed • Followed up	
Setting	List where the intervention was conducted and assessed ie. hospital, clinic, community and/or university setting.	
Intervention/indicator	Describe the intervention in as much detail as possible e.g. medication type, dose, duration, intervals.	
Comparison/control	Describe the comparison in as much detail as possible e.g. medication type, dose, duration, intervals.	
Outcomes	List what the study measured (e.g. weight, BMI, HbA1c) as primary outcomes and secondary outcomes. If the outcomes are not relevant to your systematic review, list these as measured but not relevant to your systematic review.	
Does the study have a clearly focused question and/or PICO?	Yes Partial No Not reported	Consider if the question is 'focused' in terms of: – the population studied – the intervention given or exposure - the comparison(s) – the outcomes considered
Inclusion Criteria	Yes No Not reported	
Exclusion Criteria	Yes No Not reported	
If there were specified inclusion/ exclusion criteria, were these appropriate?	Yes Partial No N/A	Consider if: - the eligibility criteria used to specify the patients, interventions/ exposures and outcomes of interest.
Is a cohort study the appropriate design to answer this question?	Yes Partial No	Consider if a cohort study is a good way of answering the question under the circumstances.
Were the outcomes measured appropriate?	Yes Partial No Not reported	Consider if the outcomes measured are appropriate and important outcome.
Was there sufficient duration of follow-up for outcomes to occur?	Yes Partial No Not reported	May need to check with clinicians regarding what is sufficient duration for important events to occur. An acceptable length of time should be decided before quality/risk of bias assessment begins.

INTERNAL VALIDITY – HAS THIS STUDY BEEN CONDUCTED RIGOROUSLY IN ORDER TO REDUCE BIAS?			
SELECTION BIAS	**Other than the exposure under investigation, were the groups selected from similar populations?**	Yes Partial No Not reported	Consider: - whether the different sources would affect outcomes e.g. one group recruited from hospital(s) the other from the community. - time periods i.e. historical cohort - whether there is a large difference in participation rate between the two arms of the study.
	Was the exposed cohort truly representative?	Yes Partial No Not reported	This item is assessing the representativeness of exposed individuals in the community relevant to the study's PICO, not the representativeness of the sample of individuals in the general population. Consider: - whether truly representative in the community (least bias) - whether somewhat representative (some bias) - whether selected group of users (bias) - no description of the derivation of the cohort (most bias)
	Is it clear that the outcome of interest was not present at the start of study?	Yes Partial No Not reported	In the case of mortality studies, outcome of interest is still the presence of a disease/ incident, rather than death. That is to say that a statement of no history of disease or incident is least biased.
PERFORMANCE BIAS	**Aside from the exposure, were the groups treated the same?**	Yes Partial No Not reported	To be sure it's the exposure which is responsible for the effect.
DETECTION BIAS	**Was exposure measured in a standard, valid and reliable way?**	Yes Partial No Not reported	Where exposure measures require any degree of subjectivity, some evidence should be provided that the measures used are reliable and have been validated prior to their use in the study. Consider whether ascertainment of exposure was determined by: - secure record (eg surgical records) (least bias) - structured interview - written self report (bias) - no description (most bias)
	Were outcome assessors blind to the exposure?	Yes Partial No Not reported	Consider: - If the outcome is objective (e.g. death) then blinding is less critical. - If the outcome is subjective (e.g. symptoms or function) then blinding of the outcome assessor is critical.
	Were all outcomes measured in a standard, valid and reliable way?	Yes Partial No Not reported	Where outcome measures require any degree of subjectivity, some evidence should be provided that the measures used are reliable and have been validated prior to their use in the study. For some outcomes (e.g. fractured hip), reference to the medical record is sufficient to satisfy the requirement for confirmation of the fracture. This would not be adequate for vertebral fracture outcomes where reference to x-rays would be required. Consider whether outcomes were determined through: - independent blind assessment or confirmation of the outcome by reference to secure records (x-rays, medical records, etc.) (least bias) - record linkage (e.g. identified through codes on database records) - self report (i.e. no reference to original medical records or x-rays to confirm the outcome) (bias) - no description (most bias)

	Were outcomes assessed objectively and independently?	Yes Partial No Not reported	Independence of assessment is important where the result of one outcome may effect the interpretation of another. When outcomes are objectively assessed, their independence from each other is less important.
ATTRITION BIAS	**What percentage of the individuals recruited into each arm of the study were lost to follow up?**	X% treatment X% control/ comparison Not reported	Consider: - if all patients who entered the trial were properly accounted for and attributed at its conclusion. - why patients dropped out, as well as how many. - the drop out rate may be expected to be higher in studies conducted over a long period of time. - if comparisons were made between participants followed-up and those lost to follow up, by exposure status.
	What percentage of the individuals were not included in the analysis?	X% treatment X% control/ comparison Not reported	Consider: - if analysis was as per protocol or intention to treat - number of crossovers - reason for crossover
REPORT BIAS	**Is the paper free of selective outcome reporting?**	Yes Partial No Not reported	Consider: - if all the planned outcomes were measured - if all the measured outcomes were reported - if any additional or composite outcomes were measured. This is difficult to determine if there isn't a protocol.
CONFOUNDING	**Are the cohorts comparable on the basis of design or analysis?**	Yes Partial No Not reported	Consider - either exposed and non-exposed individuals must be matched in the design and/or confounders must be adjusted for in the analysis. - statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. Note: If the relative risk for the exposure of interest is adjusted for the confounders listed, then the groups will be considered to be comparable on each variable used in the adjustment.
OTHER INTERNAL VALIDITY/BIAS	**Were there any conflicts of interest in the writing or funding of this study?**	Yes No Not reported	Consider: - if any of the authors are/were employed, sponsored etc by pharmaceutical companies, or have other financial/other ties - if any commercial companies were involved in funding, writing, editing, data analysis or manuscript approval
	Was the study sufficiently powered to detect any differences between the groups?	Yes Partial No Not reported	Consider: - if an adequate sample size calculation was undertaken - if the required sample size recruited and retained - for which outcomes the study was powered - if confidence intervals include a clinically important difference, the study was underpowered NB this is less important if significant differences were found.
	If statistical analysis was undertaken, was this appropriate?	Yes Partial No Not reported N/A	Consider: - whether the authors performed any statistical tests or just presented figures - if the statistical analysis was planned a priori - if the data were analysed accordingly to the study protocol. - the type of data and the statistical tests used. (Please refer to the CCE workbook as required) - use of parametric versus non-parametric tests; whether the data has been checked for normality - if the tests used are obscure, why did the authors used them and have they included a reference. - if point estimates and measures of variability were presented for the primary outcome

		- if subgroups were analysed appropriately - if potential confounders were identified and taken into account in the analysis - if there was any adjustment made for multiple testing - if missing data was handled appropriately
Comments	*Add any other relevant comments, including if this is likely to influence the results of the study*	
What is the overall risk of bias?	Low Moderate High Insufficient information	*Low - All of the criteria have been fulfilled or where criteria have not been fulfilled it is very unlikely the conclusions of the study would be affected.* *Moderate - Some of the criteria have been fulfilled and those criteria that have not been fulfilled may affect the conclusions of the study.* *High - Few or no criteria fulfilled or the conclusions of the study are likely or very likely to be affected.* *Insufficient information – not enough information provided on methodological quality to be able to determine risk of bias.*

Cited in full as: Monash Centre for Health Research and Implementation (MCHRI) Evidence Synthesis Program template for critical appraisal of a cohort study (2014), MCHRI – Monash University and Monash Health, Melbourne, Australia (*adapted from* Critical Appraisal Templates (2010) Centre for Clinical Effectiveness, Southern Health, Melbourne, Australia AND Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle–Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. In: Proceedings of the 3rd Symposium on Systematic Reviews Beyond the Basics: Improving Quality and Impact. Oxford; 2000. p. 3-5).

Reporting checklist for protocol of a systematic review and meta analysis.

Based on the PRISMA-P guidelines.

		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a - not an update
Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	20
Amendments			

	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	9
Support			
Sources	#5a	Indicate sources of financial or other support for the review	20
Sponsor	#5b	Provide name for the review funder and / or sponsor	n/a - no specific funding
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	20
Introduction			
Rationale	#6	Describe the rationale for the review in the context of what is already known	4-8
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	8
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	9-10

Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	11
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	11
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	11
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	11-12
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	12-14
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	13-14
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	13-14

Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	12
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	14
Data synthesis	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	14-15
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	14-15
Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	14
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	n/a - not planned
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	12

Notes:

- 1b: n/a - not an update

- 5b: n/a - no specific funding
- 16: n/a - not planned The PRISMA-P elaboration and explanation paper is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 07. July 2021 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

Lactogenic hormones in relation to maternal metabolic health in pregnancy and postpartum: protocol for a systematic review

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Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Reproductive medicine, Obstetrics and gynaecology
Keywords:	Diabetes & endocrinology < INTERNAL MEDICINE, Diabetes in pregnancy < DIABETES & ENDOCRINOLOGY, Maternal medicine < OBSTETRICS

Lactogenic hormones in relation to maternal metabolic health in pregnancy and postpartum: protocol for a systematic review

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Supplementary materials 1 – search strategy

Supplementary materials 2 – critical appraisal template

Keywords: diabetes, gestational; diabetes mellitus; obesity, maternal; prolactin; placental lactogen

ABSTRACT

Introduction: Maternal metabolic disease states (such as gestational and pre-gestational diabetes, and maternal obesity) are reaching epidemic proportions worldwide and are associated with adverse maternal and fetal outcomes. Despite this, their aetiology remains incompletely understood. Lactogenic hormones, namely human placental lactogen and prolactin, play often overlooked roles in maternal metabolism and glucose homeostasis during pregnancy and (in the case of prolactin) postpartum, and have clinical potential from a diagnostic and therapeutic perspective. This manuscript presents a protocol for a systematic review which will synthesise the available scientific evidence linking these two hormones to maternal and fetal metabolic conditions/ outcomes.

Methods and analysis: Medline (via OVID), CINAHL and EMBASE will be systematically searched for all original observational and interventional research articles, published prior to 8 July 2021, linking human placental lactogen and/ or prolactin levels (in pregnancy and/ or up to 12 months postpartum) to key maternal metabolic conditions/ outcomes (including pre-existing and gestational diabetes, markers of glucose/ insulin metabolism, postpartum glucose status, weight change, obesity and polycystic ovary syndrome). Relevant fetal outcomes (birthweight and placental mass, macrosomia and growth restriction) will also be included. Two reviewers will assess articles for eligibility according to pre-specified selection criteria, followed by full text review, quality appraisal and data extraction. Where possible, meta-analysis will be performed, otherwise a narrative synthesis of findings will be presented.

Ethics and dissemination: Formal ethical approval is not required as no primary data will be collected. The results will be published in a peer-reviewed journal and presented at conference meetings, and will be used to inform future research directions.

PROSPERO registration details CRD42021262771.

ARTICLE SUMMARY

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Novel and relevant research area linking lactation hormones to maternal metabolic health, with particular relevance to pregnancies affected by obesity and/ or diabetes
- Protocol is for the first systematic review in this area
- Employs rigorous, standardised methodology; and will involve an exhaustive literature search and quality appraisal
- Limitations include the anticipated heterogeneity in study designs, most of which will likely be observational in nature and hence unable to establish causality.

INTRODUCTION

Pregnancy entails profound maternal physiological and metabolic adaptations to accommodate the needs of the growing fetus, and to prepare for lactation. An increase in insulin resistance of 50-60% between pre-pregnancy and the late third trimester is a physiologic change in every pregnancy (regardless of glucose tolerance), and is essential to prioritise the delivery of glucose across the placenta for fetal development [1]. This is paralleled - in a normal pregnancy - by adaptive changes in the islets of the maternal endocrine pancreas to allow increasing insulin synthesis and secretion, including an increased beta-cell mass. Overall, this results in maintenance of maternal glucose homeostasis [1].

Gestational diabetes mellitus (GDM) may develop when there is failure to balance insulin secretion with the composite of pre-pregnancy and pregnancy-induced insulin resistance, and is an increasingly prevalent condition (affecting between 2 and 38% of pregnant women worldwide) [2]. GDM is associated with multiple adverse maternal and fetal outcomes, including macrosomia, pre-eclampsia and gestational hypertension, polyhydramnios, stillbirth, and neonatal hypoglycaemia; as well as an increased lifetime risk of obesity and dysglycaemia in the offspring [3]. In women with pre-existing diabetes mellitus (type 1 or type 2), superimposed pregnancy-induced insulin resistance exacerbates established pre-gestational insulin resistance and/ or deficiency, with similar potential complications.

Lactogenic hormones, chiefly human placental lactogen (hPL) and prolactin (PRL), are well-recognised for their roles in the antenatal preparation of the breast for lactation, and – in the case of PRL – in establishing and maintaining lactation after delivery. However, these hormones also have central roles in maternal metabolism: during gestation, both contribute to insulin resistance but are also likely to act as stimuli for the adaptation of maternal pancreatic islet function. Postpartum, the hormonal control of lactation (primarily mediated by PRL) may

fundamentally alter carbohydrate and lipid metabolism and adipocyte biology, guarding lactating postpartum women against progression to type 2 diabetes [4].

Human placental lactogen is a peptide hormone produced by the placenta. It is detectable as early as 6 weeks' gestation and increases across gestation, peaking at around 30 weeks. The secretion rate of hPL near term is about 1g/ day (a rate considerably greater than that of any other protein hormone) [5] and the peak concentration of hPL is at least 25-fold that of PRL [4]. hPL binds with high affinity to the PRL receptor, and is increasingly recognised as playing a major role in the modulation of maternal metabolism to meet the energy requirements of the growing fetus [6]. It is also involved in lactogenesis I (secretory initiation), supporting alveolar and ductal growth in the breast in preparation for milk production [5].

As one of the major 'diabetogenic' hormones of pregnancy (alongside placental growth hormone, progesterone, cortisol, and PRL), hPL increases maternal insulin resistance and reduces maternal glucose utilisation, elevating maternal blood glucose levels (supporting transplacental glucose transfer and adequate fetal nutrition) [4]. However, this appears to be matched by parallel upregulation of insulin secretory capacity. In rodent models, placental lactogens significantly increase glucose-induced insulin secretion, beta-cell proliferation and survival in isolated pancreatic islets [7-9]. In humans, in vitro evidence using human islet cell tissue suggests that hPL also acts (likely via the PRL receptor) on the endocrine pancreas to promote maternal beta-cell function, enhancing insulin synthesis and glucose-stimulated insulin secretion [9]. The net effect of this is – in a healthy pregnancy – maintenance of maternal normoglycaemia.

Human placental lactogen also increases lipolysis and release of free fatty acids (FFAs). With maternal fasting, hPL release increases the availability of FFAs to the mother for use as fuel; sparing glucose and amino acids for placental transport and fetal nutrition [10]. hPL is also likely to play a role in inducing and maintaining the state of physiological hyperleptinaemia

but relative leptin-resistance seen in pregnancy, which provides maternal appetite stimulus even with increasing adipose deposition [4]. Human placental lactogen (and PRL) also seem to increase appetite and food intake via other mechanisms, with widespread distribution of PRL receptors in the hypothalamus and induction of hyperphagia after intracerebroventricular administration suggesting a central mode of action [11].

Being placentally-derived, hPL is also positively correlated with birthweight and placental mass; with potential clinical application in the antenatal prediction of macrosomia and/ or fetal growth restriction in both metabolically-normal and abnormal pregnancies [12].

Prolactin is a peptide hormone produced by lactotrophs in the anterior pituitary gland, and has close structural homology to hPL. Basal serum PRL increases progressively during normal pregnancy, with peak values in late gestation approximately 10-fold higher than pre-conception [4]. Whilst best known for its lactogenic effect on the female mammary gland, PRL also alters insulin sensitivity and lipid metabolism. PRL may induce insulin resistance outside of pregnancy (as demonstrated in non-pregnant prolactinoma patients with pathological PRL elevation) [13]; and, like hPL, is likely to contribute to the insulin resistant state of pregnancy, ensuring the availability of glucose for the fetal-placental compartment. However, the physiological contribution of PRL to glucose tolerance in pregnancy and postpartum is thought to differ from other states of relative or absolute hyperprolactinaemia [4]. In vitro evidence suggests that PRL (like hPL) can directly enhance insulin secretion from human islets, although the latter hormone may have the dominant effect during human pregnancy due to its higher concentrations [9]. It is worth noting that rodent evidence for the effect of PRL on maternal beta-cell function during pregnancy is striking: knockout mice specifically lacking PRL receptors on pancreatic beta-cells have normal glucose tolerance outside of pregnancy, but become progressively glucose intolerant with gestation due to corresponding failure of beta-cell proliferation – essentially, developing GDM [14, 15].

Postpartum, physiological hyperprolactinaemia is the key endocrine change responsible for the initiation and maintenance of lactation. Prolactin concentrations during lactation are intermediate between those in the non-pregnant state and those in late pregnancy, and the pulsatile nature of secretion (lost during pregnancy) is restored. PRL surges occur following nursing, and peaks are higher in women who exclusively breastfeed their infants than in those who supplement with formula or only feed formula. In women who do not breastfeed, PRL falls to non-pregnant concentrations within 3 weeks postpartum [4].

Lactation – under the chief control of PRL – is a unique metabolic state associated with an elevation of plasma FFAs, and with the mobilisation of lipids from diet and adipose stores to the breast for milk production. Observational evidence suggests that lactation is associated with maternal metabolic benefits, with consistent findings of lower rates of persistent postpartum dysglycaemia and progression to type 2 diabetes in women who breastfeed compared with those who do not (both in the general population [16] and following GDM pregnancy [17]). As such, PRL may link effective and sustained lactogenesis to improved maternal metabolic status postpartum. Whether this is primarily mediated by improved insulin secretory capacity or reduced insulin resistance remains unclear, as there are putative biological mechanisms for both in the postpartum context [4, 18, 19]. Regardless, lactation may present a particular window of opportunity for women with postpartum insulin resistance (relevant to many women following a GDM pregnancy) to significantly improve long-term health outcomes by improving insulin secretion and/or sensitivity. Indeed, some authors have argued that lactation (quite apart from its other benefits to mother and offspring) may be seen as a therapeutic intervention in this patient cohort, analogous to the prescription of an insulin-sensitising medication [4].

It is also increasingly apparent that the relationship between impaired glucose/ insulin metabolism and poor lactation outcomes may be bidirectional. Whilst lactation outcomes are not the focus of this review, women with obesity and/ or diabetes are at increased risk of

lactogenesis delay and persistent poor milk supply [20, 21], reasons for which may include a suboptimal PRL response to infant suckling [22] and impaired insulin-receptor dynamics at the level of the lactocyte [23]. Authors linking PRL to glucose dynamics during lactation have suggested that "good beta-cell plasticity" in metabolically-healthy women may exert a permissive effect on lactation, allowing PRL to play its primary evolutionary role [18]. As such, the women who stand to benefit most from the metabolic benefits of sustained lactation may face the most barriers to achieving it. A more complete understanding of lactogenic hormone action, and how it is altered in metabolically-abnormal pregnancies, is essential to promote and support lactation in this population.

Narrative reviews (which constitute the majority of the existing work in this area, and have produced many of the current mechanistic hypotheses) are often incomplete or reach subjective conclusions. Systematic reviews focused on key physiological questions are under-utilised in contemporary endocrine literature, and provide an opportunity to move toward extensive synthesis with objective, evidence-based conclusions. This review aims to systematically examine the relationship between hPL and PRL and maternal metabolism in pregnancy and postpartum, particularly in relation to common gestational metabolic conditions; as well as the association between hPL and PRL and key fetal outcomes. It also aims to provide mechanistic insights and to examine the clinical implications of these findings, from both a diagnostic and therapeutic perspective.

SYSTEMATIC REVIEW QUESTION

In pregnant women (participants) what is the relationship between hPL/ PRL levels (exposures) and

(a) maternal gestational metabolic status/ outcomes?

(b) relevant fetal outcomes?

(c) maternal metabolic outcomes up to 12 months postpartum?

METHODS/ DESIGN

Rigorous international gold-standard methodology will be adopted in this review, which will conform to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [24]. This review has been registered with the international prospective register of systematic reviews (PROSPERO), registration number CRD42021262771. We used the PRISMA-P checklist when writing this protocol paper [25]. Any future amendments to this protocol will be reported on PROSPERO and published with the results of the review.

ELIGIBILITY CRITERIA

Selection criteria using a modified version of the Participant, Exposure, Comparison, Outcome and Study Type (PECOT) framework [26] (table 1), established a priori, will be used to determine the eligibility of articles to include in this review. All articles published prior to 8 July 2021 will be eligible, but only articles with full text available in English will be included.

It should be noted that the review aims to elucidate the *relationship* between maternal serum hPL/PRL levels and metabolic/ fetal conditions/ outcomes, without assuming causality or directionality. The designation of hPL and PRL levels as 'exposure' and the listed outcomes as 'outcomes' is somewhat arbitrary and may not apply to all studies: some may work in the opposite direction. For example, studies that enrol women with pre-existing diabetes or GDM (relevant metabolic exposure) and look at PRL and hPL levels across gestation (outcome) would warrant inclusion. It is acknowledged by the reviewers that the relationship between lactogenic hormones and maternal metabolism is likely bidirectional; and the inclusion criteria will reflect this.

Table 1: Modified PECOT framework for study inclusion/ exclusion

	Participants (P)	Exposure (E)	Comparison (C)	Outcomes (O)	Study types (T)
Inclusion criteria	Pregnant women Women up to 12 months postpartum (regardless of lactation status)	Endogenous maternal serum hPL* (recorded at least once during pregnancy) **OR** Endogenous maternal serum PRL (recorded at least once during pregnancy and/ or up to 12 months postpartum)	Studies with any /multiple control group/s or no control group will be included	**Maternal:** Diabetes status during pregnancy and up to 12 months postpartum (pre-existing diabetes [Type 1 or Type 2], IGT or GDM; adequately defined)** Metabolic indices (continuous measurements) related to maternal glucose/ lipid metabolism (e.g. glucose measurements on OGTT; insulin secretion, sensitivity/ resistance indices; beta-cell function) during pregnancy or postpartum Obesity, gestational weight gainPostpartum weight change Polycystic ovary syndrome Lipid profile **Infant:** Birthweight (absolute / centiles, macrosomia), growth restriction or placental mass in pregnancies affected by GDM or pre-gestational DM**	Longitudinal cohort Case control Cross-sectional studies Randomised controlled trials Clinical observational human trials (eg. infusion/ clamp studies) if methods were used to determine a maternal metabolic outcome of interest Systematic reviews (to be examined for eligible articles)
Exclusion criteria	Non-pregnant populations Males Pathological / iatrogenic elevation of PRL (e.g. prolactinoma, medication-induced hyperprolactinaemia) or hPL (e.g. molar pregnancy) Studies focused on multiple pregnancy	hPL/PRL levels in other fluids (e.g. amniotic fluid, breastmilk), in fetus or infant, or in cord blood hPL/PRL administered exogenously Trials examining an intervention/ procedure (e.g. amniocentesis, induction of labour, drug treatment, IV glucose or insulin infusion, prolonged fasting) with hPL/PRL levels as outcome Trials focused on ART and ART outcomes Trials examining 'lactation' as exposure without PRL measured, OR where PRL measured but not	None	Diabetes status during pregnancy and up to 12 months postpartum inadequately defined** Birthweight, placental weight or growth restriction in pregnancies **not** affected by GDM or pre-gestational DM Outcomes unrelated to named key maternal metabolic or infant outcomes, e.g. placental function / perfusion / blood flow without mention of weight or FGR [e.g. Doppler indices alone] pre-eclampsia miscarriage/ pregnancy loss fetal structural abnormalities/ congenital malformations	Animal studies In vitro/ tissue culture studies Narrative reviews Commentaries/ letters Case reports, case series Conference abstracts Expert opinion Protocol papers

		directly analysed relative to metabolic outcomes		diabetic retinopathy lactation outcomes / parameters (milk transfer, milk production, infant weight change during breastfeeding)	

NOTES:

*alternative name, *human chorionic somatomammotropin*, also included in search (and studies eligible for inclusion)

** regarding classification of diabetes type:

INCLUDE studies referring clearly to Type 1 or Type 2 diabetes, or to gestational diabetes, or to impaired glucose tolerance

INCLUDE studies which refer to 'insulin-dependent', 'juvenile-onset' or 'insulin-requiring' diabetes (inside or outside of pregnancy) ONLY IF the supporting data clearly suggests Type 1 diabetes

EXCLUDE studies which refer to 'diabetic' pregnancies, 'diabetes', 'chemical diabetes', or 'diabetes mellitus' in pregnancy without further definition; or to 'pre-gestational' diabetes without further definition, or to 'insulin treated' diabetes without further clarification

EXCLUDE studies which define diabetes **only** according to White's classification (A/B/C/D) for diabetes in pregnancy.

If one group within a study is considered adequately-defined and another inadequately-defined; INCLUDE the study but only extract data for the groups meeting definition requirements

Abbreviations: hPL, human placental lactogen; PRL, prolactin; ART, assisted reproductive technologies; GDM, gestational diabetes mellitus; FGR, fetal growth restriction; IGT, impaired glucose tolerance.

SEARCH STRATEGY

A systematic search strategy using relevant search terms, in accordance with the selection criteria (Table 1) has been developed (see *supplementary material 1*), in consultation with expert subject librarians. A combination of keywords and database-specific subject headings will be used. The following electronic databases will be searched:

- MEDLINE via OVID
- MEDLINE ePub ahead of print, in-process, in-data review and other non-indexed citations via OVID
- CINAHL plus
- EMBASE

Bibliographies of relevant studies identified by the search strategy, and relevant reviews/ meta-analyses, will also be manually searched for identification of additional eligible studies.

Given that we intend to conduct an in-depth synthesis of a large body of research spanning several decades, only peer-reviewed published data with all results available will be considered

eligible for inclusion (conference abstracts will be excluded, and grey literature will not be searched).

INCLUSION OF STUDIES

References will be screened and managed using EndNote x9 and Covidence software. Two reviewers will scan the titles, abstracts and keywords of every record retrieved by the search strategy, assessing eligibility according to the inclusion and exclusion criteria in Table 1 (and in consultation with a third reviewer where required). A pilot test of the selection criteria will be conducted on 20-30 article titles and abstracts in order to refine and clarify the criteria prior to the formal commencement of screening.

If initial information suggests that an article meets the selection criteria for eligibility, the full text will be retrieved for further assessment by two reviewers. Disagreement between reviewers as to whether a study meets inclusion criteria will be resolved by discussion, with referral to a third reviewer if consensus cannot be reached. Studies excluded based on full text review will be tabulated along with reasons for their exclusion. Following PRISMA guidelines [24], a flow diagram will be created to illustrate the selection process.

QUALITY APPRAISAL OF THE EVIDENCE

Methodological quality of the included studies will be assessed by two independent reviewers using criteria established *a priori*, outlined in the Monash Centre for Health Research and Implementation (MCHRI) Evidence Synthesis Program critical appraisal template [27], see *supplementary material 2*. Individual quality items will be investigated using a descriptive component approach. Assessment will be based on criteria relating to external validity (population, setting, clarity of study objectives, inclusion and exclusion criteria, appropriateness of study design, and follow-up) and internal validity (selection, performance and detection bias, attrition, exposure and outcome measurement, reporting bias and potential confounders). Other domains for assessment will include potential conflicts of interest, study

power, and appropriateness/ quality of statistical methodology. Any disagreement or uncertainty will be resolved by discussion among review authors. Using this approach, each study will be allocated a risk of bias rating.

DATA EXTRACTION

Data will be extracted from all included studies by two independent reviewers, using a specifically-developed data extraction form. Pilot testing of the form will be conducted using 3-5 studies of different formats to ensure all required data are captured, particularly given the anticipated heterogeneity in study design. Key anticipated domains for extraction are shown in Table 2. Relevant data which are not reported in published studies will be requested from corresponding authors.

Table 2. Key domains for data extraction

Study	First author Journal Country and year of publication Study design Follow-up duration
Participants	Number of participants Participant characteristics (at baseline) - Baseline (pre-pregnancy) metabolic conditions, if present - Mean age - Parity - Ethnicity - Singleton/ multiple pregnancy - Gestation at enrolment/ recruitment - Mean BMI - Delivery mode - Breastfeeding status If control group present, control characteristics (at baseline) - Mean age - Parity - Ethnicity - Singleton/ multiple pregnancy - Gestation at enrolment/ recruitment - Mean BMI - Delivery mode - Breastfeeding status
Exposure* (lactogenic hormone)	Hormone measured (hPL/ PRL/both) Number of timepoints Time points (pregnancy), with concentration and units of hormone at each time point Time points (postpartum), with concentration and units of hormone at each time point Assay methodology used
Key maternal metabolic outcome(s)* of interest *Glucose status in pregnancy (pre-existing diabetes mellitus of any type OR gestational diabetes)*	Key maternal "outcomes" assessed (from list)* For diabetes in pregnancy - Pre-existing (T1/T2DM) or gestational

Postpartum glucose status *Continuous metabolic indices related to maternal glucose/ lipid metabolism e.g. measures of* *-fasting glucose* *-1h and 2h OGTT glucose* *-insulin secretion* *-insulin sensitivity* *-insulin resistance* *-beta-cell function* *(during pregnancy or postpartum)* *Gestational weight gain* *Obesity* *Postpartum weight change* *Polycystic ovary syndrome* *Lipid profile (total cholesterol, HDL and LDL cholesterol, triglycerides)*	- Gestation at diagnosis - Method used for diagnosis (eg OGTT) - Diagnostic criteria if stated - Treatment (diet/ oral medications/ insulin); and treatment commencement timepoint For postpartum glucose status - Time point - Method used for diagnosis (eg OGTT) - Diagnostic criteria, if stated - Prevalence of persistent dysglycaemia postpartum Relationship of said outcome(s) to hPL/PRL levels (as t-test result, odds ratio, regression coefficient etc) - Unadjusted - After adjustment (with list of covariates included in model/s) Conclusions regarding the above
Key infant metabolic outcome(s)* of interest (for pregnancies affected by GDM or pre-existing diabetes) *Birthweight (absolute/ centiles)* *Macrosomia* *Growth restriction* *Placental mass*	Key infant outcomes assessed (from list) Relationship of said outcome to hPL/PRL levels (as t-test result, odds ratio, regression coefficient etc) - Unadjusted - After adjustment (with list of covariates included in model/s) Conclusions regarding the above

*Due to the likely bidirectional nature of the lactogenic hormone/ maternal metabolism relationship, some studies will consider hPL/PRL as 'exposure' and a metabolic parameter (e.g. postpartum glucose tolerance) as 'outcome'. Others may consider a metabolic parameter (e.g maternal pre-gestational diabetes) as 'exposure' with hPL/PRL levels during pregnancy, in comparison to healthy controls, as 'outcome'. The extraction template will accommodate both.

Abbreviations: hPL, human placental lactogen; PRL, prolactin; BMI, body mass index; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; OGTT, oral glucose tolerance test; HDL, high density lipoprotein; LDL, low density lipoprotein; GDM, gestational diabetes mellitus

STATISTICAL ANALYSIS

Analysis for the two lactogenic hormones of interest, hPL and PRL, will be undertaken separately. Key exposure / outcome associations for each hormone will be determined based on the number of studies available. It is anticipated that hPL will be analysed primarily in relation to maternal metabolic / glycaemic status during pregnancy, and to fetal outcomes (birthweight, macrosomia, growth restriction, placental mass) in pregnancies affected by diabetes. For PRL, it is anticipated that key outcomes will be maternal metabolic / glycaemic status and related maternal metabolic indices (measures of insulin secretion, sensitivity and

beta-cell function) both during pregnancy and postpartum. After data extraction, the reviewers will determine whether meta-analysis is appropriate (based on the number of studies for each hormone/outcome relationship and the heterogeneity of their designs and participant groups). If meta-analysis is possible, Review Manager statistical software will be used for analysis with random effects models employed to generate weighted mean differences. Statistical heterogeneity will be assessed using the I^2 test, with I^2 values > 50% indicating moderate to high heterogeneity. Sensitivity analyses will be performed where applicable to explore the effects of studies with high risk of bias on the overall results. Subgroup analyses will also be performed where possible (for example; by type of diabetes). Where meta-analysis is not possible, a narrative synthesis of results will be performed.

Data will be presented in summary tables and in narrative format to describe the populations, exposures and key outcomes of the included studies. Forest plots and funnel plots will be used to present results from meta-analyses (where applicable) and publication bias assessments, respectively. Meta-analysis results will be reported according to PRISMA guidelines [24].

ETHICS AND DISSEMINATION

This project will collate aggregate data from published studies (or aggregate data provided by study investigators upon request), and thus ethical approval will not be required.

Findings will be disseminated via publications in peer-reviewed journals and presentations at scientific meetings. If deemed appropriate, findings will also be communicated to relevant stakeholders to guide clinical practice and public health actions in this area.

DATA AVAILABILITY STATEMENT

No data have been generated or analysed in this manuscript.

PATIENT AND PUBLIC INVOLVEMENT STATEMENT

It was not feasible or appropriate to involve patients or members of the public in the design, planning or conduct of the planned research.

DISCUSSION

The proposed review will be the first, to our knowledge, to systematically collate and synthesise the existing scientific literature linking two key lactogenic hormones, hPL and PRL, to maternal metabolic health in pregnancy and postpartum (and, by extension, to infant outcomes). Systematic reviews which evaluate biomarkers or aim to explore physiological questions are rare in the endocrine literature, and represent an under-utilised opportunity to move beyond subjective, narrative work towards inclusive, extensive reviews with the potential for objective and evidence-based conclusions.

Whilst these hormones have long been recognised for their roles in the antenatal preparation of the breast for lactation and (in the case of PRL) for the postnatal initiation and maintenance of lactation, their metabolic roles have been relatively under-appreciated. Both hormones contribute to the insulin resistance associated with the pregnant state, but also potentially have central roles in the adaptation of the maternal pancreas during gestation, stimulating beta-cell adaptation and increasing beta-cell mass and insulin secretion [1, 9]. During a normal pregnancy, this may allow compensation for pregnancy-induced insulin resistance, resulting in overall maintenance of euglycaemia.

Despite likely playing a key role in the regulation of glucose and insulin dynamics during pregnancy, the relationship between hPL levels and the pathophysiology of GDM remains unclear. Several studies have investigated possible links, with some reporting no association between maternal hPL levels and GDM status [28-31], and others reporting higher hPL in GDM subjects than controls [32, 33], particularly if insulin-treated [34]. For hPL levels during pregnancies affected by pre-existing diabetes (T1DM/ T2DM), the majority of authors report

serially-higher hPL throughout gestation in diabetic women compared with controls [29, 32, 35-37], although other studies in T1DM have shown lower levels in the setting of poor control [38]. Furthermore, higher hPL levels are clearly related to increased placental weight and macrosomia, and several authors have suggested that increased levels of hPL in many diabetic pregnancies may simply reflect higher placental mass [4, 32, 35]. This does not suggest it is aetiologically unimportant, however – it is possible that the placentomegaly seen in maternal diabetes causes higher hPL levels, stimulating maternal and fetal beta-cell expansion and increasing fetal insulin production, thus promoting glycogenesis, fat deposition and further fetal growth [6].

Importantly however, this area of the literature is particularly dated, with many studies performed well prior to the 21st century and prior to contemporary diagnostic definitions of diabetes in pregnancy. As such, the exact type of maternal diabetes among study participants is often unclear (they are simply deemed to be 'diabetic', are defined according to the now-historical White's Classification of diabetes in pregnancy [39], or are termed 'insulin-dependent') [30, 32, 35-38]. Such studies provided valuable basic insights into the pathophysiology of the lactogen/maternal metabolism relationship, but comparison to the available better-described contemporary cohorts [28] is not possible. In this systematic review, a sufficiently clear definition of diabetes type (or adequate detail for this to be confidently deduced) is thus mandated for inclusion, as we believe this is a minimum requirement if our review findings are to be applicable to modern obstetric populations.

Acknowledging these challenges, a better understanding of the role of hPL in metabolically-abnormal pregnancies has potential clinical application. For example, accurate antenatal prediction of fetal macrosomia remains challenging, and current macrosomia prediction strategies (including physical examination and ultrasound assessment) are both resource-intensive and imprecise. There is thus a clear requirement for maternal serum biomarkers in improving antenatal macrosomia prediction, particularly in women at high risk of the outcome

(such as those with pre-gestational diabetes or GDM). Whilst several candidate maternal biomarkers have been assessed for their association with birthweight or macrosomia (both in diabetic and non-diabetic pregnancies), evidence is mixed and uncertainties around clinical utility persist [40]. hPL (which was used clinically in some settings to assess the wellbeing of the feto-placental unit in the 1970s and 1980s prior to the widespread availability of obstetric ultrasound)[41] has recently been largely overlooked as a candidate biomarker in this capacity, but previous work suggests it may have significant potential if revisited. For instance, one 1998 study measured hPL at the time of GDM screening (n=257) and found that among the subset of women with a normal glucose challenge test but whose infants ultimately weighed >4000 g (n=11), mean hPL at the time of testing had in fact been similar to the mean hPL found in women with GDM [42]. This suggests that hPL may warrant evaluation as a biomarker for macrosomia prediction, both in women with diagnosed diabetes and those without. Such an application would require the marker to be validated in modern cohorts where the underlying aetiology of maternal diabetes was adequately understood and described.

Unlike hPL (which, as a placentally-derived hormone, is washed from the circulation following delivery), PRL has probable influence in maternal metabolism both during pregnancy and postpartum, particularly if lactation ensues. The literature here is similarly conflicting. For example, maternal serum PRL levels during GDM pregnancy have been examined by several groups, with the majority reporting similar levels to normal pregnancies [28, 31, 43]. However, more recent studies have directly contradicted this. Two groups have shown that higher PRL levels in the first [44] and third [45] trimester of pregnancy were associated with reduced glucose tolerance on OGTT, with both groups suggesting that PRL may be independently involved in GDM pathogenesis. A third study has demonstrated an opposite result, showing an inverse association between third trimester PRL and GDM risk [46]. This lack of consensus highlights the need for effective evidence synthesis followed by further research.

Postpartum, lactation (under the chief control of PRL) appears to confer maternal metabolic benefits, but the mechanism by which this occurs is unclear. One group found that maternal serum PRL in late pregnancy was significantly higher in women who progressed to normal glucose tolerance postpartum than in those who progressed to postpartum prediabetes/ diabetes; and that higher antepartum PRL independently predicted improved postpartum insulin secretion capacity. That group suggested that these findings may reflect a postpartum extension of the beneficial effects of PRL on beta-cell mass and islet adaptation that are thought to occur during gestation. Another group, who measured PRL postpartum, presented different findings and discussion: women with higher circulating PRL in the context of lactation in their study had reduced beta-cell function and lower insulin secretion indices; but were less insulin resistant [18]. Authors have suggested that this improvement in insulin resistance may result from the mobilisation of muscle and liver lipids into breast milk under the control of PRL [4], an action that may be particularly beneficial in women who are insulin resistant at baseline (women with recent GDM are known to have increased intramyocellular lipid content, IMCL, at 4-6 months post-delivery compared with controls) [47].

There is thus a clear need for a systematic review of the literature in this field – both lactogenic hormones clearly have central roles in the regulation of maternal metabolism (both during pregnancy and postpartum, and for women with normal and abnormal pregnancies). However, to date the evidence has not, to our knowledge, been effectively synthesised.

Some limitations of the review process should be noted. Firstly, owing to the intentionally-broad scope of the review, included studies will be heterogeneous in their design, methodology and research questions. In the analysis phase, hPL and PRL will thus be considered separately and studies will be grouped according to similar outcomes; but it is possible that marked heterogeneity will preclude meaningful conclusions and/ or statistical meta-analysis. Secondly, some of the basic clinical work on hPL and PRL levels in normal and diabetic pregnancies is now very dated, extending back to the 1970s and 1980s. Whilst robust and worthy of inclusion,

differences in experimental design and (in particular) the classification and treatment of maternal diabetes will present challenges when comparing such studies to modern cohorts. As such, clear requirements for the adequacy of maternal diabetes definitions have been stipulated in our inclusion and exclusion criteria. Where possible, we will endeavour to conduct a subgroup analysis by publication year range or otherwise perform a narrative comparison between older and newer studies. We will also extract and tabulate variables such as the exact GDM diagnostic criteria used, and the assay methodology employed in each case; as such details are likely to vary according to era of publication (in particular, many older studies involve the routine use of radioimmunoassay, now largely superseded by modern enzyme-linked immunoassay techniques). Finally, as previously described, the relationship between lactogenic hormones and maternal metabolism is almost certainly bidirectional, whereby some studies examine the effects of lactogenic hormones (exposure) on metabolic conditions (outcome), whilst in others, exposure and outcome are reversed. The review is designed to capture both, but – particularly in the postpartum context – the bidirectional nature of the relationship can bias observational studies. While this cannot be directly addressed in our review methodology, it will be acknowledged in the synthesis and interpretation of the findings.

CONCLUSION

In summary, this systematic review will rigorously and systematically collate and synthesise current evidence linking the key lactogenic hormones hPL and PRL to maternal metabolic health in pregnancy and postpartum (and thus to related infant outcomes). Both hormones have key roles in the maintenance of glucose homeostasis during pregnancy, including direct actions on the beta-cells of the maternal endocrine pancreas. However, the exact roles of these hormones – particularly in metabolically abnormal pregnancies – remain unclear, and evidence is conflicting. Further, hPL may have untapped potential clinical application in the antenatal

prediction of macrosomia, while the hormonal control of lactation, led by PRL, may regulate glucose and lipid metabolism and help to guard postpartum women against persistent dysglycaemia. Through this review process, the available scientific evidence will be synthesised to clarify these relationships and inform future research and practice in the field of maternal metabolic and endocrine health.

Author contributions: KR is the project lead, conceptualised and designed the protocol, wrote the first draft of the manuscript, and will coordinate and conduct the systematic review process along with co-reviewer RG. AMM has contributed to the design of the search strategy and will provide support with evidence synthesis. AM, AJ, and HJT reviewed and edited the manuscript, and AM, AJ, and HJT will provide oversight and supervision for the systematic review process. All authors contributed substantial intellectual input to the manuscript in line with ICMJE criteria for authorship and have approved the final version for publication.

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1 (pregnan* or gestation* or post?partum or post-partum or lactat* or breastfe*).ti,ab.

2 exp pregnancy/

3 postpartum period/

4 lactation/

5 Breast Feeding/

6 1 or 2 or 3 or 4 or 5

7 prolactin.ti,ab.

8 prolactin/

9 placenta* lactogen*.ti,ab.

10 placental lactogen/

11 somato-mammotropin.ti,ab.

12 somato?mammotropin.ti,ab.

13 7 or 8 or 9 or 10 or 11 or 12

14 ((pregnan* or gestation* or matern* or post?partum or post-partum or birth or f?etal or baby or infant* or newborn* or neonat*) adj1 weight*).ti,ab.

15 (placenta* adj1 weight*).ti,ab.

16 (placenta* adj1 mass*).ti,ab.

17 (diabet* or glucose or obes* or metabolic).ti,ab.

18 polycystic ovar*.ti,ab.

19 exp diabetes mellitus/

20 glucose intolerance/

21 exp diabetes, gestational/

22 obesity, maternal/

23 birth weight/

24 fetal weight/

25 polycystic ovary syndrome/

26 pregnancy outcome/

27 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26

28 6 and 13 and 27

29 exp animals/ not humans.sh.

30 28 not 29

Embase Classic+Embase <1947 to current>

1 (pregnan* or gestation* or post?partum or post-partum or lactat* or breastfe*).ti,ab.

2 exp pregnancy/

3 lactation/

4 breast feeding/

5 1 or 2 or 3 or 4

6 prolactin.ti,ab.

7 prolactin/

8 placenta* lactogen*.ti,ab.

9 placenta lactogen/

10 somato-mammotropin.ti,ab.

11 somato?mammotropin.ti,ab.

12 6 or 7 or 8 or 9 or 10 or 11

13 ((pregnan* or gestation* or matern* or post?partum or post-partum or birth or f?etal or baby or infant* or newborn* or neonat*) adj1 weight*).ti,ab.

14 (placenta* adj1 weight*).ti,ab.

15 (placenta* adj1 mass*).ti,ab.

16 (diabet* or glucose or obes* or metabolic).ti,ab.

17 polycystic ovar*.ti,ab.

18 exp diabetes mellitus/

19 glucose intolerance/

20 pregnancy diabetes mellitus/

21 maternal obesity/

22 birth weight/

23 fetus weight/

24 ovary polycystic disease/

25 pregnancy outcome/

26 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25

27 5 and 12 and 26

28 (exp animal/ or exp invertebrate/ or nonhuman/ or animal experiment/ or animal tissue/ or animal model/ or exp plant/ or exp fungus/) not (exp human/ or human tissue/)

29 27 not 28

CINAHL PLUS

S26	S6 AND S12 AND S25
S25	S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24
S24	(MH "Pregnancy Outcomes")
S23	(MH "Polycystic Ovary Syndrome")
S22	(MH "Fetal Weight")
S21	(MH "Birth Weight")
S20	(MH "Obesity, Maternal")
S19	(MH "Diabetes Mellitus, Gestational")
S18	(MH "Glucose Intolerance")
S17	(MH "Diabetes Mellitus+")
S16	polycystic ovar*
S15	diabet* or glucose or obes* or metabolic
S14	placenta* N1 (weight* OR mass*)
S13	(pregnan* or gestation* or matern* or post?partum or postpartum or birth or f?etal or baby or infant* or newborn* or neonat*) N1 weight*
S12	S7 OR S8 OR S9 OR S10 OR S11
S11	somatomammotropin
S10	(MH "Placental Hormones")
S9	"placenta* lactogen*"
S8	(MH "Prolactin")
S7	prolactin
S6	S1 OR S2 OR S3 OR S4 OR S5
S5	(MH "Breast Feeding")
S4	(MH "Lactation")
S3	(MH "Postnatal Period+")
S2	(MH "Pregnancy+")
S1	pregnan* or gestation* or post?partum or postpartum or lactat* or breastfe*

Template for critical appraisal of a cohort study
Document evidence from the article in quotation marks.

Study ID		
Study citation		
EXTERNAL VALIDITY – IS THIS STUDY AND ITS RESULTS GENERALIZABLE TO MY SYSTEMATIC REVIEW QUESTION?		
Patient/population/ participants	Describe whether they were gender specific, had a particular condition or the general population, age and any other relevant characteristics (e.g. BMI)	
Control population (if appropriate)	Describe whether they were gender specific, had a particular condition or the general population, age and any other relevant characteristics (e.g. BMI)	
N	Where possible, list the number of participants that were: • Screened • Enrolled • Allocated/randomised • Assessed • Followed up	
Setting	List where the intervention was conducted and assessed ie. hospital, clinic, community and/or university setting.	
Intervention/indicator	Describe the intervention in as much detail as possible e.g. medication type, dose, duration, intervals.	
Comparison/control	Describe the comparison in as much detail as possible e.g. medication type, dose, duration, intervals.	
Outcomes	List what the study measured (e.g. weight, BMI, HbA1c) as primary outcomes and secondary outcomes. If the outcomes are not relevant to your systematic review, list these as measured but not relevant to your systematic review.	
Does the study have a clearly focused question and/or PICO?	Yes Partial No Not reported	Consider if the question is 'focused' in terms of: – the population studied – the intervention given or exposure - the comparison(s) – the outcomes considered
Inclusion Criteria	Yes No Not reported	
Exclusion Criteria	Yes No Not reported	
If there were specified inclusion/ exclusion criteria, were these appropriate?	Yes Partial No N/A	Consider if: - the eligibility criteria used to specify the patients, interventions/ exposures and outcomes of interest.
Is a cohort study the appropriate design to answer this question?	Yes Partial No	Consider if a cohort study is a good way of answering the question under the circumstances.
Were the outcomes measured appropriate?	Yes Partial No Not reported	Consider if the outcomes measured are appropriate and important outcome.
Was there sufficient duration of follow-up for outcomes to occur?	Yes Partial No Not reported	May need to check with clinicians regarding what is sufficient duration for important events to occur. An acceptable length of time should be decided before quality/risk of bias assessment begins.

INTERNAL VALIDITY – HAS THIS STUDY BEEN CONDUCTED RIGOROUSLY IN ORDER TO REDUCE BIAS?			
SELECTION BIAS	**Other than the exposure under investigation, were the groups selected from similar populations?**	Yes Partial No Not reported	Consider: - whether the different sources would affect outcomes e.g. one group recruited from hospital(s) the other from the community. - time periods i.e. historical cohort - whether there is a large difference in participation rate between the two arms of the study.
	Was the exposed cohort truly representative?	Yes Partial No Not reported	This item is assessing the representativeness of exposed individuals in the community relevant to the study's PICO, not the representativeness of the sample of individuals in the general population. Consider: - whether truly representative in the community (least bias) - whether somewhat representative (some bias) - whether selected group of users (bias) - no description of the derivation of the cohort (most bias)
	Is it clear that the outcome of interest was not present at the start of study?	Yes Partial No Not reported	In the case of mortality studies, outcome of interest is still the presence of a disease/ incident, rather than death. That is to say that a statement of no history of disease or incident is least biased.
PERFORMANCE BIAS	**Aside from the exposure, were the groups treated the same?**	Yes Partial No Not reported	To be sure it's the exposure which is responsible for the effect.
DETECTION BIAS	**Was exposure measured in a standard, valid and reliable way?**	Yes Partial No Not reported	Where exposure measures require any degree of subjectivity, some evidence should be provided that the measures used are reliable and have been validated prior to their use in the study. Consider whether ascertainment of exposure was determined by: - secure record (eg surgical records) (least bias) - structured interview - written self report (bias) - no description (most bias)
	Were outcome assessors blind to the exposure?	Yes Partial No Not reported	Consider: - If the outcome is objective (e.g. death) then blinding is less critical. - If the outcome is subjective (e.g. symptoms or function) then blinding of the outcome assessor is critical.
	Were all outcomes measured in a standard, valid and reliable way?	Yes Partial No Not reported	Where outcome measures require any degree of subjectivity, some evidence should be provided that the measures used are reliable and have been validated prior to their use in the study. For some outcomes (e.g. fractured hip), reference to the medical record is sufficient to satisfy the requirement for confirmation of the fracture. This would not be adequate for vertebral fracture outcomes where reference to x-rays would be required. Consider whether outcomes were determined through: - independent blind assessment or confirmation of the outcome by reference to secure records (x-rays, medical records, etc.) (least bias) - record linkage (e.g. identified through codes on database records) - self report (i.e. no reference to original medical records or x-rays to confirm the outcome) (bias) - no description (most bias)

	Were outcomes assessed objectively and independently?	Yes Partial No Not reported	Independence of assessment is important where the result of one outcome may effect the interpretation of another. When outcomes are objectively assessed, their independence from each other is less important.
ATTRITION BIAS	**What percentage of the individuals recruited into each arm of the study were lost to follow up?**	X% treatment X% control/ comparison Not reported	Consider: - if all patients who entered the trial were properly accounted for and attributed at its conclusion. - why patients dropped out, as well as how many. - the drop out rate may be expected to be higher in studies conducted over a long period of time. - if comparisons were made between participants followed-up and those lost to follow up, by exposure status.
	What percentage of the individuals were not included in the analysis?	X% treatment X% control/ comparison Not reported	Consider: - if analysis was as per protocol or intention to treat - number of crossovers - reason for crossover
REPORT BIAS	**Is the paper free of selective outcome reporting?**	Yes Partial No Not reported	Consider: - if all the planned outcomes were measured - if all the measured outcomes were reported - if any additional or composite outcomes were measured. This is difficult to determine if there isn't a protocol.
CONFOUNDING	**Are the cohorts comparable on the basis of design or analysis?**	Yes Partial No Not reported	Consider - either exposed and non-exposed individuals must be matched in the design and/or confounders must be adjusted for in the analysis. - statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. Note: If the relative risk for the exposure of interest is adjusted for the confounders listed, then the groups will be considered to be comparable on each variable used in the adjustment.
OTHER INTERNAL VALIDITY/BIAS	**Were there any conflicts of interest in the writing or funding of this study?**	Yes No Not reported	Consider: - if any of the authors are/were employed, sponsored etc by pharmaceutical companies, or have other financial/other ties - if any commercial companies were involved in funding, writing, editing, data analysis or manuscript approval
	Was the study sufficiently powered to detect any differences between the groups?	Yes Partial No Not reported	Consider: - if an adequate sample size calculation was undertaken - if the required sample size recruited and retained - for which outcomes the study was powered - if confidence intervals include a clinically important difference, the study was underpowered NB this is less important if significant differences were found.
	If statistical analysis was undertaken, was this appropriate?	Yes Partial No Not reported N/A	Consider: - whether the authors performed any statistical tests or just presented figures - if the statistical analysis was planned a priori - if the data were analysed accordingly to the study protocol. - the type of data and the statistical tests used. (Please refer to the CCE workbook as required) - use of parametric versus non-parametric tests; whether the data has been checked for normality - if the tests used are obscure, why did the authors used them and have they included a reference. - if point estimates and measures of variability were presented for the primary outcome

		- if subgroups were analysed appropriately - if potential confounders were identified and taken into account in the analysis - if there was any adjustment made for multiple testing - if missing data was handled appropriately
Comments	*Add any other relevant comments, including if this is likely to influence the results of the study*	
What is the overall risk of bias?	Low Moderate High Insufficient information	*Low - All of the criteria have been fulfilled or where criteria have not been fulfilled it is very unlikely the conclusions of the study would be affected.* *Moderate - Some of the criteria have been fulfilled and those criteria that have not been fulfilled may affect the conclusions of the study.* *High - Few or no criteria fulfilled or the conclusions of the study are likely or very likely to be affected.* *Insufficient information – not enough information provided on methodological quality to be able to determine risk of bias.*

Cited in full as: Monash Centre for Health Research and Implementation (MCHRI) Evidence Synthesis Program template for critical appraisal of a cohort study (2014), MCHRI – Monash University and Monash Health, Melbourne, Australia (*adapted from* Critical Appraisal Templates (2010) Centre for Clinical Effectiveness, Southern Health, Melbourne, Australia AND Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle–Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. In: Proceedings of the 3rd Symposium on Systematic Reviews Beyond the Basics: Improving Quality and Impact. Oxford; 2000. p. 3-5).

Reporting checklist for protocol of a systematic review and meta analysis.

Based on the PRISMA-P guidelines.

		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a - not an update
Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	20
Amendments			

	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	9
Support			
Sources	#5a	Indicate sources of financial or other support for the review	20
Sponsor	#5b	Provide name for the review funder and / or sponsor	n/a - no specific funding
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	20
Introduction			
Rationale	#6	Describe the rationale for the review in the context of what is already known	4-8
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	8
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	9-10

Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	11
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	11
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	11
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	11-12
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	12-14
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	13-14
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	13-14

Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	12
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	14
Data synthesis	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	14-15
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	14-15
Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	14
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	n/a - not planned
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	12

Notes:

- 1b: n/a - not an update

- 5b: n/a - no specific funding
- 16: n/a - not planned The PRISMA-P elaboration and explanation paper is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 07. July 2021 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai