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The Role of Hexokinase Domain Containing Protein-1 in Glucose Regulation During Pregnancy

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Abstract

Purpose of Review: Gestational diabetes mellitus (GDM) is a common pregnancy complication conferring an increased risk to the individual of developing type 2 diabetes. As such, a thorough understanding of the pathophysiology of GDM is warranted. Hexokinase domain containing protein-1 (HKDC1) is a recently discovered protein containing hexokinase activity which has been shown to be associated with glucose metabolism during pregnancy. Here, we discuss recent evidence suggesting roles for the novel HKDC1 in gestational glucose homeostasis and the development of GDM and overt diabetes.

Recent Findings: Genome wide association studies identified variants of the *HKDC1* gene associated with maternal glucose metabolism. Studies modulating HKDC1 protein expression in pregnant mice demonstrate that HKDC1 has roles in whole-body glucose utilization and nutrient balance, with liver-specific HKDC1 influencing insulin sensitivity, glucose tolerance, gluconeogenesis, and ketone production.

Summary: HKDC1 has important roles in maintaining maternal glucose homeostasis extending beyond traditional hexokinase functions, and may serve as a potential therapeutic target.

Keywords

Hexokinase; HKDC1; glucose metabolism; gestational diabetes

Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance that is first discovered during pregnancy in an individual with no known history of overt diabetes previously [1]. It represents one of the most common metabolic disturbances during pregnancy [2], with an estimated prevalence of 9% to 26% (average of 18%) per the Hyperglycemia and Adverse Pregnancy Outcomes study [3], and as of 2017, GDM is estimated to affect one in seven births worldwide [4]. Hyperinsulinemic clamp studies performed on healthy lean women

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indicated that during pregnancy insulin sensitivity is reduced by 56% and basal endogenous glucose production increases by 30% [5,6]; hence, the ability for pancreatic beta cells to adapt to these conditions is critical for maintaining euglycemic homeostasis. GDM results, at least in part, from the inability of pancreatic beta cells to respond adequately to increasing insulin requirements during pregnancy, resulting in varying degrees of hyperglycemia [1]. Risk factors for the development of GDM include obesity [7], excessive gestational weight gain [8], ethnicity [9], advanced maternal age [10], consumption of a western diet [11], family or personal history of GDM [12], and specific genetic variations [13–15].

The development of GDM can have adverse consequences to both mother and child. In the setting of GDM, the fetus is subjected to increased transport of glucose, amino acids, and fatty acids which greatly stimulates insulin production within the baby, promoting fetal overgrowth and macrosomia leading to shoulder dystocia during childbirth [16,17], but may also lead to fetal beta cell dysfunction [18]. Toward the mother, GDM presents both short- and long-term consequences. Perinatally, GDM increases the risk for preeclampsia, preterm birth, and Cesarean section [19]. In the long-term, GDM increases the probability of developing chronic hyperglycemia, with 60% of women with GDM developing type 2 diabetes (T2DM) in their lifetime [20], with a 2–3% yearly risk of conversion, [20] and each subsequent pregnancy conferring a three-fold risk of developing T2DM [16]. It has also been reported that women with GDM have a 63% increased risk of cardiovascular disease [21]. It is therefore critical that GDM is effectively diagnosed and treated accordingly. Furthermore, a thorough understanding of the pathophysiology and genetic risk for developing GDM is warranted.

Given the high prevalence of GDM in the maternal population, genome wide association studies (GWAS) are critical for identifying genetic loci associated with increased susceptibility to developing GDM. However, the number of GWAS studies performed remains small and further research is needed. Thus far, it has been found that genetic variations in *IGFBP2*, *CDKAL1*, *GLIS3*, *CDKN2A/2B*, *HHEX/IDE*, *TCF7L2*, *MTNR1B*, *HNF1A*, *GCK*, and *HKDC1* are associated with GDM in multiple population analyses [14–15, 22–27]. A specific genetic variant of *glucokinase (GCK)*, one of the well-characterized hexokinases, was found to increase the odds of developing GDM in European and Thai cohorts within the HAPO study (*GCK* rs1799884) [25], suggesting a link between the HK family of enzymes and GDM for the first time.

A hexokinase (HK) is an enzyme that phosphorylates hexose sugars, primarily glucose, trapping the sugar intracellularly and committing it to use in a variety of metabolic processes based on cellular needs [28]. Four HKs have been extensively classified – HK1-3 primarily mediate the phosphorylation of glucose for cellular metabolism, while GCK has a higher K_m value and functions more so as a glucose sensor [29]. Interestingly, in the mid-2000s, phylogenetic analyses were conducted in an effort to better understand the evolution of GCK and the diversification of the HKs [30]. Within these analyses, a novel HK-like gene called hexokinase domain containing protein-1 (HKDC1) was uncovered. *HKDC1* is located on chromosome 10 adjacent to *HK1*, and encodes a 100 kDa protein product sharing 70% sequence identity to HK1 [30,31]. Review of the human EST database indicated that

HKDC1 is widely expressed, and given that HKs are critical for glucose metabolism and homeostasis, these initial findings suggested that HKDC1 may be an additional HK.

Since its recent discovery, HKDC1 has continued to gain interest from a clinical standpoint, with studies linking this novel HK to glucose metabolism [31–33] and cancer progression, metastasis, and poor disease prognosis [34–46], which suggests that HKDC1 may serve as a potential therapeutic target. In this review, we discuss recent GWAS analyses and biological studies that have collectively led to our present understanding of HKDC1's roles in glucose utilization and homeostasis during pregnancy, and that further suggest a role for HKDC1 in the development of GDM and possibly T2DM.

HKDC1 contains hexokinase activity

Comparison of the amino acid sequences of *HKDC1* and the other HKs indicates the presence of conserved amino acid residues in the N- and C-terminal domains, which are the predicted areas of glucose and ATP binding, respectively [30]. Since HKs traditionally transfer a phosphate group from ATP to glucose, the presence of conserved glucose and ATP binding sites on *HKDC1* suggests that HKDC1 has hexokinase activity. To test this hypothesis, Guo et al [47] performed hexokinase activity assays utilizing lysates from INS-1 rat pancreatic β-cells transduced with adenovirus containing *HKDC1* from a human cytomegalovirus promoter. At baseline, INS-1 cells contain minimal hexokinase activity. Utilizing different amounts of glucose, INS-1 cell lysates both transduced with and containing high levels of HKDC1 protein demonstrated enhanced hexokinase activity across the range of glucose concentrations while exhibiting expression levels of the other HKs that were unaffected by HKDC1 expression. Furthermore, this study showed that HKDC1 has a lower K_m than GCK. Overall, these findings suggest that HKDC1 contributes to overall cellular HK activity through either direct HK activity and/or modulation of the activity levels of the other HKs. To more directly test if HKDC1 has intrinsic HK activity, Guo et al [47] then utilized hexokinase activity assays *in vitro* using purified HKDC1 and found that HKDC1 contained 20% of the specific activity as HK1. Taken together, these studies indicate that HKDC1 contains intrinsic HK activity and therefore is a fifth known HK.

HKDC1 is widely expressed in human tissues

In Irwin et al [30], the authors utilized a human EST database to suggest that *HKDC1* is widely expressed in many tissues, with the highest levels of gene expression within the pharynx, thymus, colon, esophagus, and eyes. Following this, Ludvik et al [31] next assessed *HKDC1* mRNA levels in human tissue samples from the Genotype-Tissue Expression Consortium. Similarly, *HKDC1* expression was found to be widely and differentially expressed in many human tissues, confirming these prior data. Collectively, *HKDC1* expression levels were highest in the colon, small intestine and kidney, as well as in several endocrine organs including the pituitary, testis and thyroid. Further assessment of localization within tissues using immunohistochemistry demonstrated that HKDC1 protein expression was restricted to the intestinal epithelial cells in the colon, hepatocytes in the liver, and the renal tubule within the kidney. Interestingly, *HKDC1* gene expression was absent in adipose tissue and skeletal muscle. Additionally, a comparison of *HKDC1*

expression in humans as compared to mouse tissues suggested a similar pattern of expression.

In a further study, Khan et al [48] utilized surgical specimen samples to assess HKDC1 protein expression broadly across many human tissues. They found that similar to the colon, HKDC1 was highly expressed in the small intestine and in particular, this expression was localized to the brush border. Strong protein expression was also notable in the exocrine pancreas, myenteric plexus, thyroid, and within alveolar macrophages. Overall, a large number of other tissues also demonstrated HKDC1 protein expression, albeit at low to moderate levels compared to the aforementioned tissues. Correlating with mRNA assessment, HKDC1 protein was absent in adipose tissue and skeletal muscle. Collectively, these studies demonstrate that HKDC1 is widespread in human tissues.

Genetic variation in HKDC1 and maternal glucose metabolism

HKDC1 was originally identified as a putative fifth human hexokinase in 2007, however, its functional role was initially unknown [30]. In the years since its discovery, genetic studies have played a pivotal role in uncovering the physiological functions of HKDC1, including its involvement in regulating maternal glucose metabolism during pregnancy.

An association between gene variants in *HKDC1* and maternal glucose metabolism was first identified by Hayes et al [27] through analysis of select participants (mothers and offspring) within a large-scale genome-wide association study (GWAS) investigating the genetic architecture underlying glycemic traits during pregnancy. This GWAS—the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study—included DNA and phenotypic data from 25,505 pregnant women in 15 centers across nine countries and assessed cohort-specific and meta-analyses of genome-wide single nucleotide polymorphism (SNP) data to identify common genetic variants associated with glycemic traits during pregnancy [49]. The authors here [27] analyzed a subset of HAPO participants, including 7,463 DNA samples from pregnant women from Afro-Caribbean, Hispanic, Northern European, and Thai ethnic backgrounds. Glycemic traits were measured at approximately 28 weeks of gestation and included one- and two-hour plasma glucose levels collected during an oral glucose test, as well as maternal fasting plasma glucose and C-peptide levels. After all statistical adjustments, the strongest association in this GWAS was revealed to be 2-hour plasma glucose (2HPG) with *HKDC1* – a finding that ignited interest in its characterization.

The HAPO GWAS identified 2HGP as significantly associated with genetic variants in locus of 10q22.1, a small 400-kb region spanning several genes, including *HKDC1*, which is comprised of a 3,653 base pair region adjacent to the highly similar hexokinase *HK1* [http://useast.ensembl.org/Homo_sapiens/Gene/Summary?db=core]. At this location, *HKDC1* SNP rs4746822 reached genome-wide significance in a meta-analysis combining the four ancestry groups as well in several replication cohorts (Table 1). Analysis from this study also identified several other 2HPG-associated variants with locations both within and proximal to this region, including histones H3K27ac and H3K27me. This data indicates the presence of active regulatory elements contributing to *HKDC1's* expression [50]. These results linked *HKDC1* to a potential physiological function during gestation for the first

time, and the authors concluded that *HKDC1* may play an important role in regulating glycemic control during pregnancy [51].

Findings from the HAPO GWAS study were utilized in addition to several other genetic databases in order to further elucidate the genetic mechanisms underlying *HKDC1* during a follow-up analysis, which identified four common SNPs in *HKDC1* predicted to impact the expression of the protein, namely, rs4746822, rs10762264, rs2394529, and rs9645501 (Table 1) [47,52,53]. All four genetic variants were located proximal to the coding region of *HKDC1*, providing further evidence of a model in which genetic variations in multiple regulatory elements alters the regulation of *HKDC1* expression. Functional studies revealed that the four SNPs are regulatory variants in four separate enhancers that have a cooperative effect of reducing *HKDC1* expression in women with higher gestational glucose levels [47].

Following these discoveries, several smaller GWAS studies have been conducted in order to verify the association between *HKDC1* and 2HPG in additional ancestry groups. Because elevated gestational 2-hour plasma glucose levels are indicative of an inadequate response to a pregnancy-induced increase in insulin resistance, several studies sought to evaluate the association of the four previously described genetic variants in *HKDC1* with GDM [54]. One small GWAS seeking to replicate the results of the HAPO study in a Han Chinese population, an ancestry not included in the original HAPO cohort, found a significant association between *HKDC1* gene variant rs4746822 and GDM (Table 1) [55]. Another study used a cohort entirely comprised of pregnant women within a Southern Indian population. The authors found a strong association between *HKDC1* and GDM in variants rs4746822 and rs10762264 in addition to a modest association in variant rs2394529 (Table 1) [56]. While statistical power limitations prevented any evidence of a coordinated effect between the variants, the study calculated the GDM risk of each individual variant to be 1.95 (1.24–3.16, 95% CI), 1.71 (1.12–2.61, 95% CI), and 1.64 (1.05–2.57, 95% CI) times higher, respectively.

In summary, known variants in the *HKDC1* gene, have been linked to changes in glycemic control during pregnancy and GDM. In addition to identifying *HKDC1* in the context of maternal glucose metabolism, these genetic studies have served as a foundation for elucidating the underlying mechanisms of HKDC1 in GDM.

Assessment of HKDC1 function in glucose homeostasis during pregnancy

Global HKDC1 knockdown

As the first study to utilize a mouse model to investigate the role of HKDC1 in glucose metabolism during pregnancy, Ludvik et al [31] generated an *HKDC1* global knockout mouse with a gene trap system using embryonic stem cells obtained from the Knockout Mouse Repository (www.komp.org). Utilizing a breeding approach between mice that were heterozygous for *HKDC1* ($HKDC1^{+/-}$), no offspring that were homozygous for *HKDC1* knockout ($HKDC1^{-/-}$) were identified in any of the litters, whereas the remaining offspring occurred at the expected 2-to-1 Mendelian ratio of heterozygous ($HKDC1^{+/-}$) to wild type ($HKDC1^{+/+}$) mice. Heterozygous $HKDC1^{+/-}$ exhibited an approximately 50% reduction in total HKDC1 protein expression and these mice were utilized for further analyses [31].

To assess the effect of *HKDC1* knockdown on glucose metabolism during pregnancy, 8- to 12-week-old pregnant mice were utilized during pregnancy, at day 15 of gestation, which is the peak of insulin resistance during mouse pregnancy [57]. Compared to wild-type littermate controls, pregnant *HKDC1*^{+/−} mice exhibited significantly increased glucose excursion at multiple time points during an oral glucose tolerance test (OGTT). However, non-pregnant female and male *HKDC1*^{+/−} mice at 8- to 12-weeks of age did not exhibit a difference in glucose tolerance compared to age-matched controls. In a further study, similar to pregnant mice, aged *HKDC1*^{+/−} mice at 28-weeks of age also demonstrated impaired glucose tolerance during an OGTT. These results, summarized in Table 2, suggest that HKDC1 is important for maintaining glucose homeostasis under conditions of metabolic stress, such as in pregnancy and aging.

What precise function does HKDC1 have in glucose regulation during pregnancy? *HKDC1*^{+/−} mice did not display any differences in baseline phenotype, random blood glucose, fasting glucose and insulin, insulin action or gluconeogenesis as compared to wild-type controls. Additionally, these mice did have liver glycogen levels that trended lower than littermate controls. To shed light on this question, Ludvik et al [31] utilized an oral bolus of [U-^{13}C]glucose in the OGTT, and the overall quantity of radiolabeled glucose in various tissues was analyzed. Following the OGTT, *HKDC1*^{+/−} mice were found to have significantly lower tissue uptake of glucose compared to wild type mice. This finding correlates with increased blood glucose excursion seen during an OGTT. Though these radiolabeled glucose experiments were not conducted in pregnant mice, the authors suggest that HKDC1 may be important for whole-body glucose utilization and energy storage, particularly under conditions of enhanced metabolic stress, which includes pregnancy [58,59]. These data also support the GWAS-derived observation that variants in *HKDC1* are associated with maternal 2-hour glucose levels after an OGTT at 28 weeks' gestational age in humans.

Modulation of hepatic HKDC1 protein expression

Utilizing an adult-onset hepatocyte-specific HKDC1 gain/loss mouse model, Khan et al [32] (Table 2) assessed the importance of liver HKDC1 on glucose metabolism during pregnancy. Here, *HKDC1* was knocked out of the adult mouse liver using AAV-Cre constructs in *HKDC1*^{fl/fl} mice (www.komp.org). Overexpression of HKDC1 in the mouse liver was achieved by injecting mice with a human *HKDC1* adenoviral construct. Mice were examined both pre-pregnancy and at day 17–18 of gestation. Pregnant mice exhibit insulin resistance that peaks toward the latter half of gestation, however the authors showed that overexpression of liver-specific HKDC1 during gestation reverses this phenomenon. In contrast, knockout of hepatic HKDC1 in pregnant mice significantly impairs glucose tolerance. In agreement with these findings, pregnant mice overexpressing hepatic HKDC1 produced significantly less glucose during a pyruvate challenge, whereas hepatic HKDC1 knockdown mice had more pronounced hepatic gluconeogenesis. Complementing this observation, expression of genes involved in *de novo* gluconeogenesis negatively correlated with the level of hepatic HKDC1 expression. At a molecular level, the authors further found that with enhanced HKDC1 expression during pregnancy, there was enhanced phosphorylation and subsequent activation of protein kinase B (Akt) in response to

insulin as compared with wild-type mice. Additionally, these mice overexpressing liver HKDC1 exhibited significantly greater plasma levels of nonesterified fatty acids and ketones in the fasting state, suggesting enhanced β-oxidation. Taken together, this study demonstrates that liver-specific HKDC1 has roles in influencing whole-body nutrient balance during pregnancy through modulation of insulin sensitivity, glucose tolerance, hepatic gluconeogenesis, and ketone production.

Potential roles for HKDC1 in glucose homeostasis

From the mouse studies described, we have gathered that HKDC1 is critical for glucose homeostasis predominately under conditions of enhanced metabolic stress, which includes pregnancy. Does HKDC1's roles in glucose homeostasis extend beyond the traditional functions of HKs? HKDC1's localization to the outer mitochondrial membrane may be important, as overexpression of liver-specific HKDC1 significantly modulates mitochondrial tasks including reducing glycolytic capacity, maximal respiration, glucose oxidation, and mitochondrial membrane potential [33]. Hence, excess HKDC1 expression induces alterations in mitochondrial dynamics within hepatocytes. Whether liver-specific HKDC1 protein expression levels are altered during pregnancy in relation to specific genetic variants of *HKDC1* is not yet known.

A common trend in these described mouse models is the role of HKDC1 in glucose metabolism during pregnancy, which is a time of increased cellular stress. Prior work by Evstafieva et al [60] found that HKDC1 gene transcription is greatly upregulated under cellular stress due to the upregulation of Activating Transcription Factor 4 (ATF4). ATF4 is a central factor in modulating the cellular integrated stress response to allow for cells to adapt to and endure stressors [61–63]. Inhibition of the mitochondrial respiration chain or endoplasmic reticulum (ER) stress causes an upregulation of *HKDC1*, however, in the presence of the same stress along with inhibition of ATF4 by RNA interference, *HKDC1* gene expression is attenuated. Examination of whether specific *HKDC1* gene variants modulate mitochondrial function to different extents during pregnancy, and hence regulate the extent to which glucose homeostasis is maintained, has not been performed.

HKDC1 association with hemoglobin A1c

The Accelerating Medicines Partnership Type 2 Diabetes Knowledge Portal project (AMP-T2DKP) analyzes human genetic data from T2DM patients for the purpose of identifying novel targets for therapeutic development. Here, a GWAS using a multi-parent advanced generation inter-cross (MAGIC) plus population of T2DM suggests a genetic link between at least 45 *HKDC1* variants and hemoglobin A1c [https://t2d.hugeamp.org/region.html] (p values from $1.35e^{-62}$ for variant rs4745982 to 0.009705 for variant rs10998736; sample size of 82,442 individuals with T2DM). A majority of these identified variants are suggested to alter transcription factor binding motifs, thus altering gene transcription of *HKDC1*. Amongst the T2DM population studied above, the variants of *HKDC1* identified differ from those discussed that are associated with 2-hour plasma glucose levels during pregnancy. Along with the aforementioned data from GWAS analyses of certain population cohorts detailing associations of *HKDC1* with 2-hour plasma glucose levels during an OGTT, these

studies collectively indicate a potential role for HKDC1 in overall glucose metabolism and homeostasis, possibly linking distinct variants of *HKDC1* to GDM and T2DM.

Conclusion

Novel hexokinase HKDC1 phosphorylates glucose and is widely expressed in many human tissues, similarly to the family of HKs. However, the published data suggests that HKDC1 has functions extending beyond the traditional role of an HK and is important for regulating glucose homeostasis during pregnancy. GWAS analyses have pinpointed specific genetic variations that are connected with 2-hour plasma glucose levels post-glucose load. Closer analyses of these nucleotide polymorphisms demonstrated that they may affect regulation of *HKDC1* gene expression, leading to reductions in *HKDC1* expression in women found to have higher gestational glucose levels. The finding that lower levels of *HKDC1* may result in gestational hyperglycemia is congruent with mouse model studies showing that in times of cellular stress such as pregnancy, reducing HKDC1 protein expression is associated with worsened insulin resistance, reduced glucose utilization and tolerance, and increased gluconeogenesis – which are reversed with increasing HKDC1 levels. It may be possible that genetic variations in *HKDC1* may prevent adequate HKDC1 protein expression or function during pregnancy, which may cause individuals with these variants to become more susceptible to the development of GDM.

The precise mechanisms by which HKDC1 affects gestational glucose metabolism are still not fully understood. Do genetic variants which alter *HKDC1* gene expression also translate into significantly different HKDC1 protein levels? Furthermore, why precisely does HKDC1's roles in glucose metabolism occur only in times of cellular stress? Therefore, more work aimed at identifying the pathways and precise molecules affected by HKDC1 expression is needed. Presently, it is clear that HKDC1 is involved in modulation of glucose homeostasis during pregnancy, and given the multitude of complications that GDM poses for mother and child, a thorough understanding of the mechanisms of action of HKDC1 deserves to the elucidated for the benefit of human health and potentially seeking therapeutics targeting HKDC1 action.

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Table 1:

Genetic variants in *HKDC1* and their associations

Reference	SNP Variant	Related trait	*P* value	Ethnicity	Participants	Trait description
Hayes et al. [27]	rs5030937	gravid 2HPG	1.02E-22	Hispanic (n=817), Afro-Caribbean (n=1075), Northern European (n=4393 *), Thai (n=1,178)	7,463 pregnant women	2-h plasma glucose collected after OGTT at ~28 weeks gestation
	rs4746822	gravid 2HPG	6.30E-16			
Guo et al. [47]	rs4746822	gravid 2HPG	4.41E-13	Africa, Americas, Asia, Europe	4,437 pregnant women from Hayes et al. cohort	2-h plasma glucose collected after OGTT at ~28 weeks gestation
	rs10762264		3.75E-12			
	rs2394529		1.88E-12			
	rs9645501		8.61E-06			
Kanthimathi et al. [56]	rs4746822	GDM	0.004	South Indian	500 pregnant women with GDM, 510 non-GDM	Following OGTT during pregnancy, plasma glucose values exceed: • Fasting 5.1 mmol/L • 1-h 10.0 mmol/L • 2-h 8.5 mmol/L
	rs10762264		0.002			
	rs2394529		0.020			
	rs9645501		0.059			
Tan et al. [55]	rs4746822	gravid 2HPG	0.016	Han Chinese	334 pregnant women, 367 controls	Following OGTT during 24–28 weeks gestation, plasma glucose values exceed: • Fasting 5.1 mmol/L • 1-h 10.0 mmol/L • 2-h 8.5 mmol/L

*
Includes external replication analysis

Table 2:

Effects of modulation of HKDC1 protein expression during pregnancy on glucose homeostasis

Reference	Experiment	Location	Method of Analysis	Timing of Analysis	Effects on glucose homeostasis
Ludvik et al. [31]	50% HKDC1 knockdown	All tissues	Oral glucose load	Day 15 of gestation	↓ glucose tolerance ↓ tissue glucose uptake ↔ insulin sensitivity ↔ gluconeogenesis
Khan et al. [32]	HKDC1 knockout	Liver	Intraperitoneal glucose load	Day 17–18 of gestation	↓ glucose tolerance ↑ hepatic gluconeogenesis ↔ insulin sensitivity ↔ Akt phosphorylation△
	HKDC1 overexpression	Liver	Intraperitoneal glucose load	Day 17–18 of gestation	↑ glucose tolerance ↑ insulin sensitivity ↑ Akt phosphorylation△ ↑ ketone body production✝ ↓ hepatic gluconeogenesis

△ Akt phosphorylation assessed in the liver, adipose tissue, and skeletal muscle of pregnant mice

✝ Ketones assessed in pregnant mice during fasting