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Hyperthyroidism impairs pancreatic beta cell adaptations to late pregnancy and maternal liporegulation in the rat

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Abstract *Aims/hypothesis:* Hyperthyroidism modifies lipid dynamics (increased oxidation), impairs insulin action and can suppress insulin secretion. We therefore examined the impact of hyperthyroidism on the relationship between glucose-stimulated insulin secretion (GSIS) and insulin action, using late pregnancy as a model of physiological insulin resistance that is associated with compensatory insulin hypersecretion to maintain glucose tolerance. Our aim was to examine whether hyperthyroidism compromises the regulation of insulin secretion and the ability of insulin to modulate circulating lipid concentrations in late pregnancy. *Materials and methods:* Hyperthyroidism was induced by tri-iodothyronine (T_3) administration from day 17 to 19 of pregnancy. GSIS was assessed during an IVGTT and during hyperglycaemic clamps in vivo and in vitro, using step-up and -down islet perfusions. *Results:* Hyperthyroidism in pregnancy elevated the glucose threshold for GSIS and impaired GSIS at low and high glucose concentrations in islet perfusions. In the intact animal, insulin secretion (after bolus glucose) was more rapidly curtailed following removal of the glucose stimulus to secretion. In contrast, GSIS was maintained during protracted hyperglycaemia (hyperglycaemic clamps) in the hyperthyroid pregnant state in vivo. *Conclusions/interpretation:* Hyperthyroidism in vivo during late pregnancy blunts GSIS in subsequently isolated and

perfused islets at low and high glucose concentrations. It also adversely affects GSIS under conditions of an acute glucose challenge in vivo. In contrast, GSIS is maintained during sustained hyperglycaemia in vivo, suggesting that in vivo factors can rescue GSIS. The ability of insulin to suppress systemic lipid levels during hyperglycaemic clamps was impaired. We therefore suggest that higher circulating lipids may preserve GSIS under conditions of sustained hyperglycaemia in the hyperthyroid pregnancy.

Keywords Insulin resistance · Insulin secretion · Islet perfusions · Pregnancy · T_3

Abbreviations ΔG_{0-30} : incremental blood glucose values integrated over the 30-min period after the bolus injection of glucose · GIR: glucose infusion rate · GSIS: glucose-stimulated insulin secretion · ΔI_{0-30} : incremental plasma insulin values integrated over the 30-min period after bolus injection of glucose · ΔI_{0-60} : incremental plasma insulin values integrated over the period of chronic glucose infusion · ΔI_{60-140} : incremental plasma insulin values integrated over the 80-min period of islet perfusion · k : rate of glucose disappearance · T_3 : tri-iodothyronine · TAG: triacylglycerol

Introduction

In healthy individuals, there is a feedback loop between insulin sensitivity and insulin secretion [1, 2]. This may involve common factors contributing to both insulin resistance and pancreatic beta cell function, or factors influencing insulin sensitivity, pancreatic beta cell function and the feedback mechanisms that link them [3]. Inadequate compensatory hypersecretion of insulin to counter insulin resistance is critical to the pathology of type 2 diabetes [3]. Pregnancy is a progressive state of maternal insulin resistance [4–6] (reviewed in [7]), which in healthy pregnancies is accompanied by reciprocal increases in insulin secretion (see [5, 8]). Gestational diabetes occurs in women who secrete insufficient insulin to compensate for lowered in-

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sulin sensitivity during the third trimester [9] (reviewed in [3]).

In addition to glucoregulation, insulin suppresses adipocyte lipolysis (see [10]) and hepatic VLDL-triacylglycerol (TAG) secretion [11–13]. Elevated plasma NEFA concentrations predict the transition from IGT to type 2 diabetes [14, 15], suggesting that impaired pancreatic beta cell function is related to inadequate suppression of lipolysis. Despite maternal hyperinsulinaemia, pregnancy is also associated with hypertriacylglycerolaemia [16–19]. Insulin hypersecretion is reduced in concert with normalisation of maternal TAG levels by the administration of the fibrate WY14,643 in pregnancy [5]. Hyperthyroidism, which elicits adipocyte insulin resistance with regard to anti-lipolysis [20], can cause glucose intolerance in susceptible individuals [21] and has deleterious effects on pregnancy outcome [22]. The exact relationships between adipocyte insulin resistance, elevated NEFA and/or TAG, and pancreatic beta cell dysfunction during pregnancy are not yet established. We therefore investigated the impact of hyperthyroidism on the feedback between insulin sensitivity and pancreatic beta cell function in late pregnancy in relation to the suppression by insulin of systemic lipid levels. It is well known that hyperthyroidism modifies lipid dynamics (increased lipolysis and oxidation), impairs insulin action, and can also suppress insulin secretion [20, 23–28]. Our aim was to examine whether hyperthyroidism compromises the regulation of insulin secretion and the ability of insulin to modulate fuel homeostasis in late pregnancy, which itself is characterised by a form of insulin resistance that is associated with altered lipid dynamics, but can be countered by enhanced insulin secretion. We have previously argued that insulin hypersecretion during pregnancy may be geared to regulation of maternal lipid handling, and our present study shows that this action is impaired by hyperthyroidism. Our new data also show that glucose intolerance can be induced by hyperthyroidism in pregnancy due to a diabetogenic effect of hyperthyroidism on the islet itself. We further demonstrate that this adverse effect of hyperthyroidism in late pregnancy can be reversed in vivo, if there is sustained delivery of glucose, which has been shown by others to repartition fatty acid metabolism from oxidation to esterification.

Materials and methods

Materials Reagents were purchased from Roche Diagnostics (Lewes, Sussex, UK) or Sigma (Poole, Dorset, UK). Kits for determination of insulin (by ELISA, using rat insulin as standard) and glucose (by a glucose oxidase method) were purchased from Mercodia (Uppsala, Sweden) and Roche Diagnostics. Wako kits for the spectrophotometric determination of NEFA and TAG levels were purchased from Alpha Labs (Eastleigh, Hants., UK).

Animal treatment Studies were conducted in adherence to the UK Animal Scientific Procedures Act (1986). Female Wistar rats (Charles River, Margate, Kent, UK) were

maintained at $21 \pm 2^\circ\text{C}$ (12-h light–dark cycle), with free access to standard diet (Special Diet Services, Witham, Essex, UK; 52% carbohydrate, 15% protein, 3% lipid, 30% non-digestible residue [by weight]; 10.92 kJ metabolisable energy/g) and water. Rats were time-mated by the appearance of sperm plugs (day 0 of pregnancy). Pregnant rats with less than eight fetuses were excluded.

Pregnant rats were rendered hyperthyroid by subcutaneous injection of tri-iodothyronine (T_3 ; 1 mg/kg body weight per day; 3 days from day 17 to 19 of pregnancy); euthyroid control rats were injected with hormone solvent (10 mmol/l NaOH, 0.03% BSA) [20, 29]. This dose and period of T_3 treatment were selected on the basis of previous studies that demonstrated T_3 -induced changes in metabolic function and gene expression, together with cardiac hypertrophy (results not shown). Although not measured in this series of experiments, the dosage and period of treatment typically generate serum T_3 concentrations of 15–20 nmol/l [29] compared with normal T_3 concentrations of about 1.5 nmol/l [20, 30, 31]. We elected to administer this treatment specifically during late pregnancy to avoid the potential effect of hyperthyroidism in modulating the expansion of beta cell mass that typically occurs during mid-pregnancy in response to the development of maternal insulin resistance, and because adipose-tissue lipid metabolism is geared towards lipolysis rather than lipogenesis during late pregnancy. For in vivo studies, animals were fitted with chronic indwelling jugular cannulae (for infusion and sampling) with 6–8 days' recovery. Cannulae were filled with saline (154 mmol NaCl/l) and plugged until the day of the experimental studies. Hyperglycaemic clamps and IVGTT were completed in conscious, unrestrained rats in the postabsorptive (6-h starved) state.

IVGTT An intravenous glucose bolus (0.5 g glucose/kg body weight) [10, 32] was administered via a chronic indwelling cannula. Blood samples (100 μl) were withdrawn before glucose injection and at 2, 5, 10, 15 and 30 min after glucose injection. Aliquots (50 μl) were deproteinised and the supernatant fractions assayed for glucose. The remainder was centrifuged ($10,000 \times g$, 4°C), and plasma assayed for insulin. Insulin and glucose responses during the glucose tolerance test were used for calculation of the incremental insulin or glucose values integrated over the 30-min period after glucose injection (ΔI_{0-30} and ΔG_{0-30} , respectively). The rate of glucose disappearance (k) was calculated from the slope of the regression line obtained with log-transformed glucose values from 5 to 15 min after glucose administration.

Islet perfusion Rats were injected with sodium pentobarbital (60 mg/ml NaCl [154 mmol/l]; 1 ml/kg body weight i.p.). Once locomotor activity had ceased, pancreases were excised for islet preparation (collagenase digestion) and the rats killed. Isolated islets were collected into HEPES-buffered Hanks' balanced salts solution containing 5% BSA. Insulin release was measured in a perfusion system, as described previously [5, 33]. In this system, 50 islets

were housed in small chambers on Millicell culture plate inserts (Sigma, Poole, Dorset, UK). Islets were perfused in basal medium (KRB containing HEPES [20 mmol/l] pH 7.4, BSA [5 mg/ml] and glucose [2 mmol/l]) for 60 min at a flow rate of 1 ml/min at 37°C prior to collection of fractions. Glucose concentrations were then modified as indicated. Fractions (2 ml) were collected at 2-min intervals and assayed for insulin and glucose. Insulin responses during the perfusion were used for calculation of the incremental insulin values integrated over the 80 min period of the perfusion (ΔI_{60-140}). In addition, to quantify insulin secretory responses, areas under the insulin curves were calculated for discrete 16-min periods: $\Delta 1$, during which the perfusate glucose concentration was increased from 2 to 8 mmol/l; $\Delta 2$, during which the perfusate glucose concentration was increased from 8 to 16 mmol/l; $\Delta 3$, where the perfusate glucose concentrations were lowered from 16 to 2 mmol/l; and $\Delta 4$, when perfusate glucose had been restored to basal.

Hyperglycaemic clamps After 30 min equilibration, a 25% glucose solution was infused to maintain glycaemia at about 11 mmol/l in order to cause glucose-stimulated insulin secretion (GSIS) and thereby evaluate beta cell function in vivo. Blood and plasma samples, obtained at 5-min intervals thereafter for 60 min, were assayed for glucose, insulin, NEFA and TAG. Blood glucose and plasma insulin, NEFA and TAG values obtained between 30 and 60 min after initiation of glucose infusion were used for calculation of mean steady-state concentrations. Insulin responses were used to calculate the incremental values integrated over the period of glucose infusion (ΔI_{0-60}). The steady-state rate of glucose infusion (GIR) was calculated as the mean GIR during the last 30 min of glucose infusion.

Table 1 Body weights, food intakes, and postabsorptive blood glucose and plasma insulin concentrations of pregnant euthyroid or hyperthyroid dams, and the number and weights of their fetuses

	Euthyroid	Hyperthyroid
Body weight at day 17 of pregnancy (g)	327±5 (12)	315±6 (11)
Body weight at day 19 of pregnancy (g)	363±8 (12)	328**±6 (11)
Mean food intake from days 17 to 19 of pregnancy (g)	71.6±3.2 (12)	62.4±3.7 (11)
Mean fetal number per dam	14±1 (7)	13±1 (6)
Mean fetal weight (g)	2.3±0.1 (7)	2.8*±0.1 (6)
Basal blood glucose (mmol/l)	3.0±0.2 (6)	3.7**±0.2 (8)
Basal plasma insulin (μU/ml)	31±5 (6)	38±5 (8)

Blood glucose and plasma insulin concentrations were determined using commercial kits. Data are means±SEM, with the numbers of rats or litters in parentheses. Mean fetal weight per litter was calculated from the individual weights of all fetuses in each litter. Mean fetal weight was calculated as the average of mean fetal weights of each litter, with numbers of litters given in parentheses * $p<0.05$ and ** $p<0.01$ for differences between euthyroid and hyperthyroid pregnant rats

Statistical analysis Results are means±SEM, with the numbers of litters, rats or islet preparations in parentheses. Statistical analysis was performed by ANOVA followed by Fisher's post hoc tests for individual comparisons or Student's *t*-test as appropriate (Statview, Abacus Concepts, Berkeley, CA, USA). A *p* value <0.05 was considered to be statistically significant.

Results

Hyperthyroidism during late pregnancy impairs maternal weight gain, but augments fetal weight Body weights and food intakes over the 3-day period of T_3 treatment are shown in Table 1. Body weight progressively increased from day 0 to 19 of pregnancy (results not shown; see [34]). Body weights of euthyroid dams increased from day

Fig. 1 Effects of hyperthyroidism during late pregnancy on glucose tolerance and insulin secretion in response to an acute glucose challenge in vivo. Blood samples were drawn from euthyroid or hyperthyroid 19-day pregnant rats before and at intervals after a single intravenous glucose bolus, for measurement of blood glucose and plasma insulin concentrations. Blood glucose (a) and plasma insulin (b) concentrations at times relative to administration of glucose bolus in euthyroid (open symbols) or hyperthyroid (closed symbols) 19-day pregnant rats. c Rates of glucose disappearance (*k* values), calculated from the slopes of the regression lines obtained with log-transformed glucose values from 5 to 15 min after glucose administration. d, e Glucose and insulin responses during the acute glucose challenge were used to calculate the incremental blood glucose and plasma insulin values integrated over the 30-min period after the injection of glucose (ΔG_{0-30} and ΔI_{0-30} , respectively). Results are means±SEM for six euthyroid and eight hyperthyroid pregnant rats. * $p<0.05$ and *** $p<0.001$ for differences between euthyroid and hyperthyroid pregnant rats

Table 2 Mean perfusate glucose concentrations and integrated AUC for insulin for discrete 16-min periods during islet perfusions

	Mean glucose (mmol/l)		ΔI ($\mu\text{U ml}^{-1}\text{min}^{-1}$)		Decline in ΔI elicited by hyperthyroidism (%)
	Euthyroid	Hyperthyroid	Euthyroid	Hyperthyroid	
$\Delta 1$	6.4 $\pm$ 0.2 (9)	5.9 $\pm$ 0.1 (4)	132.3 $\pm$ 33.3 (9)	20.5* $\pm$ 9.0 (4)	85
$\Delta 2$	13.8 $\pm$ 0.4 (9)	14.0 $\pm$ 0.1 (4)	868.7 $\pm$ 122.9 (9)	162.7*** $\pm$ 63.7 (4)	81
$\Delta 3$	4.5 $\pm$ 0.2 (9)	4.5 $\pm$ 0.3 (4)	1190.0 $\pm$ 220.6 (9)	521.9* $\pm$ 249.5 (4)	56
$\Delta 4$	2.4 $\pm$ 0.2 (9)	2.3 $\pm$ 0.1 (4)	554.0 $\pm$ 137.5 (9)	221.4* $\pm$ 105.2 (4)	60

Mean perfusate glucose concentrations and AUC for insulin were calculated as described (see Islet perfusion). The percentage decline in the insulin AUC for the discrete 16-min periods elicited by hyperthyroidism are also shown. Data are means $\pm$ SEM, with the numbers of pregnant rats in parentheses

* $p < 0.05$ and *** $p < 0.001$ for differences between euthyroid and hyperthyroid pregnant rats

17 to 19 of pregnancy by 11% (Table 1). Although no statistically significant effect of T_3 administration on food intake was observed, body weight gain was markedly impaired (by 64%; $p < 0.01$) (Table 1). Fetal numbers were not significantly affected by hyperthyroidism (Table 1). In contrast, mean fetal weight (calculated as the average of mean fetal weights for each litter) was increased by 22% by hyperthyroidism (Table 1) and, as a consequence, mean total litter weight was increased by 13% ($p < 0.05$).

Hyperthyroidism during late pregnancy causes postabsorptive hyperglycaemia and decreased rates of glucose disappearance after an acute glucose challenge in vivo Basal (postabsorptive) glucose concentrations were increased (by 23%; $p < 0.01$) in hyperthyroid compared with euthyroid pregnant dams (Table 1). To assess glucose handling in vivo, glucose was administered as a single i.v. bolus. Blood glucose profiles during IVGTT are shown in Fig. 1a. Glucose disappearance rates (k values) were significantly decreased (by 40%; $p < 0.001$) by hyperthyroidism (Fig. 1c). However, incremental glucose values integrated over the 30-min period after glucose injection (ΔG_{0-30}) were not statistically affected by hyperthyroidism (Fig. 1d).

GSIS in vivo in response to acute glucose challenge is impaired by hyperthyroidism during late pregnancy Insulin concentrations in the postabsorptive state were unaffected by hyperthyroidism (Table 1). Plasma insulin profiles during IVGTT are shown in Fig. 1b. Incremental insulin values integrated over the 30-min period after glucose injection (ΔI_{0-30}) (Fig. 1e) were significantly lowered by hyperthyroidism in late pregnancy. The plasma insulin: blood glucose concentration ratio in the hyperthyroid group was significantly lower than that of the euthyroid group at 10 min (euthyroid 66.6 $\pm$ 11.1 $\mu\text{U}/\mu\text{mol}$ [$n=6$]; hyperthyroid 22.3 $\pm$ 4.5 $\mu\text{U}/\mu\text{mol}$ [$n=8$]; $p < 0.05$) and 15 min (euthyroid 35.2 $\pm$ 7.1 $\mu\text{U}/\mu\text{mol}$ [$n=6$]; hyperthyroid 12.3 $\pm$ 2.7 $\mu\text{U}/\mu\text{mol}$ [$n=8$]; $p < 0.05$) after the i.v. glucose bolus. This response suggested a more rapid return of insulin concentrations to postabsorptive values relative to the decline in glycaemia, introducing the possibility that hyperthyroidism might modify insulin secretion in response to glucose at submaximal concentrations and/or result in more rapid uncoupling of insulin secretion from the glucose stimulus.

Hyperthyroidism during late pregnancy increases the glucose threshold and decreases glucose responsiveness of GSIS with perfused islets We evaluated in detail the impact of hyperthyroidism on the relationship between GSIS and rising or falling extracellular glucose concentra-

Fig. 2 Hyperthyroidism during late pregnancy suppresses GSIS by isolated perfused islets. Islets isolated from euthyroid (open symbols) or hyperthyroid (closed symbols) 19-day pregnant rats were pre-perfused in medium containing 2 mmol glucose/l for 60 min at a flow rate of 1 ml/min at 37°C. Portions (2 ml) were collected at 2-min intervals and stored at -20°C prior to assays for insulin and glucose. **a, b** Perfusion profiles for insulin and glucose. Areas under the insulin curves calculated for the period from 60 to 140 min (ΔI_{60-140}) are shown (**c**) for euthyroid (open bar) or hyperthyroid (closed bar) 19-day pregnant rats. Results are means $\pm$ SEM for nine islet preparations from euthyroid pregnant rats and four islet preparations from hyperthyroid pregnant rats. The significance of differences in response between preparations from hyperthyroid and euthyroid pregnant rats have been omitted for clarity in the perfusion profiles, but are quantified in Table 2. * $p < 0.05$ for differences in ΔI_{60-140} values between islets from hyperthyroid and euthyroid pregnant rats

Table 3 Steady-state blood glucose and plasma insulin concentrations, and incremental AUC for insulin (ΔI_{0-60}) during sustained glucose challenge in vivo in euthyroid or hyperthyroid 19-day pregnant rats

	Euthyroid	Hyperthyroid
Steady-state blood glucose (mmol/l)	10.1 $\pm$ 0.2 (6)	11.4*** $\pm$ 0.1 (6)
Steady-state plasma insulin (μ U/ml)	462 $\pm$ 34 (6)	553 $\pm$ 50 (6)
Steady-state plasma insulin : steady-state blood glucose concentration ratio (μ U/ μ mol)	48.0 $\pm$ 9.3 (6)	48.4 $\pm$ 5.6 (6)
ΔI_{0-60} (μ U l ⁻¹ min ⁻¹)	4469 $\pm$ 828 (6)	5785 $\pm$ 1334 (6)

Blood glucose and plasma insulin values were measured for seven samples taken between 30 to 60 min after initiation of glucose infusion for calculation of steady-state concentrations. Insulin responses during the hyperglycaemic clamp were used for calculation of the incremental insulin values integrated over the period of glucose infusion (ΔI_{0-60}). Data are means $\pm$ SEM, with the numbers of rats in parentheses

*** p <0.001 for difference between euthyroid and hyperthyroid pregnant rats

tions using step-up and step-down islet perfusions generating gradual rises in perfusate glucose concentrations from 2 mmol/l (basal) to the mid-physiological range (8 mmol/l), then to the high physiological range (16 mmol/l), before switching back to 2 mmol glucose/l. To compare insulin secretory responses directly, areas under the insulin curves were calculated for discrete 16-min periods. $\Delta 1$ and $\Delta 2$ (Table 2) refer to periods during which the perfusate glucose concentration was increased from 2 to 8 mmol/l and from 8 to 16 mmol/l, respectively. Insulin release profiles during islet perfusions and ΔI_{60-140} values (incremental plasma insulin values integrated over the entire period of islet perfusion) are shown in Fig. 2a, and b, respectively. Raising the perfusate glucose concentration

for 16 min to a mean assayed value of 6.4 $\pm$ 0.2 mmol/l increased rates of insulin release by islets from euthyroid pregnant rats up to 2.1-fold (p <0.001). Raising perfusate glucose concentration further to a mean assayed value of 13.8 $\pm$ 0.4 mmol/l led to 8.8-fold higher rates of insulin release compared with basal rates (p <0.01). Mean peak rates of insulin release were 180 μ U/min, and $\Delta 2$ was 6.6-fold greater than $\Delta 1$. T_3 treatment evoked a marked (71%) decline in basal rates of insulin release at 2 mmol/l glucose (euthyroid 14 $\pm$ 2 mU/min [n =9]; hyperthyroid 4 $\pm$ 1 mU/min [n =4]; p <0.01). This contrasts with the absence of an effect of hyperthyroidism on basal (postabsorptive) insulin levels in vivo, and probably reflects the loss during islet preparation of in vivo factors that elevate basal secretion (e.g. an adaptation to insulin resistance signalled to the islet in vivo). $\Delta 1$ was decreased by 85% (p <0.05) and $\Delta 2$ was decreased by 81% (p <0.001) by hyperthyroidism in late pregnancy. Thus, as illustrated in Fig. 2a, hyperthyroidism causes a marked rightward shift in the profile of insulin secretion in response to increasing perfusate glucose concentrations. In addition, hyperthyroidism suppressed mean peak rates of insulin secretion at high glucose concentrations (37%; p <0.01). Overall, the effect of hyperthyroidism during pregnancy on suppression of GSIS in isolated perfused islets is demonstrated by the 67% decline in ΔI_{60-140} values (Fig. 2b).

Hyperthyroidism during late pregnancy also suppresses GSIS on removal of the glucose stimulus with perfused islets $\Delta 3$ and $\Delta 4$ (Table 2) refer to periods where the perfusate glucose concentrations were lowered from 16 to 2 mmol/l and maintained at basal (2 mmol/l) respectively. Hyperthyroidism in late pregnancy caused 56 and 60% decreases in $\Delta 3$ and $\Delta 4$ respectively (Table 2). Restoring perfusate glucose concentrations to basal levels resulted in lower insulin secretion rates (steady-state rate of 36 $\pm$ 7 μ U/min) in the hyperthyroid pregnant group compared with the euthyroid pregnant group (Fig. 2a).

Fig. 3 Effects of hyperthyroidism on insulin secretion during a sustained glucose challenge (hyperglycaemic clamp) in 19-day-pregnant rats. Blood samples were drawn from euthyroid (open symbols) or hyperthyroid (closed symbols) 19-day-pregnant rats at intervals during a sustained intravenous glucose challenge. **a** Blood

glucose concentrations. **b** Steady-state GIR, calculated as mean GIR during the last 30 min of glucose infusion. **c** Plasma insulin concentrations. Results are means $\pm$ SEM for six euthyroid pregnant rats and six hyperthyroid pregnant rats

Hyperthyroidism during late pregnancy does not lower GSIS in response to sustained glucose challenge in vivo

We infused glucose at a fixed rate to clamp glucose at a high physiological concentration for a prolonged period (60 min) to determine whether GSIS could be sustained in hyperthyroid pregnant rats in vivo. Higher steady-state blood glucose concentrations (Table 3; see also Fig. 3a) were observed in the hyperthyroid than in the euthyroid pregnant group despite a similar GIR (Fig. 3b). Under conditions of sustained hyperglycaemia in vivo, hyperthyroidism in late pregnancy did not lower steady-state insulin levels or suppress ΔI_{0-60} (Table 3; see also Fig. 3c), contrasting with the results obtained in response to an IVGTT in vivo and during islet perfusions ex vivo involving step-up and step-down changes in perfusate glucose concentrations. The steady-state plasma insulin concentration: steady-state blood glucose concentration ratio during the hyperglycaemic clamp was unaffected by hyperthyroidism during late pregnancy (Table 3).

Liporegulation by insulin is impaired in the hyperthyroid pregnancy

NEFA profiles were obtained before and during the sustained hyperglycaemic clamps. Hyperthyroidism did not affect postabsorptive (basal) plasma NEFA concentrations in pregnant rats (Fig. 4a). In euthyroid pregnant rats, plasma NEFA fell rapidly after initiating glucose infusion, with maximal suppression within 20 min. Steady state was reached after 30–60 min, at which time plasma NEFA concentrations had been suppressed to 27% of basal values (Fig. 4a). Glucose infusion also suppressed plasma NEFA concentrations in hyperthyroid pregnant rats, but steady-state (30–60 min) plasma NEFA levels during hyperinsulinaemia in hyperthyroid pregnant rats remained at 51% of corresponding basal values, significantly higher than steady-state plasma NEFA levels in euthyroid pregnant rats (by 2-fold, $p<0.001$) (Fig. 4a). Hence, insulin

hypersecretion in the hyperthyroid pregnant state only partially compensates for maternal insulin resistance with respect to suppression of circulating NEFA concentrations.

NEFA are the major precursors for hepatic VLDL-TAG secretion. Plasma TAG values before and during the hyperglycaemic clamps are shown in Fig. 4a. Postabsorptive plasma TAG concentrations were increased two-fold ($p<0.01$) by hyperthyroidism in late pregnancy. In both euthyroid and hyperthyroid pregnant rats, plasma TAG concentrations declined after initiating glucose infusion. Steady-state (30–60 min) TAG concentrations during hyperinsulinaemia remained significantly higher (2.4-fold; $p<0.01$) in hyperthyroid than in euthyroid pregnant rats.

Discussion

Physiological insulin resistance occurs during late pregnancy [35]. This is normally accompanied by a lowered glucose threshold for insulin secretion and increased insulin secretion at high glucose concentration [5, 36, 37], which compensates for the development of insulin resistance so that glucose tolerance is maintained. We found that hyperthyroidism during late gestation targeted both of these stable pancreatic beta cell adaptations to pregnancy. Our perfused islet studies revealed a marked rightward shift in insulin secretion in response to increasing glucose concentration, as demonstrated by a significant reduction in the value for ΔI , indicating an increased glucose threshold for insulin secretion in response to the induction of hyperthyroidism during pregnancy. In addition, hyperthyroidism blunted GSIS after an acute glucose challenge in vivo and at both mid and high glucose concentrations with perfused islets in vitro. Finally, hyperthyroidism in late pregnancy led to more rapid suppression of insulin secretion following removal of the glucose stimulus to secretion after bolus glucose administration in the intact animal.

To test whether more rapidly curtailed insulin secretion in hyperthyroid pregnant rats after acute glucose challenge in vivo reflected depletion of islet insulin stores, we examined the response of GSIS in vivo to a sustained glucose challenge. In marked contrast to data obtained after transient increases in extracellular glucose, no impairment of GSIS was evident under conditions of sustained glucose delivery (hyperglycaemic clamp); hyperthyroidism in late pregnancy did not lower steady-state insulin levels or suppress ΔI_{0-60} during sustained glucose infusion in vivo. Although abnormal glucose tolerance is observed in hyperthyroidism in the non-pregnant state [38] and postabsorptive glycaemia and steady-state glucose levels during glucose infusion were higher in hyperthyroid compared with euthyroid pregnant rats, the steady-state plasma insulin concentrations: steady-state blood glucose concentrations ratio during sustained glucose infusion was unaffected by hyperthyroidism (Table 3). This indicates that insulin secretion in hyperthyroid pregnant rats is appropriate for the level of glycaemia when glucose concentrations are maintained at relatively high levels. The lowered k values seen after acute glucose challenge in the hyperthyroid pregnant

Fig. 4 Influence of hyperthyroidism on plasma NEFA and TAG concentrations before and at steady state during a sustained glucose challenge (hyperglycaemic clamp) in 19-day-pregnant rats. Blood samples were drawn from euthyroid (open bars) or hyperthyroid (closed bars) 19-day-pregnant rats at intervals after a sustained intravenous glucose challenge for measurement of plasma NEFA (a) and TAG (b) concentrations. Steady-state concentrations of plasma NEFA and TAG were calculated as means of values for seven samples taken from 30 to 60 min after initiation of glucose infusion. Results are means $\pm$ SEM for six euthyroid pregnant rats and six hyperthyroid pregnant rats. ** $p<0.01$ and *** $p<0.001$ for differences between euthyroid and hyperthyroid pregnant rats

group therefore reflect the suppression by hyperthyroidism of insulin secretion *in vivo* as glucose concentrations decline to the low physiological range. Steady-state NEFA levels during hyperinsulinaemia remained higher in the hyperthyroid pregnant group and plasma TAG levels both in the basal state and during steady-state hyperinsulinaemia were also elevated in hyperthyroid compared with euthyroid pregnant rats.

Our *in vivo* data thus show that high rates of GSIS, albeit inadequate to normalise plasma lipids, can be maintained at sustained high glucose levels in the hyperthyroid pregnant state, but insulin secretion is impaired and curtailed during transient hyperglycaemia *in vivo*. Thus, *in vivo* factors specific to sustained (but not transient) hyperglycaemia are able to 'rescue' the impairment of GSIS induced by hyperthyroidism in late pregnancy.

Islet lipid deprivation abolishes GSIS and impaired GSIS after fasting can be reversed by exogenous lipid [39–41]. Artificial lowering of malonyl-CoA levels enhances mitochondrial fatty acid oxidation at high glucose concentrations, reduces partitioning of fatty acid into lipid esterification products and suppresses GSIS in clonal beta cells [42]. Suppression of GSIS has been attributed to curtailment of lipid signalling to GSIS [42]. As hyperthyroidism increases fatty acid oxidation [43–45], we propose that the inability to initiate or sustain GSIS during transition periods of increasing or declining glycaemia *in vivo* in the hyperthyroid pregnancy reflects the accelerated removal of a lipid esterification product that functions as a 'coupling factor' for GSIS. Thus, the inability of hyperinsulinaemia to suppress plasma lipid levels under conditions of the hyperglycaemic clamp in the hyperthyroid state allows sustained delivery of lipid in addition to glucose to the islet, preserving GSIS, because these conditions allow regeneration of lipid esterification products within the islet.

In the present study, hyperthyroidism during late gestation was associated with an increase in fetal weight. The inability to sustain insulin secretion during transient glucose challenge *in vivo* and/or impaired maternal liporegulation will increase the supply of glucose to the fetus. Fetal macrosomia in response to prolonged hyperglycaemia is also observed during gestational diabetes. It is therefore proposed that the elevated basal glycaemia, together with glucose intolerance due to the blunting and/or curtailing effect of hyperthyroidism on insulin secretion in response to acute hyperglycaemia *in vivo* in late pregnancy, may underlie the observed increase in fetal weight in hyperthyroid pregnancy.

In summary, we show that insulin secretion during sustained hyperglycaemia *in vivo* is not impaired by hyperthyroidism during pregnancy, but the insulin secretory response to glucose is blunted at low and high glucose concentrations during islet perfusions *ex vivo* and more rapidly curtailed after acute glucose challenge *in vivo*. Since hyperthyroidism in pregnancy also impairs the ability of insulin to suppress systemic lipid levels, we suggest that higher circulating lipids can preserve GSIS in the hyperthyroid pregnancy, provided glucose delivery remains at high levels to suppress islet lipid oxidation and/or allow

the formation of lipid esterification products that optimise GSIS.

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