Physiological modulation of the uptake and fate of glucose in brown adipose tissue

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Glucose utilization indices (GUI values) and rates of fatty acid synthesis in interscapular brown adipose tissue (IBAT) varied during the diurnal cycle in virgin and late-pregnant rats permitted unrestricted access to food. In virgin rats, peak GUI values and lipogenic rates were observed at the end of the dark (feeding) phase, but were not sustained during the light phase. Whereas peak GUI values were comparable with those observed during re-feeding after 24 h starvation, maximum rates of IBAT fatty acid synthesis in virgin rats during the diurnal cycle were only approx. 25 % of those measured during re-feeding after 24 h starvation. Despite hyperphagia, GUI values during the diurnal cycle in late-pregnant rats fed *ad libitum* were generally lower

than those of age-matched virgin controls. The percentage of pyruvate dehydrogenase complex present in the active form (PDH_a) was also significantly decreased. Suppression of GUI and PDH_a was not paralleled by suppression of fatty acid synthesis. IBAT GUI values in late-pregnant rats during chow re-feeding *ad libitum* after 24 h starvation were only 25 % of those of corresponding virgin controls, and stimulation of fatty acid synthesis was also dramatically attenuated. The suppression of IBAT GUI values after re-feeding in pregnancy was not due to depletion of GLUT 4 protein. The results are discussed in relation to the importance of glucose as a precursor for fatty acid synthesis in IBAT.

INTRODUCTION

Adipose tissue is regarded as a major target tissue for insulin action in terms of stimulation of glucose uptake. The rapid stimulation of glucose uptake into target tissues by insulin is mediated by GLUT 4, the glucose-transporter isoform specific to tissues responding to insulin [1,2]. From a quantitative viewpoint, glucose uptake and phosphorylation by white adipose tissue under insulin stimulation is minor [3]. In contrast, brown adipose tissue (BAT) can exhibit extremely high rates of glucose utilization under physiological insulin stimulation [4], and, perhaps more importantly, an impaired ability of insulin to stimulate the uptake and phosphorylation of glucose by BAT has been suggested to contribute to the whole-body insulin resistance observed in a number of physiological and pathological situations [4–6].

The increase in glucose utilization linked to a physiological or maximal increment in plasma insulin concentrations during euglycaemic–hyperinsulinaemic clamp is significantly decreased in 19-day-pregnant rats [4]. The massively increased rates of glucose uptake/phosphorylation observed in BAT of virgin rats during re-feeding after prolonged (48 h) starvation [7] are comparable in magnitude with those observed during euglycaemic–hyperinsulinaemic clamp at insulin concentrations (250 μ units/ml of plasma) in the high physiological range (see [4,7]). Although peripheral insulin resistance during euglycaemic–hyperinsulinaemic clamp has been consistently observed in late pregnancy [8,9], the maintenance of glycaemia by glucose infusion during hyperinsulinaemic clamp does not mimic the normal presentation of carbohydrate as a component of a mixed diet, nor does the infusion of exogenous insulin reproduce the phasic release of insulin observed in response to nutrient secretagogues. Consequently, the potential importance of the insulin resistance at the level of glucose uptake/phosphorylation by BAT of late-pregnant rats demonstrated

during euglycaemic–hyperinsulinaemic clamp has not been evaluated in relation to the changes in rates of glucose uptake/phosphorylation which may be elicited by changes in dietary status. In addition, there is no information regarding the alterations in the metabolism of BAT of pregnant rats which might preclude a normal stimulation of glucose metabolism by insulin, for example, stable modification of the capacity for glucose transport (glucose-transporter protein content) and/or pyruvate oxidation [pyruvate dehydrogenase (PDH) activity]. Although insulin resistance in an animal model of type II diabetes mellitus has been attributed in part to depletion of GLUT 4 protein content in insulin-responsive tissues [10] and GLUT 4 protein content in BAT decreases in response to prolonged insulinopenia (48 h starvation and 7-day streptozotocin-diabetes) [11], the expression pattern for GLUT 4 protein content in BAT has not been examined during the non-interventional insulin resistance associated with late pregnancy. Furthermore, the effects of pregnancy on the capacity of BAT for the oxidative decarboxylation of pyruvate to acetyl-CoA, and therefore the potential use of pyruvate as oxidative or lipogenic substrate, have not been described.

The aim of the present study was to quantify and assess the potential importance of the effects of late pregnancy on BAT glucose uptake/phosphorylation under varying nutritional conditions associated with physiological fluctuations in plasma insulin concentrations. In addition, the study investigated the effects of late pregnancy on GLUT 4 protein expression and insulin-sensitive targets (the PDH complex and the lipogenic pathway) distal to glucose transport.

MATERIALS AND METHODS

Materials

Enzymes, coenzymes and substrates were purchased from Boehringer Mannheim, Lewes, East Sussex, U.K., or Sigma Chemical

Abbreviations used: BAT, brown adipose tissue; GLUT 4, the insulin-regulatable glucose transporter isoform; GUI, glucose utilization index; IBAT, interscapular BAT; PDH, pyruvate dehydrogenase; PDH_a, the active form of PDH; PDH_b, the inactive form of PDH.

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Co., Poole, Dorset, U.K. Radiochemicals were purchased from Amersham International, Amersham, Bucks., U.K. Standard rodent diet (52 % carbohydrate, 15 % protein, 3 % fat and 30 % non-digestible residue; all by weight) was obtained from Special Diets Services, Witham, Essex, U.K.

Rats

Albino Wistar rats were maintained on 12 h-light/12 h-dark cycles (light or dark from 10:00 h) and housed in a room maintained at $22 \pm 2^\circ\text{C}$. Pregnant rats were studied at 19–20 days of gestation, the duration of pregnancy being determined as described in [12]. The control rats were age-matched virgin females. The rats sampled during the dark (D) phase of the diurnal cycle were maintained on a reverse 12 h-light/12 h-dark cycle (dark from 10:00 h) to permit direct comparison with rats sampled during the light (L) phase. Mean food intakes of virgin rats fed *ad libitum* over the light and dark phases were 2.26 ± 0.25 and 19.33 ± 0.62 g respectively. Chow intakes of late (19 day)-pregnant rats fed *ad libitum* over the light and dark phases were 5.13 ± 0.64 and 23.62 ± 1.82 g respectively. Additional groups of virgin and pregnant rats were starved for 6 or 24 h, or starved for 24 h and then re-fed on standard rodent diet *ad libitum*.

Interscapular BAT (IBAT) was rapidly removed and dissected free of adhering connective tissue or white adipose tissue. Villarroja and colleagues [13] have documented progressive increases in IBAT tissue weight and lipid content during pregnancy in the rat: in the present series of experiments, representative IBAT weights (mg) and percentages of delipidated solid per 100 mg of wet tissue wt. were 245 ± 17 mg (12) and 51.6 ± 2.0 % (50) respectively in virgin rats and 497 ± 52 mg (9) and 41.9 ± 2.2 % (9) respectively in 19–20-day-pregnant rats, values similar to those obtained by Villarroja and colleagues in virgin and 20-day-pregnant rats (see [13]).

Flux measurements

Values of glucose utilization indices (GUI values) in IBAT were calculated from rates of uptake and phosphorylation of 2-deoxy[^3H]glucose [3] in conscious unrestrained rats each fitted with a chronic indwelling cannula for injection and sampling as described in [4,7,14]. The times of sampling given in Table 1 refer to the time of injection of 2-deoxy[^3H]glucose, as > 80 % of the tissue uptake of 2-deoxyglucose occurs within the first 20 min after its injection [15]. Values of the correction factor for the discrimination against 2-deoxyglucose in glucose metabolic pathways (lumped constant) have been determined previously, and are not significantly affected by late pregnancy [3,4]. Coefficients of variance of blood glucose concentrations were less than 15 % over the 1 h period of sampling, and mean blood glucose concentrations are shown in Table 1. Rates of fatty acid synthesis *de novo* were estimated as ^3H incorporation from $^3\text{H}_2\text{O}$ into tissue saponifiable fatty acid as described in [16]. $^3\text{H}_2\text{O}$ (5 mCi) was administered by intraperitoneal injection at 1 h before sampling, and the times of sampling given in Table 3 refer to the time of tissue dissection.

Western blotting

Full details of the procedures for purification of crude membranes and subsequent solubilization and electrophoresis are given in [17]. GLUT 4 abundance was calculated on the basis of similar protein loading (see [17]). Affinity-purified antibodies against residues 494–509 of GLUT 4 were used for these experiments. The second antibody was ^{125}I -labelled F(ab')₂ donkey anti-rabbit

IgG. Variation between blots was assessed with standards from rat muscle, run and blotted with each gel. Western analysis was repeated on three to five separate samples in each group.

Enzyme assays

PDH complex (active form, PDH_a) was measured at 30°C in extracts of freeze-clamped IBAT as described in [18], except that Triton X-100 (2.5 %, w/v) and protease inhibitors [benzamidine (1 mM), leupeptin (10 μM) and tosyl-lysylchloromethane (0.3 mM)] were included in the extraction medium. A unit of PDH_a activity is defined as that which converts 1 μmol of pyruvate into acetyl-CoA/min at 30°C .

Statistical analysis

Statistical significance of differences was assessed by Student's unpaired *t* test. Results are given as means $\pm$ S.E.M. for the numbers of rats (*n*) specified.

RESULTS AND DISCUSSION

Impaired glucose uptake/phosphorylation by IBAT has been demonstrated at high insulin concentrations during euglycaemic-hyperinsulinaemic clamp at 19 days of gestation in anaesthetized rats [4]. The key question is whether this altered response to insulin infusion has significance for glucose homeostasis in late pregnancy within the context of physiological modulation of carbohydrate and insulin status by fasting and feeding. In the present experiments, GUI values were measured in IBAT of conscious unrestrained rats. Age-matched virgin and 19-day-pregnant rats were sampled in the post-absorptive state (after starvation for 6 h), after starvation for 24 h, during re-feeding *ad libitum* of standard laboratory diet (pelleted chow) after 24 h starvation and during the feeding (dark) phase of the diurnal cycle, nutritional conditions associated with changes in plasma insulin concentrations (see [19,20]).

GUI values in IBAT during starvation and re-feeding

GUI values in IBAT of conscious virgin rats in the post-absorptive state and after 24 h starvation (Table 1) were similar to those observed previously [7]. Provision of chow after 24 h starvation evoked dramatic increases in IBAT GUI values in virgin rats (Table 1). GUI values at 3 h after re-feeding were 16-fold ($P < 0.01$) higher than the 24 h-starved values and remained high after 6 h of re-feeding (Table 1). As demonstrated previously [4], IBAT GUI values in the post-absorptive state were unaffected by late pregnancy; however, after 24 h starvation (from day 19 of pregnancy) IBAT GUI values were only 23 % ($P < 0.05$) of those of 24 h-starved controls (Table 1). Chow re-feeding on day 20 of pregnancy after 24 h of starvation evoked a 17-fold ($P < 0.01$) increase in IBAT GUI values within 3 h which was maintained for a further 3 h (Table 1).

GUI values during chow re-feeding in late pregnancy were only approx. 25 % ($P < 0.01$) of the re-fed controls (Table 1), indicating marked attenuation of feeding-induced stimulation of IBAT glucose uptake/phosphorylation. Blood glucose concentrations during re-feeding did not differ between virgin and late-pregnant rats (Table 1), whereas plasma insulin concentrations are significantly higher in late-pregnant rats compared with virgin rats (see [19]). Experiments using the euglycaemic-hyperinsulinaemic clamp technique indicate that an increase in plasma insulin stimulates glucose uptake/phosphorylation by IBAT [4]. The present data therefore support the concept of insulin resistance at the level of glucose uptake/phosphorylation in IBAT in late pregnancy.

Table 1 GUI values in IBAT and blood glucose concentrations during changes in nutritional status in virgin or late-pregnant rats

Results are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. Statistically significant effects of chow re-feeding *ad libitum* after 24 h starvation are shown by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Values obtained at 6 or 12 h into the dark (D) phase that are significantly different from the value at the onset of the dark phase (D-0) are indicated by: † $P < 0.05$; †† $P < 0.01$. Statistically significant effects of late pregnancy are shown by: ‡ $P < 0.05$; ‡‡ $P < 0.01$; ‡‡‡ $P < 0.001$.

Nutritional status	GUI (ng/min per mg wet wt.)		Blood glucose concn. (mM)	
	Virgin	Pregnant	Virgin	Pregnant
Post-absorptive	25.0 $\pm$ 5.4 (3)	12.5 $\pm$ 4.0 (4)	4.68 $\pm$ 0.13 (5)	3.37 $\pm$ 0.38‡ (5)
Starved (24 h)	14.2 $\pm$ 3.5 (5)	3.3 $\pm$ 0.5‡ (6)	3.77 $\pm$ 0.48 (3)	2.89 $\pm$ 0.11 (6)
Re-fed (3 h)	225.9 $\pm$ 49.0** (5)	56.7 $\pm$ 16.3***‡‡ (7)	5.13 $\pm$ 0.25* (5)	4.84 $\pm$ 0.22*** (7)
Re-fed (6 h)	159.5 $\pm$ 27.2*** (5)	35.5 $\pm$ 7.6***‡‡‡ (9)	4.66 $\pm$ 0.26 (5)	4.81 $\pm$ 0.32*** (9)
D-0	20.6 $\pm$ 5.9 (7)	5.9 $\pm$ 2.0‡ (4)	5.06 $\pm$ 0.27 (7)	3.60 $\pm$ 0.14‡‡‡ (4)
D-6	28.4 $\pm$ 6.6 (6)	45.5 $\pm$ 15.7† (5)	5.26 $\pm$ 0.17 (7)	4.51 $\pm$ 0.19††† (5)
D-12	101.7 $\pm$ 22.5†† (5)	20.1 $\pm$ 6.6‡ (4)	5.81 $\pm$ 0.23 (5)	3.98 $\pm$ 0.28‡‡ (4)

Table 2 Western-blot analyses of GLUT 4 expression in IBAT of fed, 24 h-starved or 24 h-starved/2 h re-fed virgin or late-pregnant rats

Autoradiograms were quantified by scanning densitometry. Results are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. Statistically significant effects of 24 h-starvation are shown by: * $P < 0.05$. There were no statistically significant effects of re-feeding after 24 h-starvation or of late pregnancy.

Nutritional status	GLUT 4 protein abundance (% of fed control)	
	Virgin	Pregnant
Fed <i>ad libitum</i>	100.0 $\pm$ 4.0 (10)	107.6 $\pm$ 4.6 (10)
Starved (24 h)	88.2 $\pm$ 3.4* (4)	96.4 $\pm$ 9.6 (3)
Re-fed (2 h)	92.9 $\pm$ 4.5 (3)	89.2 $\pm$ 4.0 (5)

GUI values in IBAT of virgin rats during unrestricted feeding

We wished to assess the potential contribution of brown fat to whole-body glucose clearance under conditions where changes in dietary intake are voluntary and to determine whether late pregnancy influences brown-fat glucose utilization under these circumstances. GUI values in IBAT were therefore measured in conscious rats permitted continuous free access to standard laboratory chow during the light and dark phases of the diurnal cycle. Late-pregnant rats allowed access to food *ad libitum* were hyperphagic compared with virgin rats fed *ad libitum* (see the Materials and methods section), but, as with virgin rats, most (> 82%) of the total daily food intake occurred during the dark phase.

IBAT GUI values measured in virgin rats fed *ad libitum* at D-0 were not significantly different from those observed in the post-absorptive state (Table 1). Interestingly, IBAT GUI values did not increase significantly during the first 6 h of the dark phase, cf. re-feeding after starvation (Table 1), despite significant increases

in plasma insulin concentrations [20]. Values of GUI at D-9 (23.1 ± 5.3 ng/min per mg; $n = 5$) were also not significantly higher than those at D-0 (20.6 ± 5.9 ng/min per mg; $n = 7$). Marked and significant (4.4-fold; $P < 0.01$ versus D-9) increases in IBAT GUI values were, however, observed at D-12 (Table 1). These increases were concomitant with a rapid rate of food consumption (approx. 7.6 ± 1.6 g of chow from D-9 to D-12), compared with a food consumption of 11.7 ± 1.2 g of chow over the first 9 h of the dark phase. GUI values measured at D-12 were not significantly different from those observed after 6 h of re-feeding after 24 h starvation, but were only approx. 45% of the peak (3 h) re-fed value. Limited food consumption (1.08 ± 0.24 g of chow, $n = 8$) occurred over the first 3 h of the light phase, at the end of which period IBAT GUI values had declined to basal [GUI values of 28.0 ± 6.6 ng/min per mg ($n = 7$) at L-3]. Additional results (not shown) indicated that IBAT GUI values remained relatively constant in virgin rats fed *ad libitum* from L-3 to L-12 [range 20.6 ± 5.9 (7) ng/min per mg at L-12/D-0 to 38.6 ± 9.5 (7) ng/min per mg at L-6]. Mean GUI values over the entire period from L-3 to D-9 (25.8 ± 2.5 ng/min per mg, $n = 48$) were < 12% of those observed at 3 h after re-feeding.

IBAT GUI values in 19-day-pregnant rats were similar at D-0 and in the post-absorptive state (Table 1). Late-pregnant rats consumed 9.9 ± 2.2 g ($n = 5$) of chow during the period from D-0 to D-6 [cf. 6.8 ± 1.5 g ($n = 7$) of chow in the virgin controls]. Despite this overall increase in food intake, GUI values in late-pregnant rats fed *ad libitum* never significantly exceeded those of the virgin controls fed *ad libitum* (Table 1). IBAT GUI values measured mid-way through the dark phase (D-6) were comparable in late-pregnant and virgin rats fed *ad libitum* but IBAT GUI values of 19-day-pregnant rats fed *ad libitum* at D-0 and D-12 were only 29% and 20% respectively of those of virgin rats fed *ad libitum* at D-0 and D-12 (Table 1).

GLUT 4 expression in late pregnancy

Starvation (48 h) elicits substantial loss of IBAT GLUT 4 protein content [11]. We sought to establish whether accelerated IBAT

Table 3 Rates of fatty acid synthesis in IBAT during changes in nutritional status in virgin or late-pregnant rats

Details of the times of sampling are given in Table 1. Results are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. Statistically significant effects of re-feeding after 24 h starvation are shown by: ** $P < 0.01$; *** $P < 0.001$. Values during the dark phase that are statistically significantly different from the D-0 value are shown by: †† $P < 0.01$. Statistically significant effects of late pregnancy are shown by: ‡ $P < 0.05$; ‡‡ $P < 0.01$; ‡‡‡ $P < 0.001$.

Nutritional status	Rate of fatty acid synthesis (μ g-atoms of 'H'/h per g wet wt.)	
	Virgin	Pregnant
Post-absorptive	28.9 $\pm$ 4.6 (5)	16.0 $\pm$ 2.4‡ (6)
Starved (24 h)	10.9 $\pm$ 2.1 (3)	8.0 $\pm$ 2.2 (4)
Re-fed (3 h)	195.5 $\pm$ 18.8*** (6)	34.1 $\pm$ 8.1***††‡ (9)
Re-fed (6 h)	201.8 $\pm$ 35.7** (5)	40.7 $\pm$ 16.1‡‡ (5)
D-0	36.1 $\pm$ 9.6 (8)	21.8 $\pm$ 6.9 (4)
D-6	35.9 $\pm$ 9.8 (6)	66.6 $\pm$ 8.3††‡ (5)
D-12	52.1 $\pm$ 14.0 (4)	40.7 $\pm$ 8.8 (8)

glucose uptake/phosphorylation during re-feeding after starvation was facilitated by increased GLUT 4 protein content and whether glucose uptake/phosphorylation by IBAT during re-feeding after starvation in late pregnancy was restricted by decreased GLUT 4 protein content. Starvation for 24 h led to statistically significant (12%; $P < 0.05$) depletion of IBAT GLUT 4 protein content in virgin rats (Table 2). Together with results from others [11], the data suggest that depletion of IBAT GLUT 4 protein content during starvation is of significance for glucose utilization only after periods beyond 24 h. Re-feeding (2 h) led to loss of the effect of starvation on GLUT 4 protein content, but did not increase GLUT 4 protein content relative to GLUT 4 protein content in the fed state (D-12) (Table 2). We have demonstrated [21] that decreased rates of glucose uptake/phosphorylation in IBAT after prolonged starvation are associated with a decline in GLUT 4 mRNA expression which is only partially reversed by acute re-feeding. There were no statistically significant effects of late pregnancy on IBAT GLUT 4 protein content under the nutritional conditions examined (Table 2). It is not excluded, however, that the stimulation by insulin of GLUT 4 translocation (see [22,23]) may be impaired in late pregnancy and that this may underlie, in part, the decline in rates of glucose uptake/phosphorylation.

Rates of fatty acid synthesis in IBAT during changes in nutritional status

Insulin stimulates the conversion of glucose into fatty acids in BAT and white adipose tissue (for reviews see [24,25]). Brown-adipose-tissue fatty acid synthesis (measured by using $^3\text{H}_2\text{O}$) in rats fed *ad libitum* increases progressively until day 18 of pregnancy, but is low at 20 days of pregnancy [26]. The present study demonstrated suppression of IBAT glucose uptake/phosphorylation at day 20 of pregnancy (see above). Since utilization of carbohydrate-derived substrates for fatty acid synthesis can be regulated at sites distal to GLUT 4, we

investigated whether insulin resistance at the level of IBAT glucose uptake/phosphorylation in late pregnancy extends to impaired stimulation of lipogenic flux.

A significant ($P < 0.01$) decline in fatty acid synthesis *de novo* was observed in IBAT of virgin rats sampled after 24 h of starvation (Table 3; see, e.g., [16,27]). Dramatic (18-fold) increases in fatty acid synthesis occurred after 3 h of chow re-feeding of 24 h-starved virgin rats and high rates were maintained after 6 h of chow re-feeding (Table 3). Rates of fatty acid synthesis *de novo* in IBAT of late-pregnant rats decreased ($P < 0.05$) after starvation from day 19 to day 20 of pregnancy (Table 3). Chow re-feeding of 24 h-starved late-pregnant rats significantly ($P < 0.01$) increased rates of IBAT lipogenesis after 3 h, and rates of fatty acid synthesis remained elevated after 6 h of re-feeding (Table 3). After 24 h of starvation, rates of fatty acid synthesis *de novo* in IBAT of late-pregnant rats were not significantly different from those of 24 h-starved virgin rats (Table 3), but, although the time course of the response of IBAT fatty acid synthesis to chow re-feeding after 24 h starvation was similar in late-pregnant and virgin rats, the magnitude of the response was considerably less in the former group (Table 3).

Rates of IBAT fatty acid synthesis in virgin rats at D-0 were similar to those observed in the post-absorptive state, but only approx. 20% of those observed during re-feeding after starvation (Table 3). Rates of fatty acid synthesis were not increased at D-6 (Table 3) or even at D-9 (24.4 $\pm$ 6.0 μ g-atoms of 'H'/h per g wet wt.; $n = 6$), despite substantial food consumption (see above). Although a 2-fold increase in fatty acid synthesis occurred between D-9 and D-12 [from 24.4 $\pm$ 6.0 (6) to 52.1 $\pm$ 14.0 (4) μ g-atoms of 'H'/h per g], it was less than the corresponding rise in GUI. Rates of fatty acid synthesis at L-3 were relatively low (21.2 $\pm$ 3.9 μ g-atoms of 'H'/h per g; $n = 4$). The highest mean rate of fatty acid synthesis *de novo* observed during unrestricted feeding (at D-12) was only approx. 25% of rates during re-feeding after 24 h starvation. Similarly, the mean rate of IBAT fatty acid synthesis over the period from L-3 to D-9 (29.5 $\pm$ 2.2 μ g-atoms of 'H'/h per g; $n = 14$) was only approx. 15% of those observed after re-feeding.

In late-pregnant rats, rates of fatty acid synthesis *de novo* at D-0 were 2–3-fold higher than after 24 h starvation and did not differ significantly from those observed in the post-absorptive state or during re-feeding after 24 h starvation (Table 3). Interestingly, the marked suppression of GUI values observed at D-0 and D-12 in IBAT of 19-day-pregnant rats fed *ad libitum* compared with virgin rats fed *ad libitum* (Table 1) was not paralleled by significant impairment of IBAT fatty acid synthesis (Table 3).

Decreased rates of IBAT fatty acid synthesis were observed in rats fed *ad libitum* sampled at D-12 on day 20 of pregnancy [29.1 $\pm$ 6.2 (11) μ g-atoms of 'H'/h per g] (see also [13]). A further decline occurred by day 21 of pregnancy [rates of 11.2 $\pm$ 4.8 (3) μ g-atoms of 'H'/h per g at D-12; $P < 0.05$ versus virgin rats]. These decreases may reflect a voluntary decline in food intake or an altered pattern of food intake before parturition (day 23), in addition to an impaired response to nutrient stimulation; however, alterations in food intake do not underlie the impairment of fatty acid synthesis observed during re-feeding after 24 h starvation at 20 days of gestation (where food intakes in pregnant and virgin rats are comparable; see [19]).

Comparison of rates of glucose uptake/phosphorylation and fatty acid synthesis in IBAT during changes in nutritional status

Glucose may be potentially important as a precursor for fatty acid synthesis *de novo* in IBAT (see, e.g., [7,28]). The quantitative

Table 4 Estimated potential contribution of glucose towards fatty acid synthesis in IBAT during changes in nutritional status in virgin or late-pregnant rats

Details of procedures are given in Tables 1 and 3. GUI values are expressed as potential flux from glucose to acetyl-CoA in terms of C_{16} (palmitate) equivalents in order to estimate the maximum direct contribution of glucose to fatty acid synthesis. Rates of fatty acid synthesis in Table 3 are also expressed in terms of C_{16} units to facilitate direct comparison. For this calculation, it has been assumed that 13.2 g-atoms of 'H' are incorporated per mol of fatty acid synthesized (see [24]).

Nutritional status	Estimated flux rate (μmol of C_{16} units/h per g wet wt.)			
	Virgin		Pregnant	
	GUI	Fatty acid synthesis	GUI	Fatty acid synthesis
Post-absorptive	2.08	2.19	1.04	1.21
Starved (24 h)	1.18	0.83	0.27	0.61
Re-fed (3 h)	18.81	14.81	4.72	2.58
Re-fed (6 h)	13.28	15.29	2.96	3.08
D-0	1.72	2.73	0.49	1.65
D-6	2.36	2.72	3.79	5.05
D-12	8.47	3.95	1.67	3.08

similarity between estimated (maximum) potential flux of glucose carbon towards fatty acid synthesis (expressed as μmol of C_{16} units/h per g wet wt.) and measured rates of fatty acid synthesis (similarly expressed) in IBAT of virgin rats during re-feeding after 24 h starvation (Table 4) supports the idea that glucose is a predominant lipogenic substrate for IBAT under these conditions. Any correlation between estimated and measured fluxes was less obvious during unrestricted feeding in the dark phase. The potential flux of carbon from glucose to lipid exceeds measured rates of fatty acid synthesis towards the end of the dark (feeding) phase, whereas fatty acid synthesis is clearly fuelled by precursors in addition to glucose at the onset of the dark phase (Table 4).

There is concomitant suppression of the response of glucose uptake/phosphorylation and fatty acid synthesis to re-feeding after 24 h starvation in late pregnancy. However, as in virgin rats, the estimated potential flux from glucose to fatty acid is adequate to accommodate measured rates of fatty acid synthesis under these conditions. The suppression of fatty acid synthesis during the early phase of re-feeding is greater than that which might be predicted simply from suppression of glucose uptake/phosphorylation (and potential flux from glucose to fatty acid) alone (Table 4). Conversely, rates of fatty acid synthesis in the starved state and during the feeding phase of the diurnal cycle in pregnancy are substantially higher than those that might be accommodated from the use of glucose alone (Table 4).

Insulin-sensitive targets distal to glucose uptake: the PDH complex

The mammalian PDH complex exists in an inactive (phosphorylated) form (PDH_p) and an active dephosphorylated form (PDH_a). The stimulation of fatty acid synthesis from glucose observed in rat adipose tissue *in vitro* and *in vivo* in response to insulin is associated not only with enhancement of glucose transport, but also with PDH activation [29–32]. IBAT PDH_a activities (expressed either on a wet-wt. basis or as a percentage of total PDH

Table 5 PDH_a activities in IBAT of fed, 24 h-starved or 24 h-starved/2 h re-fed virgin or late-pregnant rats

Results are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. Rats fed *ad libitum* were sampled at D-12. Statistically significant effects of starvation are shown by: ** $P < 0.01$. Statistically significant effects of chow re-feeding *ad libitum* after 24 h starvation are shown by: ††† $P < 0.001$. Statistically significant effects of late pregnancy are shown by: ‡‡ $P < 0.01$.

PDH_a activity	Nutritional status		
	Fed <i>ad libitum</i>	Starved (24 h)	Re-fed (2 h)
(units/g wet wt.)			
Virgin	1.73 $\pm$ 0.40 (13)	0.07 $\pm$ 0.02** (3)	3.08 $\pm$ 0.32††† (3)
Pregnant	0.39 $\pm$ 0.10‡‡ (10)	0.03 $\pm$ 0.01** (5)	1.46 $\pm$ 0.63 (5)
(% active)			
Virgin	26.3 $\pm$ 6.1 (13)	1.0 $\pm$ 0.3** (3)	46.9 $\pm$ 4.9††† (3)
Pregnant	6.3 $\pm$ 1.6‡‡ (10)	0.4 $\pm$ 0.1** (5)	23.7 $\pm$ 10.2 (5)
(μmol of C_{16} units/h per g wet wt.)			
Virgin	12.95	0.49	23.07
Pregnant	2.93	0.20	10.96

present in the active form) measured in extracts of freeze-clamped IBAT sampled in virgin rats fed *ad libitum* (Table 5) were approx. 2-fold higher than those measured previously in fed rats [27]. Starvation (24 h) of virgin rats led to substantial (96 %; $P < 0.01$) loss of PDH_a activity, restricting the capacity for conversion of pyruvate into fatty acid (Table 5). The dramatic stimulation of fatty acid synthesis observed during re-feeding with chow *ad libitum* after 24 h starvation was accompanied by significant PDH re-activation (Table 5). The activity of PDH_a measured at 2 h after chow re-feeding was 1.8-fold higher ($P < 0.05$) than that observed at D-12 (Table 5). IBAT PDH_a activities were significantly ($P < 0.01$) suppressed in late pregnancy in rats sampled at D-12 (to 23 % of controls; Table 5). As in virgin rats, starvation for 24 h in late pregnancy resulted in significant ($P < 0.01$) inhibition of IBAT PDH (Table 5). As a consequence, PDH_a activities in IBAT of pregnant rats after 24 h of starvation were not significantly different from those observed in IBAT of 24 h-starved virgin rats (Table 5). Re-feeding with chow *ad libitum* for 2 h in late pregnancy increased IBAT PDH_a activity, such that activities were 3.7-fold higher than in late-pregnant rats at D-12 and comparable with those observed in virgin rats at D-12. However, IBAT PDH_a activities at 2 h after re-feeding in late pregnancy were only 47 % of those observed in 2 h-re-fed virgin rats (Table 5).

Concluding remarks

Our data indicate that the contribution of brown fat to glucose homeostasis in virgin rats is critically dependent on both the pattern and rate of food consumption. If IBAT is representative of all BAT depots, our data indicate that BAT exerts its major influence on whole-body glucose homeostasis during re-feeding after starvation, rather than during unrestricted feeding *ad libitum* during the diurnal cycle (when the stimulation of IBAT GUI observed in response to food intake is relatively modest and transient). The dramatic enhancement of IBAT glucose uptake/phosphorylation observed during re-feeding after 24 h starvation

is not due to an increased content of GLUT 4 protein, and the factors responsible remain to be elucidated. Our data also indicate a general suppression of IBAT glucose utilization in late pregnancy, irrespective of the nutritional status. The conclusion that brown fat has more significance for whole-body glucose homeostasis if the food supply is interrupted than if access to food is continuous is supported by the finding that the suppression of IBAT glucose utilization observed in late pregnancy is most pronounced during re-feeding after 24 h starvation. Again, our data eliminate altered genetic expression of IBAT GLUT 4 protein in the response to pregnancy. The effects of pregnancy on IBAT glucose utilization are not confined to impairment of glucose transport: in addition, PDH_a activity in the absorptive state is suppressed.

Rates of uptake/phosphorylation of glucose are insufficient to accommodate measured rates of fatty acid synthesis *de novo* in late-pregnant rats in the fed state (at D-12; see above). However, although PDH_a is significantly suppressed in late pregnancy (indeed, to an extent remarkably similar to the suppression of glucose uptake/phosphorylation), it nevertheless retains a high activity (390 m-units/g wet wt.) which is comparable with that of liver in the fed state (114 m-units/g wet wt. in virgin rats; [27]). When expressed in terms of the potential contribution of pyruvate to fatty acid synthesis, estimated PDH_a activity in the fed state at D-12 ($2.93 \pm 0.73 \mu\text{mol}$ of C₁₆ units/h per g wet wt. at 30 °C; $n = 10$) is sufficiently high to sustain measured rates of fatty acid synthesis (see Table 4). There is evidence that circulating lactate is used in preference to glucose as a lipogenic substrate by peritoneal white adipose tissue [33], and the retention of relatively high absolute PDH_a activities in IBAT in animals fed *ad libitum* in late pregnancy highlights the potential for the utilization of pyruvate derived from circulating lactate, rather than from glycolysis *in situ*, for IBAT fatty acid synthesis under conditions where glycolysis *in situ* is suppressed.

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