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A relationship between impaired fetal growth and reduced muscle glycolysis revealed by ^{31}P magnetic resonance spectroscopy

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Summary Thinness at birth is associated with insulin resistance and an increased prevalence of non-insulin-dependent diabetes mellitus in adult life. As muscle is an important site of insulin resistance, and because thin babies have reduced muscle mass, thinness at birth may affect muscle structure and function and impair carbohydrate metabolism. We have therefore used ^{31}P magnetic resonance spectroscopy to investigate the bioenergetics of gastrocnemius and flexor digitorum superficialis muscles in 16 normoglycaemic women who had a low (≤ 23 kg/m^3) and 9 women who had a high (> 23 kg/m^3) ponderal index at birth. In the flexor digitorum superficialis study anaerobic metabolism was stressed with a constant heavy workload. Low ponderal index subjects fatigued more rapidly (3.3 vs 5.8 min); as phosphocreatine decreased, the accompanying drop in muscle pH was less than in the high ponderal index group. In the first minute of exercise phosphocreatine fell and adenosine diphosphate rose more rapidly ($p = 0.04$ and 0.03, respectively). Gastrocnemius showed a similar trend late in exercise (this exercise was more oxidative, becoming more anaerobic with increasing workload). These changes were not explained by differences in body composition, muscle mass or blood flow. The findings are consistent with a decreased lactic acid and glycolytic adenosine triphosphate production in the low ponderal index group and suggest the possibility that the mechanisms which control substrate utilisation and metabolism in adult life be programmed during prenatal life. [Diabetologia (1995) 38: 1205–1212]

Key words ^{31}P nuclear magnetic resonance spectroscopy, skeletal muscle, glucose metabolism, fetal growth, programming.

In both European and non-European populations low birthweight is linked with a higher prevalence of non-insulin-dependent diabetes mellitus (NIDDM) or glucose intolerance in adult life [1–4]. These trends result from reduced intrauterine growth rather than prematurity and are independent of adult obesity [2]. Surveys carried out in Preston, Lancashire, UK, and in Uppsala, Sweden, where detailed measurements of the body size at birth were recorded show that the baby likely to develop glucose intolerance is characterised by thinness at birth, as indicated by a low ponderal index (PI) (birthweight/length^3) [2, 5]. Thinness at birth was also related to a high prevalence of syndrome X, the co-existence of hypertension, glucose intolerance, hypertriglyceridaemia and insulin resistance [6]. A link between thinness at birth and insulin resistance in middle life was also shown when insulin tolerance tests were carried out on a sample of 103 of the Preston subjects [7]. These findings have led to the hypothesis that insulin resistance originates in fetal life and may arise as a result of the metabolic adaptation of the fetus to an adverse environment.

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Abbreviations: PI, Ponderal index (at birth); MRS, magnetic resonance spectroscopy; PCr, phosphocreatine; Pi, inorganic phosphate; FDS, flexor digitorum superficialis; PME, phosphomonoester; PDE, phosphodiester; NIDDM, non-insulin-dependent diabetes mellitus; Fid, free induction decay.

The processes which could link thinness at birth with insulin resistance in adult life are not known. Muscle is a major site of insulin resistance [8]. Muscle biopsies have shown that insulin resistance is associated with particular morphological characteristics including a greater proportion of type 2B fibres, a lesser proportion of type 1 fibres and lower capillary density [9, 10]. Babies born at term with a low PI have a reduced mid-arm circumference, which implies that they have a low muscle bulk as well as less subcutaneous fat [11]. It is therefore possible that thinness at birth is associated with abnormalities of muscle metabolism which persist into adult life and interfere with the metabolic action of insulin.

We have therefore used ^{31}P magnetic resonance spectroscopy (^{31}P MRS) to investigate the bioenergetics of skeletal muscle before, during and after exercise in a sample of the 50-year-old subjects who took part in the Preston study [7] and whose body size at birth had been recorded.

Subjects and methods

Subjects. From 1932 a standardised record was kept for each woman admitted to the labour ward at Sharoe Green Hospital, Preston, Lancashire. The record included the baby's birth and placental weights, length and head circumference. As previously described the National Health Service central register was used to trace 393 of the singleton infants born during 1935–1943 who still live in or close to the city [2]. Of these subjects 266 agreed to undergo an oral glucose tolerance test. Because NIDDM and impaired glucose tolerance were not only associated with low birthweight but also low birthweight in relation to placental weight, which is thought to be a marker of fetal malnutrition, we selected a sample from the 232 normoglycaemic men and women whose birth and placental weights were spread over the range for these two variables. The sample was chosen from the three birthweight groups (≤ 2.9, -3.4 and >3.4 kg) and the four placental weight groups (≤ 0.45, -0.6, -0.7 and ≥ 0.7 kg) used in previous analyses. Four men and four women were sampled from each of the 12 birth and placental weight groups. Because of insufficient numbers in some groups the final sample comprised 42 men and 40 women. These subjects subsequently underwent insulin tolerance tests to measure insulin resistance [7]. Insulin resistance was expressed as the half-life in minutes of the fall in blood glucose after the administration of a bolus of insulin (0.05 U/kg). Because the relationships between fetal growth and insulin resistance were stronger in women than men, we asked the 40 women to undergo nuclear magnetic resonance (NMR) spectroscopy. Two women with body mass index greater than 30 kg/m^2 were unsuitable for spectroscopy and 13 declined to participate, leaving 25 subjects. The 13 non-respondents did not differ significantly from the 25 respondents by birthweight ($p = 0.53$), placental weight ($p = 0.67$), or PI ($p = 0.86$).

Their height was measured with a portable stadiometer (CMS Weighing Equipment Ltd, London, UK) and weight with a SECA Scale (SECA Ltd, Birmingham, UK). Body mass index (BMI) was calculated as the weight (kg) divided by the height (m) squared. Skinfold thicknesses were measured by a single observer with Harpenden Skinfold Callipers; (Harpenden, Herts., UK) at the biceps, triceps, subscapular and suprailiac sites. Body fat percentage was computed according to the method of Durnin and Wormsley [12]. Blood pressure was measured with an automated recorder (Dinamap Model 18465X; Critikon, Tampa, Fla., USA) [7]. Creatinine concentration in 24-h urine samples was determined by the Jaffe reaction and muscle mass estimated with the assumption that a daily excretion of 60 mg of creatinine corresponded to 1 kg of muscle [13]. Corrected arm muscle area (CAMA) was computed by the method of Jeliffe and Jeliffe [14] from the upper arm circumference (AC) and triceps skinfold (TSF) ($CAMA = [(AC - \pi TSF)^2 - 10]/4\pi$).

Ethical committee approval for the study was obtained from the Preston District Hospital ethics committee and MRS studies were approved by the Central Oxford Research ethics committee. Each participating subject gave written informed consent.

^{31}P magnetic resonance spectroscopy

Flexor digitorum superficialis (FDS). Spectra were obtained from the dominant forearm. Investigations were carried out using a Fourier transform spectrometer (Oxford Research Systems, Coventry, UK) interfaced with a 1.9 Tesla, 30-cm bore superconducting magnet (Oxford Magnet Technology, Oxford, UK). The subject lay supine with the arm abducted to 90° and positioned in the bore of the magnet. The pulsing conditions of 2-s interpulse delay and 20-μs pulse length were chosen to optimise the ^{31}P signal. The 90° pulse at the centre of the 2.5-cm diameter surface coil was 16 μs. A spectrum composed of 128 accumulated free induction decays (fids; collection time 256 s) from resting muscle provided the baseline values for each individual exercise study. The fingers of the dominant arm pulled a weight of 1.5 kg through a distance of 5 cm at a rate of 40 times per min (0.5 W). If the subject was able to perform 5 min of exercise at this workload, the weight was then increased by 250 g (0.08 W) at 1.25-min intervals. If the subject became unable to move the weight the full distance at the required rate, the exercise was terminated (all subjects became exhausted by 11 min). Each exercise spectrum was collected over 32 s. Data were acquired for 12 min after the subject stopped exercising (4 spectra of 16 fids followed by 4 of 32 and 2 of 64). One subject did not perform the FDS study and the FDS exercise data for another subject could not be used for technical reasons.

Gastrocnemius. Subjects were positioned with the right gastrocnemius over a 6-cm diameter surface coil in a 1.9 Tesla superconducting magnet with a 100-cm bore (Oxford Magnet Technology Ltd) interfaced with a Bruker Biospec Spectrometer (Bruker, Coventry, UK). The pulse length was 80 μs (90° pulse at coil centre, 80 μs) and the interpulse delay 2 s. A 64 fid (128 s) spectrum was collected from resting muscle. Spectra composed of 32 fids (64 s) were collected throughout exercise. Exercise consisted of plantar flexion of the right foot lifting a weight (10 % lean body mass) a distance of 7 cm. After 5 min of exercise, the weight was increased by 2 % lean body mass at the beginning of each spectral accumulation until the subject could no longer perform the work required. Recovery was followed by collecting the following spectra sequentially: 4 of 8 fids, 4 of 16, 3 of 32 and 2 of 64.

Quantification. All data were processed with 6-Hz line broadening and Fourier transformation. Metabolite ratios were calculated from the peak areas of phosphocreatine (PCr), inor-

ganic phosphate (Pi), β-ATP, phosphomonoesters (PME), and phosphodiesters (PDE) [15]. For the gastrocnemius data, signal intensities were obtained by using a time domain fitting computer program (VARPRO, R de Beer, Delft, The Netherlands). The program identifies a specified number of exponentially decaying signals in the accumulated fids. The search for peaks is facilitated by using prior knowledge of the expected amplitudes, relative positions and widths of the peaks to be fitted. The signal-to-noise of the data from finger flexors (approximately 50:1 for the PCr peak in the spectra from resting muscle) was too low to be quantified using time domain fitting, so the metabolite ratios were determined as previously described using manual triangulation of the peaks, corrected for line shape. All data were corrected for magnetic saturation. The cytoplasmic pH (pHi) was determined from the chemical shift difference of the Pi and PCr peaks as previously detailed [16]. PCr is normally expressed as the ratio of PCr/(PCr + Pi) because PCr + Pi remains constant during exercise and the ratio corrects for changes in signal intensity due to any movement of the muscle away from the coil. Cytoplasmic concentrations of PCr and Pi were calculated from the concentration ratios PCr/ATP and Pi/ATP assuming an intracellular concentration for ATP of 8.2 mmol/l intracellular water (mmol/l). Free cytoplasmic ADP was calculated from the creatine kinase equilibrium reaction assuming a creatine concentration of 42.5 mmol/l of intracellular water [16].

The exercise protocols for FDS and gastrocnemius were designed to stress different metabolic pathways. The workload for FDS was large, leading to a short exercise duration (overall mean, 4.1 min) fuelled mainly by a large net PCr breakdown and by glycogenolysis/glycolysis: the pHi decreased by about 0.5 units during this time. Gastrocnemius exercise was sustained by a larger contribution from oxidative metabolism. Mean duration of exercise was twice as long as for FDS. Changes in PCr were not as great and pHi decreased by only about 0.08 units at 5 min and 0.3 units at the end of exercise.

Recovery half-times ($t_{1/2}$) were calculated from the semilog transformation of the first four data points in recovery. pHi recovery was expressed as the rate of increase in pHi during the linear portion of the recovery curve (approximately 2–8 min after the end of exercise). Initial rates of PCr resynthesis were determined by linear regression using the calculated PCr concentration at the end of exercise and first two data points in recovery. The estimated maximum rate of ATP synthesis (V_{max}) was calculated from this initial rate, using a K_m for ADP of 30 µmol/l: $V_{max} = (d[PCr]/dt)\{1 + K_m/[ADP]\}$ [17].

Near infrared spectroscopy. The half-times for tissue reoxygenation after exercise were determined using a commercial apparatus (RunMan; NIM Inc., Philadelphia, Pa., USA) to perform near infrared spectroscopy of FDS. The light source was placed over the muscle and reflected light was measured at 760 and 850 nm to distinguish oxygenated and deoxygenated haemoglobin. Exercise was performed as described above for finger flexors except that after about 1 min of exercise a cuff was inflated around the upper arm to 50 mmHg above the systolic blood pressure as exercise was continued for a further 20–30 s. There was a 5-s delayed between cessation of exercise and deflation of the cuff. The half-time of reoxygenation was determined using the point at which the cuff was deflated as the point of both maximum deoxygenation and the start of metabolic recovery.

Muscle biopsies. Muscle biopsies (100 mg) were obtained from the medial head of the gastrocnemius muscle using the conchotome technique in 22 of the subjects. The samples were trimmed and either immediately frozen in liquid nitrogen (biochemical studies) or transported in buffer (histological studies) before being frozen in liquid nitrogen. Cross-sections of 8 µm thickness were cut with a cryotome at −20 °C and stained for histological examination of muscle fibres and capillaries. Sections were stained for myosin ATPase activity after preincubation at pH 4.3, 4.5 and 9.4. The fibres were classified into slow twitch (type 1) and fast twitch (type 2) fibres. In each biopsy 500 fibres were counted. Capillaries were stained using a Ulex europaeus lectin stain and the total number in a 2-mm square counted. Muscle creatine content was measured by a fluorimetric method [18].

Statistical analysis. MRS measurements are presented as the mean ± 1 SD. The data were analysed using tabulations of means and linear regression. The statistical significance of differences between groups was calculated by Student's t-test or by least sequences regression analysis for continuously distributed variables. Variables were log transformed to normality where necessary.

Results

Because PI at birth was the birth measurement most strongly predicting insulin resistance, the 25 women were divided into two groups, those with PI below and those above the overall mean of 23 kg/m^3. Both groups were of similar age (mean, 55 vs 54 years, respectively) BMI (24.6 vs 25.6 kg/m^2), body fat percentage (39.0 vs 38.7 %) systolic blood pressure (140 vs 143 mmHg) but significantly different insulin resistance as assessed by the short insulin tolerance test (mean $t_{1/2}$ for the low PI group 16.9 vs 14.3 min for the high PI group, $p = 0.045$). They had similar total muscle mass (14.5 vs 13.3 kg) and corrected arm muscle area (39.0 vs 40.7 cm^2).

^{31}P MRS studies

Resting muscle: The pre-exercise data from the FDS and gastrocnemius muscles are shown in Table 1. The FDS studies showed no difference in resting muscle between the high and low PI groups for pHi, metabolite ratios, metabolite concentrations or phosphorylation potential. In the gastrocnemius, PCr/ATP and therefore the calculated PCr concentration was slightly but significantly higher in the low PI group.

Exercise: Table 2 shows that during FDS exercise the low PI subjects achieved a significantly shorter exercise duration than the high PI subjects (3.3 vs 5.8 min, $t = 2.35$, $p = 0.03$). FDS exercise duration also correlated with PI at birth ($r = 0.55$, $p = 0.007$; Fig. 1). There was, however, no significant relationship between PI at birth and the duration of gastrocnemius exercise although the direction of the changes was similar. Table 2 also shows changes in the intracellular concentrations of key metabolites during

Table 1. Magnetic resonance spectroscopy results from resting muscle according to ponderal index at birth

	Flexor digitorum superficialis		Gastrocnemius	
	Low PI	High PI	Low PI	High PI
n	16	8	16	9
pH	7.03 ± 0.03	7.04 ± 0.04	7.02 ± 0.02	7.02 ± 0.02
PCr/ATP	3.04 ± 0.32	3.02 ± 0.13	3.64 ± 0.26	3.43 ± 0.18[a]
PCr/(PCr + Pi)	0.90 ± 0.03	0.90 ± 0.01	0.90 ± 0.01	0.90 ± 0.02
Pi/ATP	0.33 ± 0.10	0.32 ± 0.04	0.40 ± 0.05	0.36 ± 0.09
PCr/Pi	10.1 ± 3.4	9.7 ± 1.4	9.3 ± 1.27	10.00 ± 2.48
PME/ATP	0.10 ± 0.06	0.05 ± 0.03	0.10 ± 0.13	0.07 ± 0.04
PDE/ATP	0.05 ± 0.04	0.05 ± 0.04	0.21 ± 0.14	0.17 ± 0.08
ADP (μmol/l)	7 ± 7	7 ± 3	9 ± 5	13 ± 4
(1/phosphorylation potential) × 10^6	3.6 ± 4.1	3.3 ± 1.4	4.5 ± 2.2	5.5 ± 1.6

Data are mean ± SD. [a] $p = 0.05$

Table 2. Magnetic resonance spectroscopy results from exercising muscle according to ponderal index at birth

	Flexor digitorum superficialis		p-value	Gastrocnemius		p-value
	Low PI	High PI		Low PI	High PI	
n	15	8		16	9	
Duration (min)	3.3 ± 1.5	5.8 ± 3.7	0.03	9.7 ± 3.7	11.5 ± 2.8	0.55
First min of exercise						
pHi	7.00 ± 0.15	6.89 ± 0.08	0.07	7.08 ± 0.02	7.07 ± 0.02	0.11
PCr/(PCr + Pi)	0.49 ± 0.13	0.62 ± 0.13	0.04	0.72 ± 0.06	0.74 ± 0.06	0.74
ADP (μmol/l)	58 ± 37	27 ± 11	0.03	29 ± 12	30 ± 8	0.51

Data are mean ± SD

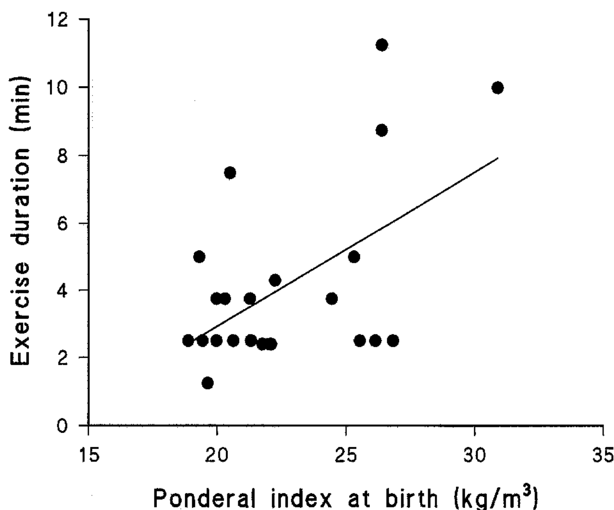

Fig. 1. Relationship between ponderal index at birth and exercise duration during FDS muscle exercise

the first min of exercise. During FDS exercise the fall in intracellular pH tended to be less in the low PI group (0.03 vs 0.15 units; Fig. 2) although this did not reach statistical significance. However, the mean PCr/(PCr + Pi) decreased to a significantly lower value in the low PI subjects (0.49 vs 0.62, $p = 0.04$; Fig. 2) resulting in a significantly higher ADP in this group (58 vs 27 μmol/l, $p = 0.03$; Fig. 2). Figure 3a shows the relationship between the pH and PCr/(PCr + Pi) changes during exercise (one point for each subject at rest and for each min of exercise). At any level of PCr depletion the fall in pH was less in the low PI group (mean gradient of pHi fall per unit PCr/(PCr + Pi) fall, 0.31 vs 0.65, $p = 0.003$). Both the fall in PCr/(PCr + Pi) and the rise in [ADP] during the first min of exercise correlated strongly with PI at birth ($r = -0.52$, $p = 0.01$ and $r = -0.71$, $p = 0.0003$, respectively). The relationship between ADP during the first min of exercise and PI is shown in Figure 4. During the gastrocnemius exercise these changes were not present in the first min of exercise (Table 2) but tended to develop as the workload increased as exercise progressed and a larger proportion of the ATP was derived from glycogenolysis/glycolysis (Fig. 3b). In regression analyses the relationships between the MRS changes in the arm or leg and PI at birth were unaffected by the inclusion of adult BMI, body fat percentage or muscle mass in the models. The MRS changes during exercise were much more closely related to PI at birth than the other birth measurements. Thus, for example, ADP during the first min of exercise did not correlate significantly with birthweight ($p = 0.15$), placental weight ($p = 0.74$), head circumference ($p = 0.36$), or gestational age ($p = 0.57$).

Recovery from exercise: Data from the post-exercise period were used to assess oxidative capacity be-

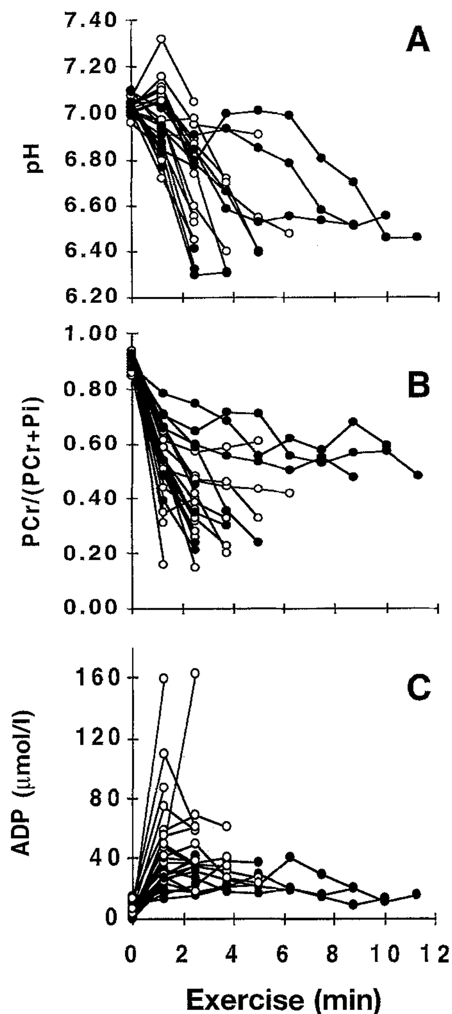

Fig. 2. (A–C) Changes in (**A**) intracellular pH, (**B**) phosphocreatine concentration (expressed as PCr/(PCr + Pi)) and (**C**) ADP concentration during finger flexor exercise. ○, Ponderal index < 23 kg/m^3; ●, ponderal index > 23 kg/m^3

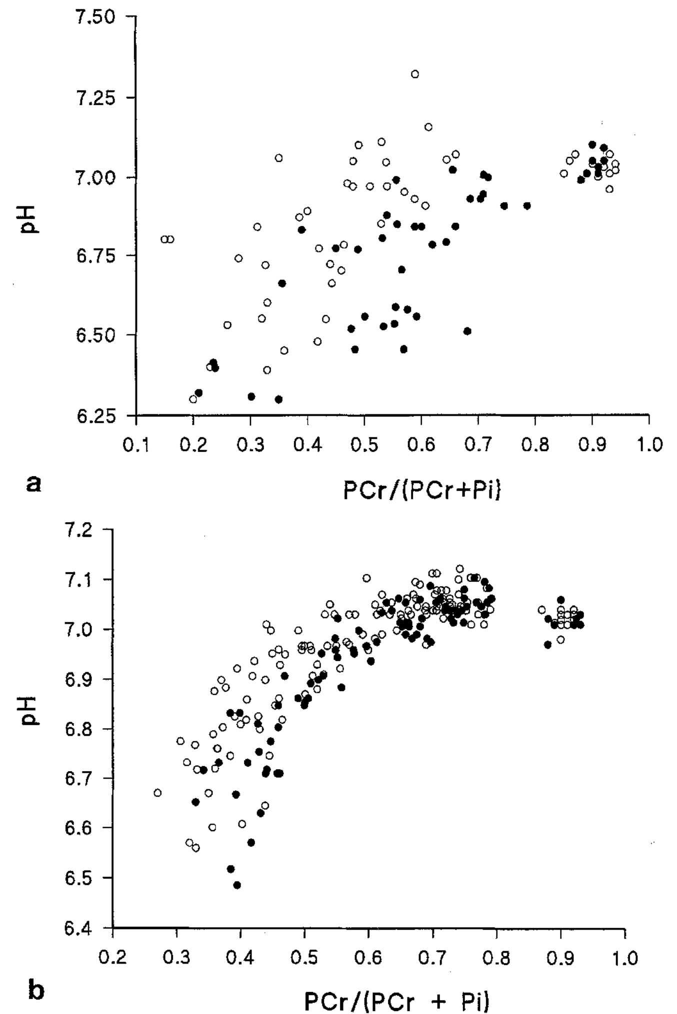

Fig. 3. (a, b) Simultaneous changes in intracellular pH and phosphocreatine (PCr/(PCr + Pi)) at rest and during (**a**) FDS and (**b**) gastrocnemius muscle exercise according to ponderal index at birth. ○, Ponderal index < 23 kg/m^3; ●, ponderal index > 23 kg/m^3. One point is shown for each subject at rest and for each min of exercise

cause PCr and ADP recovery after exercise are dependent on oxidative metabolism. As shown in Table 3, recovery half-times showed no consistent or significant differences between the two groups. The data were used to determine the maximum oxidative capacity of muscle by calculating the V_{max} for oxidative ATP production. The results were not significantly different between the low and high PI groups in either muscle.

Reoxygenation: The mean half-time for reoxygenation in FDS was significantly lower in the low PI subjects (14 vs 21 s, $p = 0.04$), suggesting that maximal arm blood flow was greater in this group. Reoxygenation half-time was also found to be significantly correlated with several of the MRS variables associated with exercise of the forearm: faster reoxygenation correlated with shorter exercise duration ($r = 0.53$, $p = 0.014$) and lower PCr and higher ADP concentrations during exercise ($r = 0.57$, $p = 0.0067$ and $r = -0.62$, $p = 0.0025$, respectively). There was no correlation between reoxygenation half-time and data from the recovery period shown in Table 3.

Fibre type and capillary density. Table 4 shows the distribution of fibre type and capillary density according to PI at birth. There was no difference between the groups in the percentage of type 1 fibres in the biopsies. The capillary density was increased in the low PI subjects, but this did not reach statistical significance ($p = 0.07$). The capillary to fibre ratio was similar in both groups.

Discussion

The results presented here demonstrate differences in the metabolism of adult skeletal muscle which correlate with PI at birth. The differences were specific

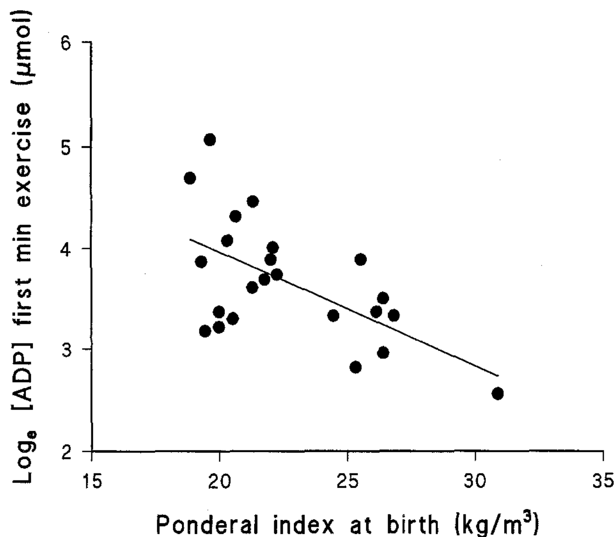

Fig. 4. Relationship between intracellular ADP concentration during the first min of FDS exercise and ponderal index at birth

to PI and did not correlate with the other birth measurements available for the women in the study. They were also independent of gestational age.

The major MRS findings during exercise were: (1) PCr depletion was greater in the low PI group but (2) the drop in pHi was not correspondingly greater, and (3) this resulted in a higher free ADP. This was seen at the start of exercise in finger flexors (in which the workload was heavy) but was only seen late in the gastrocnemius exercise (in which the workload was relatively much lighter at the beginning of the exercise but increased as the exercise progressed). In resting gastrocnemius the PCr/ATP ratio increased with decreasing PI (Table 1). This was not due to an increase in muscle creatine content because total creatine measured chemically in the muscle biopsy samples was not different in the two groups and there was no correlation with PI (low PI, 22.7 ± 5.1 μmol/g wet weight or 4.6 ± 1.4 nmol/mg protein; high PI, 20.4 ± 5.0 or 4.4 ± 1.3). It was not possible to measure ATP in the biopsy samples, but because the mean ratios of PCr/ATP, Pi/ATP, PME/ATP and PDE/ATP were all higher in the low PI group (although only PCr/ATP reached significance), it seemed likely that the absolute mean ATP concentration in gastrocnemius was lower in this group. High PCr/ATP has been reported for phosphorylase deficiency and hypothyroid muscle [19, 20] (in both conditions glycogen metabolism is affected), while in defects of oxidative metabolism PCr/ATP is low. If so, then assuming that the total creatine is not changed, the calculated ADP concentrations for gastrocnemius shown (e.g. Tables 1 and 2) are underestimated for the low PI group.

Table 4. Muscle fibre type, capillary and fibre density in the subjects

	Low PI	High PI	*p*-value
n	15	7	
Percentage type 1 fibres	69.9 ± 11	70.0 ± 8	0.90
Capillaries per mm^2	260 ± 30	231 ± 37	0.07
Fibres per mm^2	118 ± 27	106 ± 14	0.30
Capillary to fibre ratio	2.3	2.2	0.61

Data are mean ± SD

During exercise the faster depletion of PCr suggests that the rate of ATP production by other means is reduced with decreasing PI. This could be due either to decreased glycogenolysis/glycolysis or to decreased oxidative phosphorylation. It is not likely to be due to decreased muscle mass since there was no difference between estimated total muscle mass or arm muscle area between the groups and because for the gastrocnemius the load was proportional to lean body mass. Overall, the evidence is consistent with a lower rate of glycolytic ATP production. Firstly, a difference in PCr depletion was obvious under conditions in which glycolysis would be expected to contribute significantly to energy production) the beginning of the heavy finger flexor exercise and the end of the lighter gastrocnemius exercise). Secondly, if oxidative metabolism were decreased in the low PI group, slower metabolic recovery would be expected in the post-exercise recovery period because these processes rely on oxidative metabolism for ATP synthesis. ADP recovery was apparently slower in finger flexors of the low PI group, but it was normal in gastrocnemius, so there was no consistent evidence for

Table 3. Mean recovery after exercise according to ponderal index at birth. ($t_{1/2}$ of reoxygenation after limb occlusion/exercise is also shown)

	Flexor digitorum superficialis		*p*-value	Gastrocnemius		*p*-value
	Low PI	High PI		Low PI	High PI	
n	15	8		16	9	
PCr $t_{1/2}$ (s)	47 ± 29	46 ± 21	0.93	27 ± 9	21 ± 7	0.11
ADP $t_{1/2}$ (s)	16 ± 6	12 ± 3	0.10	10 ± 3	10 ± 3	0.81
PCr initial rate (mmol · l^{-1} · min^{-1})	18 ± 6	18 ± 6	0.92	25 ± 6	20 ± 4	0.06
V_{max} (mmol/ATP · l^{-1} · min^{-1})	32 ± 9	37 ± 9	0.27	39 ± 9	34 ± 6	0.14
Reoxygenation $t_{1/2}$ (s)	14 ± 8	21 ± 6	0.04	–	–	

Data are mean ± SD

abnormal recovery. In FDS, exercise duration was short for some of the low PI subjects, so blood flow may have been lower at the end of exercise than in the high PI subjects, thus tending to increase recovery times. Additionally, V_{max} values for oxidative ATP production calculated from these data are not different for the two groups, suggesting that there is no association between oxidative capacity and PI. During exercise, when glycogenolysis/glycolysis is activated, there is a decrease in pHi as lactic acid is produced. In these studies, the fall in pHi accompanying PCr depletion was not as great in the low PI group. This could have been due either to a lower rate of lactic acid production or an increased rate of proton elimination. In other studies we have shown that such changes can indeed be due to increased rate of proton efflux and that this is reflected in a more rapid recovery of pHi after exercise [17]. The recovery of pHi was the same in the two groups, suggesting that more rapid elimination of acid was not likely to be the cause of the relatively high pHi in exercise in the low PI group. These MRS findings are associated with a deficit in muscle function as shown by the shorter exercise duration achieved by the low PI subjects (Fig. 1). Although this has not previously been described, associations between insulin resistance and reduced exercise capacity have been reported [21].

It could be argued that the changes we observed reflect differences in blood flow to the tissues we studied. The near-infrared spectroscopy technique provides information about the rate of reoxygenation during recovery from exercise. There was no evidence that oxygen was limiting for oxidative ATP production because pH and ADP recovery times did not correlate with reoxygenation times. Unexpectedly, muscle which was quickly depleted of PCr (and thus had a short exercise duration) and which had the high ADP in exercise tended to reoxygenate fastest. In addition, subjects who had a low PI at birth had shorter reoxygenation times (Table 3) and evidence of a higher capillary density (Table 4). This is consistent with (although in the absence of data on oxygen consumption, it does not prove) an increase in the oxygen supply to the muscle during exercise, possibly an adaptation to the delayed or diminished glycogenolytic/glycolytic response to exercise. Increased ADP during exercise is characteristic of muscle which has a limited ability to produce ATP, such as in defects of glycogenolysis (phosphorylase or debranching enzyme deficiencies) and oxidative phosphorylation (mitochondrial myopathy). Coupled with an increased blood flow bringing additional oxygen, this high ADP might allow the muscle to increase ATP production during exercise by stimulating oxidative metabolism, helping to compensate for any deficit brought about by the decrease in glycolytic ATP synthesis.

Overall these results suggest that subjects who had a low PI at birth have normal muscle physiology if their metabolic demand can be supplied by oxidative metabolism. If, however, the metabolic demand is increased to a level which cannot be satisfied by oxidative processes, these subjects appear to be unable to meet the extra energy requirements by increasing glycolysis. The findings are unlikely to be due to decreased insulin action as insulin mediated glucose entry into cells does not appear to be rate limiting in glycolysis [22]. Moreover, there was no correlation between the NMR changes we observed and the measurements of insulin resistance in the subjects. The reduced rate of glycolysis in the low PI subjects was not due to glycogen deficiency as measurements of the glycogen content of the muscle biopsies were similar in the two groups (unpublished data). It is more likely that they represent subtle shifts in the control of the regulatory enzymes of glycogenolysis or glycolysis. Several of the hormones, for example growth hormone, or cortisol, which control substrate utilisation and metabolism in adult life also regulate fetal growth and maturation and respond to changes in fetal nutrition. Persisting changes in the levels of these hormones or in the tissue responses to them may explain the link between reduced fetal growth and adult muscle metabolism [23].

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