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Control of muscle pyruvate oxidation during late pregnancy

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Despite significant increases in circulating concentrations of lipid fuels (triacylglycerol, non-esterified fatty acids (NEFA) and ketone bodies) in late-pregnant rats sampled in the fed (absorptive) state, cardiac and skeletal muscle active pyruvate dehydrogenase (PDH_a) activities remained comparable with those observed in fed, age-matched virgin controls. Cardiac PDH_a activity was suppressed in response to acute (6 h) starvation in late-pregnant (as well as virgin) rats: this inactivation was opposed by inhibition of mitochondrial long-chain FA oxidation. Starvation (6 h) also led to PDH inactivation in skeletal muscles of late-pregnant, but not virgin, rats. Starvation for 24 h led to further suppression of cardiac PDH_a activity and was associated with significant increases in PDH kinase activities in both virgin and late-pregnant rats. Late pregnancy did not itself influence cardiac PDH kinase activity.

Pyruvate dehydrogenase complex; Pyruvate dehydrogenase kinase; Starvation; Late pregnancy; Glucose/fatty acid cycle; Pyruvate

1. INTRODUCTION

The pyruvate dehydrogenase complex (PDH) catalyses the pathway for net loss of glucose carbon in vivo, namely the oxidative decarboxylation of pyruvate and production of acetyl CoA. PDH is inactivated by phosphorylation, catalysed by PDH kinase, and reactivated by dephosphorylation, catalysed by PDHP phosphatase [1,2]. The percentage of PDH which is phosphorylated can be varied progressively over a wide range by changing the mitochondrial concentrations or molar ratios of effectors that regulate activities of PDH kinase and PDHP phosphatase [3,4]. In general, the total amount of PDH (sum of active and inactive forms) is unchanged (see e.g. [5–7]).

The glucose/fatty acid cycle was formulated as a basis for the regulation of fuel selection in starvation and diabetes, and proposes that the utilization of glucose and lipid in energy metabolism is reciprocal and not dependent [8]. The decrease in whole-body glucose utilization that occurs as a result of prolonged starvation [9] is associated with markedly decreased rates of glucose uptake/phosphorylation by oxidative muscles that are thought to result from an enhanced rate of oxidation of fatty acids (FA) and ketone bodies [9,10]. Starvation also leads to muscle PDH inactivation by phosphorylation [5–7,11–15], again via mechanisms believed to involve effects of an increased oxidation of lipid fuels [6,15,16]. The effects of inhibitors of the mitochondrial oxidation of FA [6,15,16] and of adipose-tissue lipolysis [15] are consistent with this interpretation. Direct and short-term effects of FA lead to activation of PDH kinase by increased mitochondrial [acetyl CoA]/[CoA] ratios (see [17,18]), whereas indirect and longer-term effects are achieved through an increased specific activity of PDH kinase [19–21].

Because of the requirement of certain vital tissues for glucose as the predominant fuel, there is a need for stringent regulation of rates of glucose uptake and oxidation if hypoglyceamia is to be avoided in starvation. It can be envisaged that the regulation of glucose utilization would also be important if the demand for glucose per se were to be increased. During pregnancy, the foetus has an absolute developmental requirement for glucose, and we [22] and others (e.g. [23]) have observed that late pregnancy is associated with increases in circulating ketone body and non-esterified FA (NEFA) concentrations in the fed state (for review see [24]). A concomitant marked decline in rates of glucose uptake/phosphorylation in maternal muscle [22] led us to suggest that a component of the mechanism(s) operating to conserve glucose for use by the developing foetus is the use of circulating lipid-fuels by the mother with associated sparing of glucose at the level of tissue glucose uptake [22].

Acute lipid-feeding in vivo leads to a more rapid and more marked suppression of PDH_a activity than of rates

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Abbreviations: GUI, glucose utilization index; NEFA, non-esterified fatty acids; PDH, pyruvate dehydrogenase; PDH_a, the active form of PDH; TDG, 2-tetradecylglycidate; TG, triacylglycerol.

Dedicated to Professor Sir Philip Randle F.R.S. on the occasion of the 30th anniversary of the glucose/fatty acid cycle.

of glucose uptake/phosphorylation in muscles [10,15,25]. In the Langendorff-perfused heart, the provision of exogenous FA (and presumably increased FA oxidation) also causes a more rapid and profound suppression of PDH_a activity than of glucose uptake and phosphorylation [26]. We therefore proposed that the progressively enhanced substitution of lipid for glucose as primary energy substrate could explain both the selective suppression of glucose catabolism at the level of pyruvate oxidation during early starvation, and the subsequent inhibition of glucose uptake/phosphorylation as starvation proceeds [10,15]. It would be predicted from these studies that if increased lipid-fuel oxidation underlies the mechanism for suppression of muscle glucose uptake/phosphorylation during late pregnancy, marked PDH_a inactivation in muscle would be concomitantly observed.

In the present study, we have examined the responses of muscle PDH_a activities to late pregnancy in relation to circulating lipid-fuel (NEFA, triacylglycerol (TG) and ketone body) concentrations. In addition, we have characterized the response of muscle PDH_a activity to the changes in circulating lipid supply associated with acute (6 h) and prolonged (24 h) starvation. In the heart, where the induction of PDH kinase has been demonstrated to be elicited by NEFA [20], we have also examined the effects of late pregnancy on the expression of PDH kinase.

2. MATERIALS AND METHODS

Biochemicals were from Boehringer-Mannheim Ltd., Lewes, East Sussex, UK or Sigma Chemical Co., Poole, Dorset, UK. TDG was a gift from McNeil Pharmaceuticals, Spring House, PA, USA. 2-Deoxy[1-^{3}H]glucose was from Amersham International plc, Amersham, Buckinghamshire, UK. Kits for measurements of NEFA and TG concentrations were from Alpha Laboratories, Eastleigh, Hampshire, UK.

Age-matched female Wistar rats were maintained on a 12 h-light/12 h-dark cycle (light from 10.00 h). Ad libitum-fed rats were sampled in the absorptive state (within 0.5 h of the end of the dark (feeding) phase). Time-mated pregnant rats were studied after 18–20 days of pregnancy (term is 22–24 days), the stage of pregnancy was determined as described in [27]. The mean number of foetuses per rat was 13 (range 8–21), with no statistically significant differences between the groups of late-pregnant rats sampled. Rats were permitted free access to a standard rodent diet (52% carbohydrate, 15% protein, 3% fat and 30% non-digestible residue; all by wt.; Special Diets Services, Witham, Essex, UK) or starved for 6 or 24 h (removal of food at 10.00 h). 2-Tetradecylglycidate (TDG), an inhibitor of mitochondrial long-chain FA oxidation [28,29], was administered intragastrically (2.5 mg/100 g b.wt., suspension in 0.5% (w/v) CM-cellulose) under light diethyl ether anaesthesia.

PDH complex (active form, PDH_a) and citrate synthase activities were measured in freeze-clamped muscle extracts as described previously [6], with the addition of Triton X-100 (2.5%, w/v) and protease inhibitors (benzamidine (1 mM), leupeptin (10 μM), TLCK (0.3 mM)) in the extraction medium (see [21]). PDH_a activities are expressed relative to citrate synthase to correct for possible differences in mitochondrial extraction. A unit of enzyme activity is defined as that which converts 1 μmol of substrate into product/min at 30°C. For assay of PDH kinase activity, mitochondrial pellets (after 30 min incubation of mitochondria at 30°C without respiratory substrate in the presence of 15 μM FCCP) were frozen, thawed and dispersed into 50 mM KH_2PO_4, 50 mM K_2HPO_4, 10 mM $EGTA^+$, 2 mM dithiothreitol, 1 mM benzamidine, 10 μM leupeptin, 0.3 mM TLCK, pH 7.5, and then extracted by freeze–thawing 3 times. PDH kinase activities were estimated in mitochondrial extracts by the rate of ATP-dependent inactivation of PDH complex as described in [30]. PDH kinase activity was computed as the apparent first-order rate constant [30,31]. The incubation mixture contained 0.5 mM ATP, 1 mM $MgCl_2$, 36 μg/ml oligomycin B and 100–200 mU PDH complex/ml. Samples for assay of active PDH were taken at 3–4 time intervals over up to 8 min (depending on PDH kinase activity).

The protocol for measurement of muscle glucose utilization indices (GUIs) is given in [9,10,32]. GUIs in virgin and late-pregnant rats can be equated with rates of glucose uptake/phosphorylation and compared directly since the correction factor for discrimination against 2-deoxyglucose in glucose metabolic pathways in oxidative muscles is close to unity [32,33] and unaffected by late pregnancy [34]. Each rat was fitted with a chronic indwelling cannula under Hypnorm (fentanyl citrate (0.315 mg/ml)/fluanisone (10 mg/ml); 1.0 ml/kg i.p.) and Diazepam (5 mg/ml; 1 ml/kg i.p.) anaesthesia. Rates of 2-deoxy[^{3}H]glucose uptake/phosphorylation were measured in unrestrained, conscious rats at 5–7 days after cannulation. Each measurement was initiated by the injection of a 30 μCi tracer dose of 2-deoxy[^{3}H]glucose via the indwelling cannula. Blood (100 μl) was collected via the cannula at 1, 3, 5, 10, 20, 40 and 60 min after injection of radiolabel. The experiment was then terminated by the injection of sodium pentobarbital (60 mg/kg b.wt.) through the indwelling cannula. Blood and muscle samples were treated as described in [32].

Lactate and ketone body (3-hydroxybutyrate plus acetoacetate) concentrations were measured in KOH-neutralized $HClO_4$ extracts of whole blood using enzymatic methods [35,36]. Plasma NEFA and TG concentrations were measured using kits.

Statistical significance of differences between groups was assessed by Student's unpaired t-test. Results are means ± S.E.M. with the number of observations in parentheses.

3. RESULTS AND DISCUSSION

3.1. *Cardiac PDH_a activities in the ad libitum-fed state in late pregnancy*

There was marked and statistically significant suppression (to 35% of the control value) of rates of glucose uptake/phosphorylation (assessed by the uptake and phosphorylation of radiolabelled 2-deoxyglucose) by the heart in the ad libitum-fed state in late pregnancy (rates of 98.8 ± 10.3 ng/min per mg wet wt., $n = 12$, and 34.2 ± 4.3 ng/min per mg wet wt., $n = 10$, in virgin and late-pregnant rats respectively, $P < 0.001$; see also [22]). In contrast, cardiac PDH_a activities measured in extracts of freeze-clamped hearts of ad libitum-fed, late-pregnant rats were not significantly different ($P > 0.05$) from the control (ad libitum-fed, virgin) PDH_a activities (Table I). It would appear that there is divergent regulation of glucose uptake/phosphorylation and pyruvate oxidation in the heart in late pregnancy. An acute elevation in NEFA supply is associated with suppression of glucose uptake/phosphorylation by the heart [10], and lipid fuel concentrations are elevated in late pregnancy [22–24]. We therefore examined whether late pregnancy is associated with altered regulatory characteristics of cardiac PDH_a activity with respect to lipid-fuel supply.

Table I

Cardiac PDH_a and PDH kinase activities in virgin and late-pregnant rats: effects of starvation and inhibition of mitochondrial long-chain fatty acid oxidation by 2-tetradecylglycidate (TDG)

Experimental group	PDH_a activity (mU/U of citrate synthase)				PDH kinase (expressed as pseudo-first-order rate constant (min^{-1}))	
	Virgin		Pregnant		Virgin	Pregnant
	Control	TDG-treated	Control	TDG-treated		
Fed ad libitum	70.7 ± 9.4 (5)	71.2 ± 10.1 (5)	57.4 ± 6.2 (8)	84.2 ± 12.5 (5)	0.20 ± 0.02 (3)	0.17 ± 0.01 (5)
Starved (6 h)	40.6 ± 3.3* (5)	59.9 ± 5.6^{+} (4)	24.9 ± 8.3** (5)	84.6 ± 6.7$^{\S+++}$ (4)	–	–
Starved (24 h)	1.1 ± 0.6*** (4)	–	7.2 ± 3.6*** (5)	–	0.40 ± 0.03** (3)	0.36 ± 0.02*** (5)

Cardiac citrate synthase activities (U/g wet wt. of tissue) in virgin and late-pregnant rats were 107.3 ± 5.6 (n = 22) and 98.1 ± 4.4 (n = 25) U/g wet wt., respectively. Rate constants were calculated by least squares linear regression analysis of (ln % zero time activity) against time. Experimental details are given in section 2. Results are means ± S.E.M. with the number of observations in parentheses. Statistically significant effects of late pregnancy are shown by $^{\S}P < 0.05$. Statistically significant effects of TDG treatment are shown by $^{+}P < 0.05$; $^{+++}P < 0.001$. Statistically significant effects of starvation are shown by $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

3.2. *The role of FA in the regulation of cardiac PDH_a activity in late pregnancy*

Elevated NEFA concentrations in vivo can be evoked by the administration of a single lipid meal (corn oil) plus heparin [25,37,38], and NEFA concentrations comparable to those observed after extended (15–24 h) starvation are achieved within 1 h [10,25]. Phosphorylation and inactivation of PDH is observed concomitantly [10,25]. Cardiac PDH_a inactivation during starvation occurs within 4 h of food withdrawal [16]. Although there are no marked increases in circulating NEFA and ketone body concentrations at this time [15,16], cardiac PDH inactivation can be reversed by specific inhibition of mitochondrial long-chain FA oxidation by TDG [16], and bears a close temporal correlation with an increased capacity for utilization of FA derived from circulating TG via LPL [39]. TDG administration does not increase cardiac PDH_a activities in virgin rats in the fed state, implying that rates of mitochondrial long-chain FA oxidation are low ([6]; see also Table I).

Cardiac PDH_a activity responded to acute (6 h) starvation in late pregnancy with a significant decline in activity (to 43% of the initial value, $P < 0.01$; Table I) which, as in virgin rats, was opposed by the administration of TDG over the period from 3 to 6 h of starvation (Table I). Cardiac PDH_a activities in 6 h-starved late-pregnant rats treated with TDG were not significantly different from those observed in fed late-pregnant rats treated with TDG (Table I). The decline in cardiac PDH_a activity observed in response to acute (6 h) starvation is therefore secondary to an increased rate of mitochondrial long-chain FA oxidation.

3.3. *Cardiac PDH_a and PDH kinase activities during prolonged (24 h) starvation in late pregnancy*

Essentially complete suppression of cardiac PDH_a activity was observed after 24 h of starvation in virgin rats in the present experiments (Table I; see also [15,16]). There was also marked suppression of cardiac PDH_a activity after 24 h of starvation in late pregnancy (Table I). There was as a consequence no significant difference between cardiac PDH_a activities of 24 h-starved late-pregnant and 24 h-starved virgin rats, and in both cases the capacity for pyruvate oxidation was minimal.

NEFA and ketone body concentrations comparable to those observed after 24 h starvation in virgin rats are observed within 6 h of the onset of starvation in late-pregnant rats (Table II). Tissues of late-pregnant rats that are sampled after starvation for 24 h have thus been exposed to elevated NEFA concentrations for at least the preceding 18 h (compared with a 9 h period of exposure in virgin rats, see [15]). It has been demonstrated that long-term exposure to FA leads to a stable increase in the intrinsic activity of PDH kinase in cultured cardiomyocytes [20]. It was therefore of interest to examine the expression and regulation of PDH kinase activity during late pregnancy.

PDH kinase was assayed by following the rate of ATP-dependent disappearance of active PDH in extracts of isolated cardiac mitochondria. Mean PDH kinase activities expressed as the apparent first-order rate constants are shown in Table I. PDH kinase activities measured in extracts of heart mitochondria were unaffected by late pregnancy in rats sampled in the ad libitum-fed state (Table I). PDH kinase activities in ex-

Table II

Metabolite concentrations in the fed state and during starvation in virgin and late-pregnant rats

Metabolite conc. (mM)	Ad libitum fed		Starved (6 h)		Starved (24 h)	
	Virgin	Pregnant	Virgin	Pregnant	Virgin	Pregnant
Blood ketone bodies	0.12 ± 0.02 (5)	0.36 ± 0.07§§ (9)	0.18 ± 0.07 (4)	0.81 ± 0.29 (5)	1.29 ± 0.25** (3)	2.60 ± 0.17§§*** (6)
Plasma NEFA	0.13 ± 0.03 (7)	0.32 ± 0.05§§ (13)	0.17 ± 0.04 (5)	0.51 ± 0.07§§** (4)	0.75 ± 0.12*** (5)	0.79 ± 0.06*** (6)
Plasma TG	0.75 ± 0.12 (6)	1.71 ± 0.19§§§ (9)	0.74 ± 0.05 (5)	1.67 ± 0.41 (6)	0.55 ± 0.14 (3)	1.74 ± 0.45§ (6)
Blood lactate	1.00 ± 0.16 (5)	1.68 ± 0.17§ (5)	0.68 ± 0.14 (4)	1.13 ± 0.20* (13)	0.76 ± 0.08 (4)	1.09 ± 0.24 (5)

Experimental details are given in section 2. Results are means ± S.E.M. with the number of observations in parentheses. Statistically significant effects of late pregnancy are shown by $^{\S}P < 0.05$; $^{\S\S}P < 0.01$; $^{\S\S\S}P < 0.001$. Statistically significant effects of 6 h or 24 h starvation are shown by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

tracts of mitochondria from hearts of 24 h-starved virgin rats were 2.0-fold higher than in extracts of mitochondria from hearts of ad libitum-fed virgin rats (Table I). Previous studies have demonstrated a 2 to 4-fold increase in cardiac PDH kinase activities after 48 h starvation in male rats [40]. Starvation for 24 h was associated with 2.0-fold increase in cardiac PDH kinase activities in late-pregnant rats (Table I). Thus, interestingly, despite higher lipid-fuel concentrations in both the fed and starved states, and a more rapid elevation in NEFA and ketone body concentrations in response to starvation (Table II), there were no statistically significant effects of late pregnancy on cardiac PDH kinase activities (Table I).

3.4. *PDH_a activities in oxidative skeletal muscle in pregnancy*

The regulation of PDH_a activity in skeletal muscle shares many common features with the regulation of PDH activity in heart [12]. Furthermore, by virtue of their large contribution to total body mass and because (in virgin rats) they exhibit high rates of glucose uptake/phosphorylation in the fed state [9,10], oxidative skeletal muscles are collectively quantitatively more impor-

Table III

PDH_a activities in oxidative skeletal muscles during starvation in virgin and late-pregnant rats

Experimental group	PDH_a activities (mU/U of citrate synthase)					
	Diaphragm		Adductor longus		Soleus	
	Virgin	Pregnant	Virgin	Pregnant	Virgin	Pregnant
Fed ad libitum	12.3 ± 1.3 (5)	12.1 ± 2.6 (7)	5.4 ± 0.7 (5)	7.3 ± 1.4 (7)	3.0 ± 0.7 (5)	2.7 ± 0.3 (7)
Starved (6 h)	11.3 ± 3.7 (5)	1.3 ± 0.6**§ (5)	4.6 ± 1.1 (5)	0.5 ± 0.3***§§ (5)	1.3 ± 0.4 (5)	0.9 ± 0.4** (5)
Starved (24 h)	0.9 ± 0.5*** (3)	0.5 ± 0.3** (4)	2.9 ± 1.0 (3)	1.1 ± 0.2** (4)	1.1 ± 0.2** (3)	1.3 ± 0.4* (4)

Citrate synthase activities (U/g wet wt.) for individual skeletal muscles were: diaphragm, virgin 44.2 ± 1.5 (16), pregnant 43.6 ± 1.5 (16); adductor longus, virgin 29.0 ± 1.4 (16), pregnant 26.9 ± 1.0 (16); soleus, virgin 32.7 ± 1.3, pregnant 28.0 ± 0.8 (16) ($P < 0.001$). Experimental details are given in section 2. Results are means ± S.E.M. with the number of observations in parentheses. Statistically significant effects of late pregnancy are shown by $^{\S}P < 0.05$; $^{\S\S}P < 0.01$. Statistically significant effects of 6 h or 24 h starvation are shown by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

tant for the clearance of circulating glucose than the heart. There is compelling evidence that (as in heart) PDH inactivation in response to starvation in skeletal muscle is secondary to increased lipid-fuel oxidation [10,12,15]. We therefore investigated the effects of late pregnancy on PDH_a activities in diaphragm and two well-characterised oxidative skeletal muscle (soleus and adductor longus).

PDH_a activities measured in diaphragm, soleus and adductor longus muscles of late-pregnant rats sampled in the ad libitum-fed state were not significantly different from those measured in muscles of the fed, virgin controls (Table III). In contrast (as in the heart), there is marked inhibition of glucose uptake/phosphorylation by oxidative skeletal muscle in late-pregnant rats in the fed state [22]. The retention of a relatively unimpaired capacity for pyruvate oxidation under conditions where the use of circulating glucose is suppressed is thus not specific to the heart.

We have shown previously in virgin rats that suppression of PDH_a activity in response to starvation occurs later after the onset of starvation in skeletal muscle than in heart [15]. These findings were confirmed in the present experiments, where acute (6 h) starvation did not suppress skeletal-muscle PDH_a activities (Table III) although cardiac PDH_a activity had declined (Table I). In marked contrast, starvation for 6 h in late pregnancy elicited significant suppression of PDH_a activities in all three skeletal muscles studied (Table III). This is consistent with an accelerated utilization of available lipid-fuels by skeletal muscles during progressive starvation in late pregnancy. Suppression of skeletal-muscle PDH_a activity was substantial in both virgin and late-pregnant rats after 24 h starvation, with no significant differences in skeletal muscle PDH_a activities between virgin and late-pregnant rats (Table III).

4. CONCLUSIONS

The results demonstrate an unexpected and interesting discrepancy between glucose utilization (uptake/phosphorylation) and PDH_a activity status in heart and oxidative muscles in late pregnancy in the fed state. In the heart, the divergent regulation of glucose uptake and phosphorylation and PDH_a activity status does not appear to be a consequence of altered regulatory characteristics of PDH with respect to acute or prolonged starvation. However, despite an elevation of circulating lipid supply in the fed state in late pregnancy, cardiac PDH_a activities are relatively high and PDH kinase activities remain relatively low. The retention of high PDH_a activities concomitant with the existence of low rates of glucose utilization in muscle in the fed state emphasizes the potential for use of circulating lactate + pyruvate as oxidative fuel under physiological conditions, such as pregnancy, associated with a requirement for stringent regulation of glycaemia. The potential use of glucose derivatives – rather than glucose itself – by muscle bears an interesting analogy with the use of lactate + pyruvate in preference to glucose by the liver as a precursor for glycogen and lipid synthesis (reviewed in [41]).

Substrate competition between glucose and lipid-derived fuels forms the basis of the glucose/fatty acid cycle, whereby acute changes in the relative concentration of these energy substrates can cause profound changes in metabolic fluxes in oxidative tissues [8]. Although the uptake/phosphorylation of circulating glucose by the heart is suppressed in late pregnancy [22], there was a significant (68%) elevation in blood lactate concentrations in the fed (absorptive) state in late-pregnant rats (Table II) and the heart can readily utilize circulating lactate [42]. Furthermore, exogenous lactate inhibits the uptake and phosphorylation of glucose by heart [42] and skeletal muscle [43] in vitro at the concentrations found in late-pregnant rats in the fed state in vivo (Table II). Pyruvate can oppose inactivation of PDH through inhibitory effects on PDH kinase [44,45]. It is plausible that inhibition of PDH kinase by a rise in intramitochondrial pyruvate secondary to an elevation in blood lactate may attenuate the acute effects of lipid-fuel oxidation to activate cardiac PDH kinase in the fed state during late pregnancy. In view of the failure to observe enhanced expression of PDH kinase after 24 h starvation in late pregnancy, it would be of interest to examine further whether a high intracellular pyruvate concentration can also oppose the induction of cardiac PDH kinase by FA, introducing the concept of long-term substrate interactions at the level of gene expression as an additional component of the glucose/fatty acid cycle.

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