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ARTICLE

Pathophysiology of postprandial hyperglycaemia in women with type 1 diabetes during pregnancy

H. R. Murphy · D. Elleri · J. M. Allen · J. Harris · D. Simmons · G. Rayman · R. C. Temple · A. M. Umpleby · D. B. Dunger · A. Haidar · M. Nodale · M. E. Wilinska · R. Hovorka

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Abstract
Aims/hypothesis Although maternal hyperglycaemia is associated with increased risk of adverse pregnancy outcome, the mechanisms of postprandial hyperglycaemia during pregnancy are poorly understood. We aimed to describe glucose turnover in pregnant women with type 1 diabetes, according to stage of gestation (early vs late gestation).
Methods The rates of systemic glucose appearance (R_a) and glucose disposal (R_d) were measured in ten pregnant women with type 1 diabetes during early (12–16 weeks) and late (28–32 weeks) gestation. Women ate standardised meals—a starch-rich 80 g carbohydrate dinner and a sugar-rich 60 g carbohydrate breakfast—and fasted between meals and overnight. Stable-label isotope tracers ([6,6-$^2H_2$]glucose and [U-^{13}C]glucose) were used to determine R_a, R_d and glucose bioavailability. Closed-loop insulin delivery maintained stable glycaemic conditions.
Results There were no changes in fasting R_a (10±2 vs 11±2 μmol kg^{-1} min^{-1}; p=0.32) or fasting R_d (11±2 vs 11±1 μmol kg^{-1} min^{-1}; p=0.77) in early vs late gestation. There was increased hepatic insulin resistance (381±237 vs 540±242 μmol kg^{-1} min^{-1}×pmol/l; p=0.04) and decreased peripheral insulin sensitivity (0.09±0.04 vs 0.05±0.02 μmol kg^{-1} min^{-1} per pmol/l dinner, 0.11±0.05 vs 0.07±0.03 μmol kg^{-1} min^{-1} per pmol/l breakfast; p=0.002) in late gestation. It also took longer for insulin levels to reach maximal concentrations (49 [37–55] vs 71 [52–108] min; p=0.004) with significantly delayed glucose disposal (108 [87–125] vs 135 [110–158] min; p=0.005) in late gestation.
Conclusions/interpretation Postprandial glucose control is impaired by significantly slower glucose disposal in late gestation. Early prandial insulin dosing may help to accelerate glucose disposal and potentially ameliorate postprandial hyperglycaemia in late pregnancy.

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H. R. Murphy (✉) · D. Elleri · J. M. Allen · J. Harris · A. Haidar · M. Nodale · M. E. Wilinska · R. Hovorka
University of Cambridge Metabolic Research Laboratories and NIHR Cambridge Biomedical Research Centre,
Institute of Metabolic Science,
Box 289, Addenbrooke's Hospital, Hills Road,
Cambridge CB2 0QQ, UK
e-mail: hm386@medschl.cam.ac.uk

D. Elleri · D. B. Dunger · M. E. Wilinska · R. Hovorka
Department of Paediatrics, University of Cambridge,
Hills Road,
Cambridge, UK

D. Simmons
Cambridge University Hospitals NHS Foundation Trust,
Cambridge, UK

G. Rayman
Diabetes Centre, Ipswich Hospital NHS Trust,
Ipswich, UK

R. C. Temple
Elsie Bertram Diabetes Centre, Norfolk and Norwich University Hospital NHS Trust,
Norwich, UK

A. M. Umpleby
Postgraduate Medical School, University of Surrey,
Guildford, UK

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Abbreviations

CSII	Continuous subcutaneous insulin infusion
EGP	Endogenous glucose production
IQR	Interquartile range
MCR	Metabolic clearance rate
NICE	National Institute for Health and Clinical Excellence
R_a	Rate of appearance
$R_{a\ sugars}$	Rate of appearance of simple carbohydrates
$R_{a\ total}$	Total postprandial glucose appearance
R_d	Rate of disposal
$R_{I,EGP}$	Hepatic insulin resistance
$S_{I,PERI}$	Peripheral insulin sensitivity
$t_{25\%}$, $t_{50\%}$, $t_{75\%}$	Time for 25%, 50% and 75% appearance/disappearance
t_{max}	Time to maximum concentration in plasma
TTR	Tracer:tracee ratio

Introduction

Maternal glucose is the primary metabolic substrate for fetal growth during pregnancy, both in healthy women and in women with diabetes. Data from healthy pregnant women suggest that the mother adapts to the increasing fetal demands for glucose by increasing both the total rate of appearance of glucose and the endogenous glucose production in late gestation [1]. Women with type 1 diabetes spend on average 8 h per day in hyperglycaemia in late gestation, with most hyperglycaemic excursions following meals [2]. As the maternal and fetal glucose pools are in equilibrium [3], with a rapid transfer of glucose to the fetus according to maternal glucose concentration [4], maternal hyperglycaemia is associated with an adverse pregnancy outcome, most commonly fetal growth acceleration and increased risk of large-for-gestational-age offspring [5–7]. This remains the most common complication of pregnancy in type 1 diabetes [8–10] and confers immediate risks of delivery complications and neonatal hypoglycaemia in addition to the longer-term risks of insulin resistance, obesity and type 2 diabetes [11–13].

After digestion, monosaccharides are transported into the portal vein, where the systemic appearance of glucose is determined by the balance between hepatic extraction and release into the systemic circulation. The postprandial glucose excursion is defined by interactions between total postprandial glucose appearance (from sugars and complex carbohydrates), endogenous glucose production (EGP) and glucose disposal. Defining the precise contribution of complex carbohydrate is methodologically difficult, requiring the intrinsic labelling of glucose in starch [14]. Hence, the precise mechanisms of postprandial hyperglycaemia for meals containing complex carbohydrates have not yet been elucidated.

The oral glucose isotope [U-^{13}C]glucose traces meal-derived sugars. Its use has provided insights into the appearance of meal-derived glucose for sugar-rich meals in healthy individuals [15, 16] and to a limited extent in those with type 1 diabetes [17, 18]. The systemic glucose isotope [6,6-$^{2}H_2$]glucose traces the rate of systemic appearance (R_a) and disposal (R_d) of glucose. This allows a calculation of EGP during fasting conditions and of total postprandial glucose appearance (the sum of postprandial glucose from sugars, complex carbohydrates and EGP) after meals, thereby providing clinically relevant data regarding the discrepancy between glucose appearance and disposal. Carbohydrate digestion rates and gastric emptying are complex, with both intra- and interindividual variability, and in people with type 1 diabetes are further influenced by pre-meal glucose control [19]. Pregnancy adds further challenges of tight postprandial glucose control targets (<7.8 mmol/l at 1 h) as well as dynamic alterations in EGP, insulin kinetics and gastric emptying [1, 20, 21].

Recent technological advances, in particular the accelerated development of closed-loop insulin delivery systems, have further highlighted the need for improved prandial algorithms [22, 23]. Physiologically derived insulin algorithms would benefit from an accurate measurement of glucose turnover and insulin kinetics in pregnancy for women with type 1 diabetes. The aim of this study was to facilitate the development of improved prandial insulin algorithms by quantifying glucose turnover during fasting and postprandial conditions in pregnancy in women with type 1 diabetes. Values for R_a and R_d were quantified to document changes in glucose turnover according to stage of gestation (early vs late pregnancy) following an evening meal, overnight fast and breakfast meal. In addition, gestational changes in insulin resistance and insulin kinetics were investigated.

Methods

Study protocol Ten pregnant women with type 1 diabetes were admitted to the Wellcome Trust clinical research facility (Cambridge, UK) for 24 h on two separate occasions during early pregnancy (12–16 weeks) and late gestation (28–32 weeks). The protocol was approved by the Suffolk, Norfolk and Cambridgeshire research ethics committees, and all participants provided written informed consent.

Study participants From March 2009 to March 2010, ten pregnant women (median age 31.1 [28.7–31.7] years, duration of diabetes 19 [13.5–24] years, weight 66.6 [64–73.9] kg, booking HbA_{1c} 6.9% [6.2–8.0] and BMI 24.1 [23.1–26.3] kg/m^2) were recruited. All participants were non-smokers, and half were primiparous. Inclusion criteria were diagnosis of type 1 diabetes (WHO criteria) for at least 12 months, intensive insulin therapy (multiple daily injections or pump therapy) and a viable singleton pregnancy. Women with concomitant physical or psychological conditions, poor glycaemic control (HbA_{1c} >10% [86 mmol/mol]), class II–III obesity (BMI ≥35 kg/m^2), insulin resistance (total daily insulin dose ≥1.5 U/kg), impaired renal function, autonomic neuropathy and/or gastroparesis were excluded. For women on multiple daily injections (*n*=5), basal insulin was withdrawn 24 h before admission and replaced with rapid-acting insulin analogue Aspart (Novo-Nordisk, Bagsvaerd, Denmark) delivered in three or four pre-meal boluses.

Study procedures At 13:00 hours, two intravenous sampling cannulae were inserted: one for obtaining venous blood samples and the other to infuse the glucose-appearance-mimicking glucose tracer ([6,6-$^2H_2$]glucose). A continuous subcutaneous insulin infusion (CSII) was commenced (Deltec Cozmo; Smiths Medical, St Paul, MN, USA) delivering the rapid-acting insulin analogue Aspart, with closed-loop insulin delivery used to maintain stable glycaemic conditions [22]. For women on CSII, their existing site was used, and for those previously on injection therapy, the insertion site was located on the inguinal region of the anterior abdominal wall. Prandial insulin boluses were not part of closed-loop insulin delivery; these were calculated by women according to their pre-meal capillary glucose measurement, the carbohydrate content of the meal and the insulin:carbohydrate ratio. All women were aiming for the National Institute for Health and Clinical Excellence (NICE) postprandial glycaemic control target of <7.8 mmol/l at 1 h [21]. All boluses were administered by a research nurse immediately before meals using a standard bolus delivery pattern. Full details of the prandial boluses and total daily insulin doses in early and late gestation are described in Table 1.

At 16:00 hours, a primed intravenous infusion of [6,6-$^2H_2$]glucose (1 mg/kg) was started and was continued until 12:00 the following day. The infusion rate of [6,6-$^2H_2$] glucose remained constant when no changes in systemic glucose appearance were anticipated (16:00–18:00 and 02:00–07:00 hours). Infusion rates were increased following meal consumption: –10 to 0 min 100%, 0–10 min 125%, 10–20 min 180%, 20–30 min 240%, 30–60 min 280%, 60–70 min 270%, 70–80 min 260%, 80–90 min 250%, 90–120 min 240%, 120–150 min 200%, 180–210 min 180%, 210–240 min 160%, 240–270 min 140%, 270–300 min 120%, 300–330 min 110%, 330–360 min 105% and 360–450 min 100% to mimic systemic glucose appearance in postprandial conditions and reduce swings in the tracer:tracee ratio (TTR).

At 18:00 hours, the women ate an evening meal. This was followed by an overnight fast (drinking water being permitted) until the following morning, when a breakfast meal was provided at 07:00 hours. The study ended at 12:00 on day 2.

Study meals The evening meal was penne pasta with a tomato-based vegetable sauce and grated cheese topping, freshly prepared by the study dietitian. It contained 2,497 kJ (602 kcal): 80 g carbohydrate (52% of total energy), 11 g sugars, 4 g fat (16%) and 9 g protein (32%). Breakfast, which comprised orange juice and two slices of wholegrain toast with butter and jam, contained 1,489 kJ (356 kcal): 57 g carbohydrate (62%), 25 g sugars, 11 g fat (29%) and 7.6 g protein (9%). A meal glucose isotope tracer, 0.85 g [U-^{13}C]glucose, to trace meal-derived sugars, was divided into two equal parts and dissolved in jelly (Hartley's sugar-free raspberry jelly; Premier Foods Ltd, St Albans, UK) in two 5 ml plastic cups, frozen and swallowed intact 5 min and 10 min following the start of each meal. All meals were completed within 20 min of commencement.

Laboratory assays Venous samples were obtained for measurement of plasma glucose concentration (Yellow Springs Instrument YSI 2300 STAT Plus Analyser; Farnborough, UK). Samples were centrifuged immediately with plasma kept on ice and stored at −80°C prior to further analyses. Plasma insulin concentration was measured by an immunochemiluminometric assay (Invitron, Monmouth, UK; intra-assay CV 4.7%, interassay CV 7.2–8.1%). Glucose isotope enrichment was measured using the trimethyl silyl-*O*-methyloxime derivative, by GC-MS (Agilent 5975 C inert XL EI/CI MSD; Agilent Technologies, Wokingham, UK). Ions monitored were *m/z* 319.2, *m/z* 321.2 and *m/z* 323.2 for glucose, [6,6-$^2H_2$]glucose and [U-^{13}C] glucose, respectively, as previously described [24, 25]. Samples for the determination of background glucose isotope enrichment were taken at 15:50, 15:55 and 16:00 hours and for pre-meal glucose enrichment at 15, 10, 5 and 0 min before meals. Plasma glucose, insulin and glucose enrichment were measured at 10 min intervals for 90 min after meals and at 15–30 min intervals at other times.

Calculations The glucose turnover calculations were based on the maximum likelihood method [26] modified for a Bayesian implementation in WinBUGS (MRC Biostatistics Unit, Cambridge, UK) version 1.4 [27] and utilising the

Table 1 Participant characteristics: glycaemic control and insulin doses in early and late pregnancy

Characteristic		Early gestation				Late gestation			
ID	BMI (kg/m^2)	HbA_{1c}, % (mmol/mol)	Insulin total daily dose, U (U/kg)	Prandial boluses[a]		HbA_{1c}, % (mmol/mol)	Insulin total daily dose, U (U/kg)	Prandial boluses[a]	
				Dinner	Breakfast			Dinner	Breakfast
01	23.1	6.6 (49)	50 (0.77)	5	8	6.3 (45)	68.5 (0.95)	20	20
02	22.3	6.1 (43)	35 (0.55)	7	6	5.4 (36)	54 (0.75)	10	8
03	26.4	6.5 (48)	45 (0.54)	7	9	6.4 (46)	44 (0.50)	10	18
04	22.5	7.5 (58)	44 (0.85)	8	8	6.6 (49)	84 (1.4)	20	8
05	23.2	5.7 (39)	22.2 (0.33)	6.7	8	5.4 (36)	54 (0.75)	13	17.5
06	32.0	8.4 (68)	47.8 (0.51)	11.6	11.8	6.5 (48)	63 (0.67)	14	16.5
07	24.4	6.9 (52)	53 (0.71)	10	7	5.7 (39)	62.6 (0.75)	13	14
08	23.8	5.6 (38)	60 (0.99)	10	10	5.9 (41)	72 (1.0)	12	12
09	27.8	8.7 (72)	60 (0.83)	16	14	8.0 (53)	90 (1.2)	24	12
10	26.1	8.2 (66)	35 (0.53)	13	12	7.1 (54)	50.5 (0.70)	7	10
Median	24.1	6.9 (52)	46.4 (0.63)	9	8.5	6.3 (45)	62.8 (0.75)	13	13
IQR	23.1–26.3	6.2–8.0 (44–64)	37.2–52.3 (0.52–0.84)	7–12.3	6.5–11.9	5.7–6.6 (39–49)	54.0–71.1 (0.68–1.1)	10–20	9–17.5

Values are given as median (IQR) unless stated otherwise

[a] Prandial insulin boluses were not calculated by the closed-loop algorithm. They were calculated by women according to their pre-meal capillary glucose measurement, their insulin:carbohydrate ratio, the insulin sensitivity factor and the carbohydrate content of the meal (80 g dinner, 60 g breakfast). All women were aiming for the NICE postprandial glycaemic control target of <7.8 mmol/l at 1 h

WBDiff interface (MRC Biostatistics Unit) version 1.9.4 to implement the differential equations representing the two-compartment Mari model [28].

We calculated R_a, R_d, and $R_{a\ sugars}$. R_a represents the total rate of glucose appearance in the systemic circulation. During overnight fasting conditions (02:00–07:00 hours), the R_a reflects EGP. During postprandial conditions (5 h post-meal), $R_{a\ total}$ reflects total postprandial glucose appearance, representing the sum of all meal-derived glucose (sugars and complex carbohydrates) and postprandial EGP. R_a and R_d were calculated by analysis of [6,6-$^2H_2$]glucose. $R_{a\ sugars}$ represents the rate of appearance of simple carbohydrates and was calculated from [6,6-$^2H_2$] glucose and [U-^{13}C]glucose utilising the dual-tracer approach [26].

The bioavailability of [U-^{13}C]glucose represented the total bioavailability of meal-derived sugars. The time to reach 25%, 50% and 75% of cumulative $R_{a\ sugars}$ reflects the rate of glucose appearance from meal-derived sugars from the start of meal to 5 h later ($R_{a\ sugars}\ t_{25\%}$, $R_{a\ sugars}\ t_{50\%}$ and $R_{a\ sugars}\ t_{75\%}$). Similarly, the rate of total postprandial glucose appearance was quantified by the time to reach 25%, 50% and 75% of cumulative $R_{a\ total}$ over the 5 h postprandial period.

Insulin resistance and kinetics Hepatic insulin resistance ($R_{I,EGP}$) was calculated as the product of fasting R_a and fasting plasma insulin concentration between 02:00 and 07:00 hours. Peripheral insulin sensitivity ($S_{I,PERI}$) was calculated as average R_d above fasting during the 5 h after meal divided by average plasma insulin above fasting during the first 5 h after each meal.

Insulin kinetics were assessed using a two-compartment absorption chain, model 1 in Wilinska et al. [29] with an impulse response function defined as $y = t \times \exp(-t/t_{max})/(t_{max} \times t_{max} \times \mathrm{MCR})$ where t_{max} is time to maximum insulin concentration in plasma, and MCR is the metabolic clearance rate of insulin. The plasma insulin concentration is obtained by convolution $y \times u$, where u is the insulin delivery. To account for time-varying residual insulin appearance, a linear component was added to the model-derived plasma insulin concentration in the form of $a \times t + b$. The model parameters t_{max}, MCR, a and b were estimated for the 5-h-long dinner and breakfast periods employing a non-linear, weighted, least-squares approach using SAAM II version 1.2.1 software (SAAM Institute, Seattle, WA, USA) with weights reciprocal to the square of the measured plasma insulin concentration.

Statistics The effects of the two meals (dinner and breakfast) and the stage of gestation (early and late) were contrasted using ANOVA with adjustment for maternal characteristics (age, duration of diabetes, HbA_{1c} and BMI at booking). To contrast only the effect of gestation (early vs late), paired t tests were used. Data were log-transformed where appropriate to achieve normality. Values are given as mean±SD, and if not normally distributed as median and interquartile range (IQR). Analyses were conducted on SPSS Version 15 (SPSS, Chicago, IL, USA). Values of $p<0.05$ were considered statistically significant.

Results

Glucose control Closed-loop insulin delivery was used to achieve stable and comparable glycaemic conditions prior to meal consumption and throughout both study visits [22]. Satisfactory glyacemic control was maintained with no

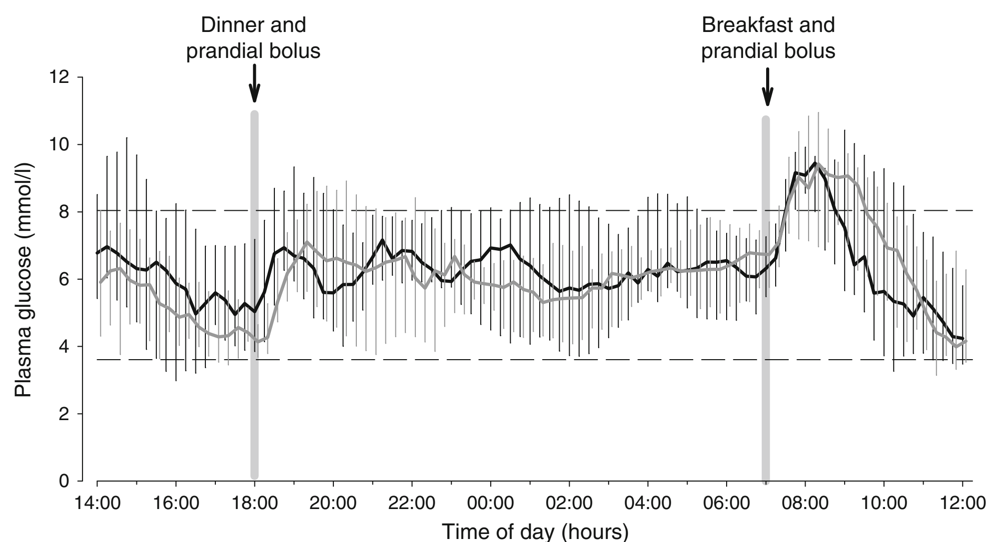

Fig. 1 Plasma glucose levels in early and late gestation. Data are median and IQR, dark grey for early gestation and light grey for late gestation

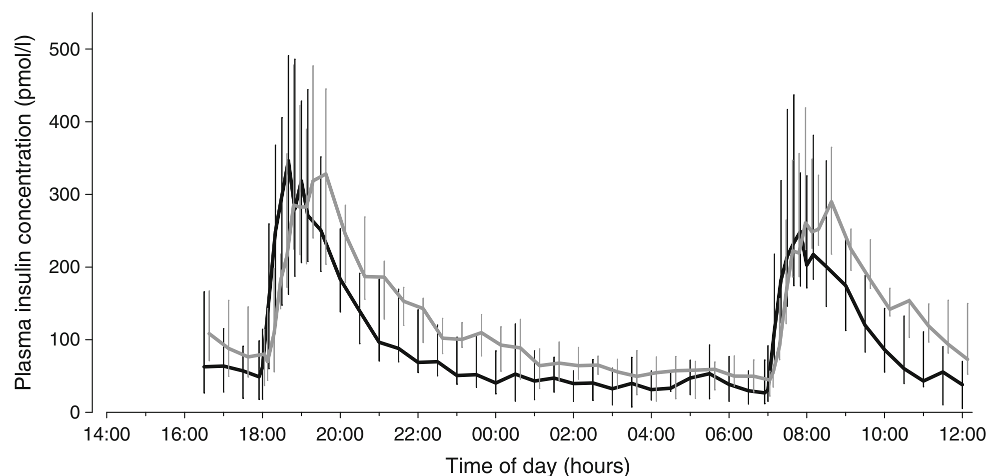

Fig. 2 Plasma insulin concentration in early and late gestation. Data are median and IQR, dark grey for early gestation and light grey for late gestation

significant differences between visits (Fig. 1). Plasma glucose levels were comparable in early (median 6.5 mmol/l, IQR 5.6–8.6) and in late (median 7.0 mmol/l, IQR 6.1–7.8; p=0.72) gestation. Stable pre-meal plasma glucose levels, required for accurate evaluation of glucose enrichment, were obtained (early vs late gestation median [IQR] glucose, pre-dinner 4.9 mmol/l [3.8–7.1] vs 5.7 [4.2–7.8] and pre-breakfast 6.1 mmol/l [5.3–7.0] vs 6.6 mmol/l [5.7–7.4]; p=0.50).

Insulin requirements Prandial insulin boluses (Table 1) increased significantly with advancing gestation (p= 0.0001). There was also a trend to increased basal insulin infusion rate (0.55±0.26 vs 0.67±0.29 U/h; p=0.15) and increased plasma insulin concentration (median [IQR], 99 [73–138] vs 129 [112–191] pmol/l; p=0.07) in late gestation (Fig. 2).

Glucose enrichment The stable-label tracer approach permitted the measurement of the systemic R_a of glucose. In postprandial conditions, $R_{a\ total}$ incorporates total meal-derived glucose (sugars and complex carbohydrates) and postprandial EGP. The [U-^{13}C]glucose TTR for sugars was indistinguishable between early and late gestation, with more intraindividual and gestational variability in [6,6-$^2H_2$] glucose TTR for glucose appearance from total carbohydrate (Fig. 3).

Glucose appearance and turnover in fasting conditions The rates of fasting glucose appearance and disposal during early and late gestation are shown in Table 2 and Figs 4 and 5. During the overnight period (02:00–07:00 hours), there was an increase in absolute EGP (early vs late gestation fasting R_a 724±151 vs 844±184 μmol/min; p=0.04), which did not persist when corrected for maternal weight gain (10±2 vs 11±2 μmol kg^{-1} min^{-1}; p=0.3). There were no gestational changes in the absolute or weight-corrected overnight glucose disposal rates (early vs late gestation fasting R_d 11±2 vs 11±1 μmol kg^{-1} min^{-1}; p=0.8). Nonetheless, higher insulin doses and plasma insulin concentrations were required to achieve a steady state in late gestation.

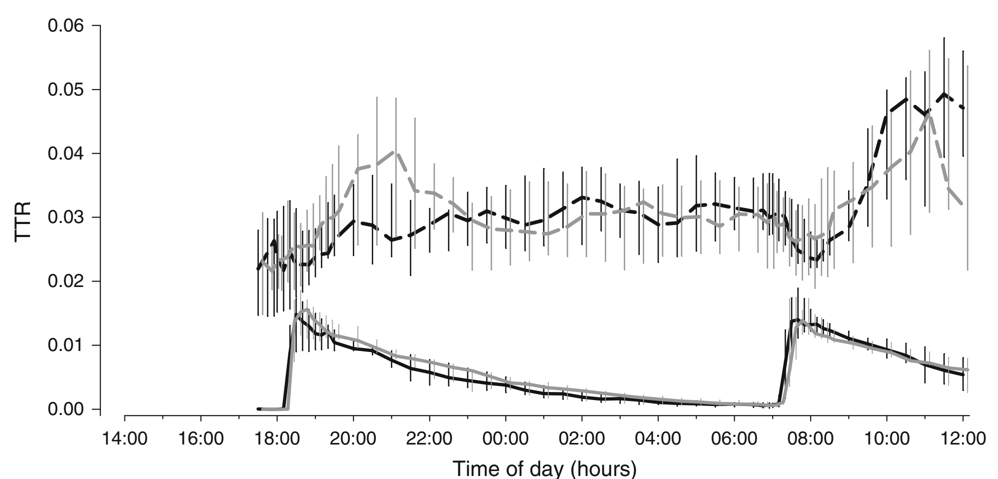

Fig. 3 TTR in early and late gestation. Data are median and IQR, dark grey for early gestation and light grey for late gestation. Dashed lines represent the [6,6-$^2H_2$]glucose tracer, tracing total absorption of carbohydrate ($R_{a\ meal}$+EGP) and solid lines the [U-^{13}C]glucose tracer, tracing sugars

Table 2 Glucose turnover, insulin sensitivity and insulin kinetics during early and late gestation in type 1 diabetes pregnancy

Variable	Units	Meal	Visit 1	Visit 2	ANOVA p value[a]	
			Mean±SD	Mean±SD	Visit	Meal
Fasting R_a (endogenous glucose production)[b]	Per kg (μmol kg^{-1} min^{-1})		10±2	11±2	0.3	–
	Absolute (μmol/min)		724±151	844±184	0.04	–
Fasting R_d[b]	Per kg (μmol kg^{-1} min^{-1})		11±2	11±1	0.8	–
	Absolute (μmol/min)		737±137	789±103	0.2	–
Surrogate marker of $R_{I,EGP}$	μmol kg^{-1} min^{-1}×pmol/l		381±237	540±242	0.04	–
Surrogate marker of $S_{I,PERI}$	μmol kg^{-1} min^{-1} per pmol/l	Dinner	0.09±0.04	0.05±0.02	0.002	0.04
		Breakfast	0.11±0.05	0.07±0.03		
Bioavailability of sugars	%	Dinner	102±6	97±9	0.4	1.0
		Breakfast	101±8	99±8		
$R_{a\ sugars}$	$R_{a\ sugars}$ $t_{25\%}$ (min)	Dinner	31±11	25±5	0.1	0.3
		Breakfast	27±8	24±9		
	$R_{a\ sugars}$ $t_{50\%}$ (min)	Dinner	64±29	51±20	0.3	0.005
		Breakfast	44±16	35±12		
	$R_{a\ sugars}$ $t_{75\%}$ (min)	Dinner	123±46	125±52	0.6	0.003
		Breakfast	76±32	78±41		
Postprandial glucose appearance $R_{a\ total}$	$R_{a\ meal}$ $t_{25\%}$ (min)	Dinner	51±10	49±15	0.7	<0.0001
		Breakfast	34±8	28±19		
	$R_{a\ meal}$ $t_{50\%}$ (min)	Dinner	109±24	97±39	0.6	<0.0001
		Breakfast	58±18	52±33		
	$R_{a\ meal}$ $t_{75\%}$ (min)	Dinner	168±30	178±49	0.5	<0.0001
		Breakfast	88±34	88±54		
Postprandial R_d	R_d $t_{25\%}$ (min)	Dinner	70±14	85±22	0.02	0.1
		Breakfast	64±8	75±15		
	R_d $t_{50\%}$ (min)	Dinner	112±22	142±34	0.003	0.07
		Breakfast	103±17	125±21		
	R_d $t_{75\%}$ (min)	Dinner	170±32	208±33	0.001	0.03
		Breakfast	150±38	186±24		
Insulin MCR	ml kg^{-1} min^{-1}	Dinner	25±3	24±11	0.4	0.5
		Breakfast	30±9	24±11		
Insulin t_{max}	min	Dinner	53±13	79±33	0.0002	0.4
		Breakfast	46±10	78±34		

[a] ANOVA was adjusted for baseline differences in maternal age, duration of diabetes, HbA_{1c} and BMI

[b] R_a and R_d were determined from 02:00 to 07:00 hours

Insulin resistance There were gestational increases in $R_{I,EGP}$ (early vs late gestation 381±237 vs 540±242 μmol kg^{-1} min^{-1}×pmol/l; p=0.04). There was also a decreased $S_{I,PERI}$ in late gestation (early vs late gestation dinner 0.09±0.04 vs 0.05±0.02 μmol kg^{-1} min^{-1} per pmol/l [p=0.002] and breakfast 0.11±0.05 vs 0.07±0.03 μmol kg^{-1} min^{-1} per pmol/l [p=0.004]).

Postprandial glucose appearance Glucose appearance in postprandial conditions is shown in Table 2. The bioavailability of simple carbohydrates was unchanged during pregnancy (102±6 vs 97±9%; p=0.4), although differences in meal composition led to a significantly more rapid appearance of glucose after breakfast (Fig. 4a) than after dinner (p=0.005 and p<0.0001 for $R_{a\ sugars}$ and $R_{a\ total}$, respectively). However, gestation did not affect the rate or timing of the postprandial R_a (early vs late gestation dinner $R_{a\ total}$ $t_{50\%}$ 109±24 vs 97±39 min and breakfast $R_{a\ total}$ $t_{50\%}$ 58±18 vs 52±33 min; p=0.6). While there are no quantitative differences in postprandial glucose appearance over the 5 h period, in early pregnancy there are two distinct peaks of glucose appearance after dinner, with a second peak between 2 and 4 h after dinner (Fig. 4b). This second peak effect is less pronounced in late pregnancy and did not occur after breakfast, with its higher proportion of simple carbohydrates.

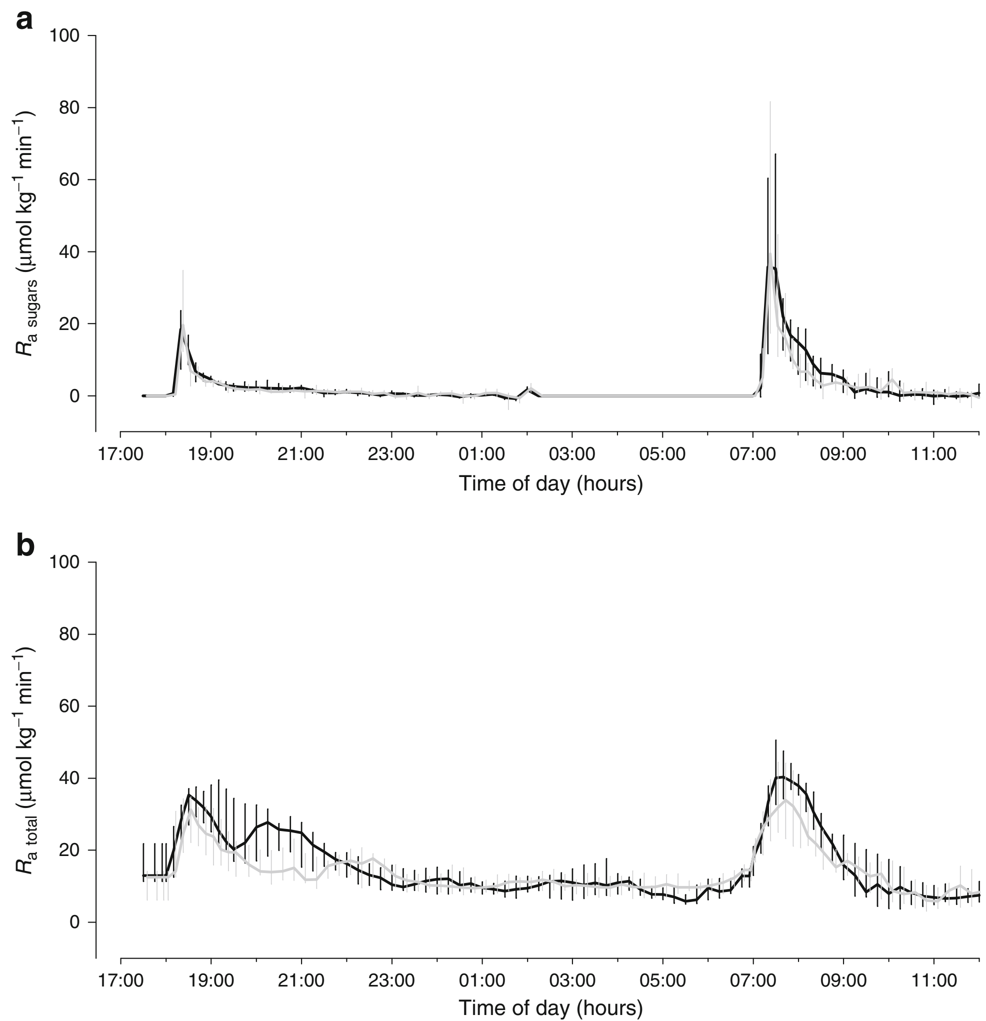

Fig. 4 Systemic R_a of glucose during early and late gestation. **a** $R_{a\ sugars}$; **b** $R_{a\ total}$. During postprandial conditions (5 h post-meal), $R_{a\ total}$ reflects total postprandial glucose appearance, representing the sum of all meal-derived glucose (sugars and complex carbohydrates) and postprandial EGP. Data are median and IQR, dark grey for early gestation and light grey for late gestation

Postprandial glucose disposal Although the fasting and postprandial glucose appearance rates were unchanged, there were significant delays in postprandial glucose disposal (Table 2, Fig. 5), after both dinner and breakfast, in late gestation (early vs late dinner R_d $t_{50\%}$ 112±22 vs 142±34 min, and breakfast R_d $t_{50\%}$ 103±17 vs 125±21 min; p=0.003). The delayed postprandial glucose disposal could not be attributed to increased insulin clearance

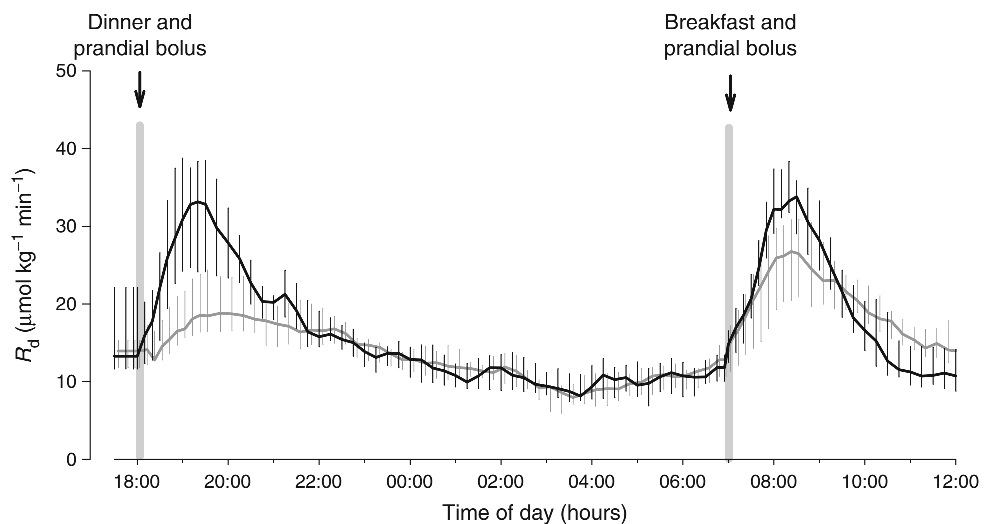

Fig. 5 Systemic glucose R_d during early and late gestation. Data are median and IQR, dark grey for early gestation and light grey for late gestation

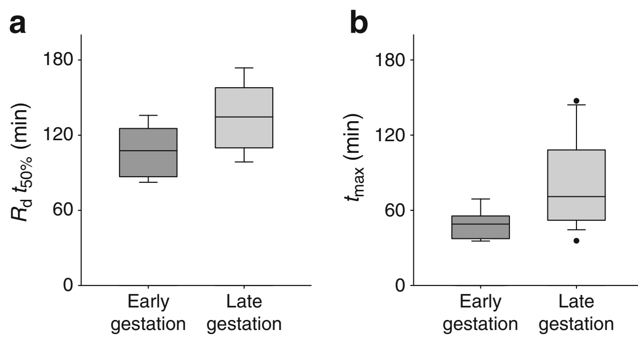

Fig. 6 Glucose disposal and plasma insulin concentration t_{max} during early and late gestation. **a** Boxplots depicting R_d $t_{50\%}$ for the dinner and breakfast meals combined. **b** Boxplots depicting insulin t_{max} for the dinner and breakfast meals combined. Data are median, IQR and range (including outliers), dark grey for early gestation and light grey for late gestation

as the metabolic clearance rate of insulin was unchanged in late gestation (p=0.4). To examine the possibility of delayed insulin absorption from the subcutaneous infusion site, we calculated insulin t_{max}. Strikingly, it took significantly longer for insulin levels to reach maximal concentrations in late gestation (early vs late insulin t_{max} dinner 53±13 vs 79±33 min and breakfast 46±10 vs 78±34 min; p=0.0002).

The similar effects of gestation on overall glucose disposal (breakfast and dinner combined) and maximal postprandial plasma insulin concentrations are shown in Fig. 6. There were significant delays both for glucose disposal (early vs late gestation R_d $t_{50\%}$ 108 [87–125] vs 135 [110–158] min; p=0.005) and for peak postprandial plasma insulin concentration (early vs late gestation insulin t_{max} 49 [37–55] vs 71 [52–108] min; p=0.004).

Discussion

We describe the first detailed physiological insights into carbohydrate metabolism, glucose turnover and insulin kinetics in pregnancy in women with type 1 diabetes. In the fasted state, glucose production and disposal rates were well matched in early and late gestation, with no differences between our study population and what had previously been described in healthy pregnancy [1]. In addition, we found no changes in glucose bioavailability or postprandial glucose appearance between early and late gestation. However, we found significant delays of approximately 30 min duration in postprandial glucose disposal during late gestation. This is likely to be due to slower achievement of a maximal postprandial plasma insulin concentration and increased peripheral insulin resistance, which together impede postprandial glucose uptake, facilitating more prolonged postprandial hyperglycaemia in late pregnancy.

A similar study using [6,6-$^2H_2$]glucose and [U-^{13}C] glucose tracers in healthy pregnant women (seven in early and five in late gestation) was performed by Kalhan et al. [1]. After a prolonged fast (14 h), they also found increases in total gluconeogenesis but no changes in EGP or glucose disposal rates, when adjusted for maternal weight gain. Similarly, fasting plasma insulin concentrations were slightly higher, but not significantly so, in late gestation. Fasting plasma glucose concentrations were notably lower (3.6–4.2 mmol/l) compared with women with type 1 diabetes (6.1 mmol/l). Another study by the same investigators comprising ten pregnant women (five healthy, four with gestational diabetes and one with type 1 diabetes) at term suggested no differences in EGP between healthy controls and women with gestational diabetes [3]. These studies focused on gluconeogenesis during fasting conditions and did not evaluate postprandial glucose control. The computational methodology required for a more complex evaluation of postprandial glucose fluxes has been more recently available [26], thereby facilitating further insights into postprandial glucose control in type 1 diabetic pregnancy.

We have demonstrated an increasing, but potentially modifiable, mismatch between postprandial glucose appearance and glucose disposal in late gestation. Despite marked differences in meal composition (sugar-rich vs starch-rich) and meal timing (07:00 vs 18:00 hours), glucose disposal was similarly delayed in late pregnancy. The particularly rapid appearance of glucose after a sugar-rich breakfast exacerbated this mismatch (R_a $t_{50\%}$ 58±18 min vs R_d $t_{50\%}$ 103±17 min), with the delay between glucose appearance and disposal of approximately 45 min in early pregnancy extended to 75 min in the third trimester (R_a $t_{50\%}$ 52±33 min vs R_d $t_{50\%}$ 125±21 min). As glucose disposal is insulin-mediated and larger prandial boluses were given in late pregnancy, this finding was unexpected and, to our knowledge, has not previously been reported.

The factors contributing to the slower development of maximal postprandial plasma insulin concentration in late pregnancy are unclear. Catalano et al. described increased insulin clearance in pregnancy, but we found no effect of gestation on the metabolic clearance rate of insulin [30]. In obese, non-pregnant individuals, delayed insulin absorption has been associated with large insulin doses and reduced subcutaneous adipose tissue blood flow [31, 32]. Sandqvist et al. reported impaired transport of insulin from the circulation to both adipose tissue and muscle in obese, insulin-resistant postmenopausal women [33]. Although the distribution of insulin to adipose tissue correlated with factors relevant to insulin resistance, the metabolic impact of impaired insulin delivery was unclear. The women in our study were not obese, they had only modest gestational weight gain (approximately 5.7 kg between study visits) and their prandial insulin boluses were not excessive for

pregnancy. Microdialysis techniques would be required to confirm delayed absorption of insulin from subcutaneous tissues to the systemic circulation.

Although insulin resistance is a well-described phenomenon of advancing gestation, whether it occurs predominantly in the liver, peripheral tissues or both has not been established in pregnancy. We found significant consistent reductions in $S_{\mathrm{I,PERI}}$, consistent across women despite their varying body weight and insulin requirements. In contrast to Catalano et al. [34], we also found consistent gestational increases in $R_{\mathrm{I,EGP}}$. As maternal insulin resistance is a prime regulator of nutritional availability for the developing placenta and fetus, increased hepatic and peripheral insulin resistance may reflect normal and physiologically necessary adaptations in healthy pregnancy but contribute to fetal growth acceleration and a risk of large-for-gestational-age offspring in pregnancies complicated by type 1 diabetes.

Clinical and therapeutic implications Although we found no systematic changes in glucose bioavailability or postprandial glucose appearance over gestation, we did observe significant interindividual and intermeal variability in the rate of total carbohydrate absorption. The 'double peak' glucose appearance after dinner in early pregnancy is very similar to that recently described after a pasta meal in non-pregnant individuals [35], suggesting that it is a characteristic feature of meals rich in complex carbohydrates. This has clinical implications for advice given to women on the timing and frequency of postprandial glucose monitoring. Half of the post-breakfast glucose appeared after 1 h (breakfast R_{a} $t_{50\%}$ 58±18 min) but took almost 2 h to do so after dinner (dinner R_{a} $t_{50\%}$ 109±24mins). Testing 1 h after meals may capture peak postprandial glucose, in some women for some meals, but fails to capture the complexity of total carbohydrate absorption between different meals or different women. In practice, continuous glucose monitoring or repeated postprandial testing may be required for optimal insulin dose adjustment [36].

In non-pregnant individuals using CSII, the administration of prandial boluses 15 min before meals is associated with improved postprandial glycaemia [37]. Taken together with our findings, this suggests that the optimal timing for prandial insulin is 15 min before meals in early pregnancy and 30–40 min before meals in late pregnancy. As pregnancy advances, it may be appropriate to advise that women replace rapidly absorbed sugar-rich meals with more slowly absorbed starch-rich alternatives. Adjunctive pharmacological therapies such as pramlintide to suppress glucagon and delay gastric emptying, or α-glucosidase inhibitors to decrease intestinal carbohydrate digestion, may also be beneficial [38], but these are not approved for use in pregnancy. Alternatively, pre-meal snack primers and/or postprandial physical activity, which enhance peripheral glucose uptake, may offer additional therapeutic options in late pregnancy [39].

We used closed-loop insulin delivery as the best available method for facilitating as near to steady-state fasting and pre-meal glucose levels as possible. However, it should be noted that there are typically no differences in the total insulin doses infused or plasma insulin concentrations during conventional CSII and closed-loop insulin delivery [22, 23]. More work is needed to extend and validate our findings and to further evaluate the risks and benefits of earlier insulin administration in pregnancy in type 1 diabetes. Investigators should also seek to determine whether there are differences in insulin absorption according to the delivery method (CSII vs subcutaneous injection) or infusion site (abdomen vs flank or limbs), and whether our findings are specific to type 1 diabetes or applicable also to pregnant women with gestational and type 2 diabetes.

Important strengths of our study include the use of closed-loop insulin delivery to maintain steady-state glycaemic conditions, stable labelled isotopes to document glucose fluxes, standardised mixed meals with a variable dietary composition (a sugar-rich breakfast and a starch-rich evening meal) and advanced computational modelling methodology [26]. Triple tracer techniques allow discrimination between EGP and glucose appearance from meals containing glucose but not complex carbohydrates [40]. Our double tracer methodology is applicable for complex carbohydrates, has comparable postprandial measurement accuracy and benefits from reduced experimental and analytical complexity [41].

Unfortunately, we have no details of maternal body composition, lipids or fat-free vs adipose tissue mass. Although our observation of approximately 100% bioavailability of [U-^{13}C]glucose is in line with previous observations [42–44], absorption of more slowly absorbed sugars (sucrose and fructose) is not necessarily assumed to be related to the absorption of the meal tracer. A limitation, of limited clinical significance, is that we were unable to separate EGP and glucose appearance from complex carbohydrates, which would have required intrinsic labelling of glucose in the starch [14]. The crucial clinically relevant information, is not how much of the postprandial glucose appearance is attributable to EGP vs meal-derived oral glucose, but rather the discrepancy between total postprandial glucose appearance and glucose disposal, which make up the postprandial glucose excursion.

In conclusion, the finding that postprandial plasma t_{max} concentration was significantly delayed in late gestation has clinical implications for the timing of insulin therapy, both in routine clinical care and in the development of closed-loop algorithms. Administering prandial insulin boluses 30–40 min before meals may reduce the mismatch between

glucose appearance and glucose disposal, attenuating post-meal hyperglycaemia in late pregnancy. These insights into the pathophysiology and management of postprandial hyperglycaemia may help women with type 1 diabetes to optimise their glycaemic control in late gestation.

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Duality of interest H.R. Murphy receives speaker honoraria from Minimed Medtronic; R. Hovorka receives speaker honoraria from Minimed Medtronic, Lifescan and Novo Nordisk, and serves on the Medtronic and Animas advisory panel; R. Hovorka, M.E. Wilinska have received licence fees from Becton Dickinson; and R. Hovorka and M.E. Wilinska have patent applications. The remaining authors declare there there is no duality of interest associated with this manuscript. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health

Contribution statement HRM and RH designed the study with input into the study protocol from all authors. JA, JH, DS, RCT and GR recruited participants. JA, JH, DE and HRM performed the studies. AMU measured glucose enrichment. HRM drafted the manuscript, and all authors assisted with revising it critically for important intellectual content. All authors contributed to the analysis and interpretation of data and approved the final version.

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