Defective regulation of energy metabolism in *mdx*-mouse skeletal muscles

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Our previous finding of a reduced energy metabolism in slow- and fast-twitch skeletal muscle fibres from the murine model of Duchenne muscular dystrophy (the *mdx* mouse) led us to examine the importance of intracellular glucose availability for a normal energy turnover. To this end, basal and KCl-stimulated (20.9 mM total extracellular K^+) rates of glucose uptake (GUP) and heat production were measured in isolated, glucose-incubated (5 mM) soleus and extensor digitorum longus muscles from *mdx* and control C57B1/10 mice, in the presence and in the absence of insulin (1.7 nM). Under all conditions and for both muscle types, glucose uptake values for *mdx* and control muscles were similar although heat production was lower in *mdx* muscles. The marked stimulation of GUP by insulin in both *mdx* and control muscles had only minor effects on heat production. In contrast, glucose deprivation or inhibition of glycolysis with 2-deoxy-D-glucose (5 mM) significantly decreased heat production in control muscles only, which attenuated, although did not suppress, the difference in basal heat production between *mdx* and control muscles. Stimulation of heat production by a short-chain fatty acid salt (octanoate, 2 mM) was significantly less marked in *mdx* than in control muscles. Increased cytoplasmic synthesis of CoA by addition of 5 mM pantothenate (vitamin B5) increased the thermogenic response to glucose more in *mdx* than in control muscles. We conclude that the low energy turnover in *mdx*-mouse muscle fibres is not due to a decrease of intracellular glucose availability, but rather to a decreased oxidative utilization of glucose and free fatty acids. We suggest that some enzyme complex of the tricarboxylic acid cycle or inefficiency of CoA transport in the mitochondria could be involved.

INTRODUCTION

Skeletal muscle fibres of the *mdx* mouse lack the plasma membrane-associated protein dystrophin. According to recent observations, they also exhibit abnormally high sarcoplasmic free Ca^{2+} concentrations (Turner et al., 1991), although not before showing dystrophic alterations (Head, 1993). Since about a quarter of the energy cost of muscle-fibre maintenance is devoted to sarcoplasmic free Ca^{2+} homeostasis in normal mice (Chinet et al., 1992) and the Ca^{2+}-clearance processes are apparently normal in *mdx* muscle cells (Takagi et al, 1992; Turner et al., 1991), we anticipated that the energy cost of sarcoplasmic Ca^{2+} homeostasis would be larger in *mdx* than in normal adult-mouse muscles. We found that, on the contrary, not only the energy cost of Ca^{2+} homeostasis but also the overall rate of energy expenditure was subnormal in slow- as well as fast-twitch *mdx* muscle fibres (Decrouy et al., 1993). Given that the muscles were incubated with the usual bicarbonate buffered Krebs-Ringer solution in which the only available substrate was glucose (5 mM), these results raised the question of whether *mdx* muscle fibres take up and/or oxidize intracellular glucose normally, and if so, whether glucose utilization and perhaps more generally energy metabolism would be impaired in *mdx*-mouse muscles. Since recent reports suggest that not only the tricarboxylic acid (TCA) cycle (Letellier et al., 1993; Chinet et al., 1994) but also some of the glycolytic steps (Pagliaro, 1993) are dependent on cytoskeletal organization (in which dystrophin undoubtedly partakes), we considered it a most important point to verify the extent to which energy metabolism might be affected in *mdx*-mouse muscles. Indeed, a defect of the energy metabolism consecutive to cytoskeletal disturbances can be considered to possibly participate in cell degeneration, as often observed in neurodegenerative illness (Beal, 1992).

In the present experiments, we therefore attempted to further investigate energy metabolism of isolated, intact skeletal muscle incubated *in vitro*. From experiments in which glucose uptake and heat production rates were measured under identical conditions, we attempted to assess more precisely the contribution of glucose to the maintenance of the energy metabolism in *mdx*-mouse muscles. The effects of insulin (stimulation of glucose uptake) and vitamin B5 (indirect stimulation of glucose oxidation via an increase of cytoplasmic CoA synthesis) were also investigated. Finally, free fatty acid (FFA) oxidation was also assessed using octanoate as a substrate.

Taken together, our results suggest that the *mdx*-mouse muscles suffer an important defect of their oxidative metabolism and raise the question whether this phenomenon may participate in the degenerative processes affecting muscles.

MATERIAL AND METHODS

Skeletal muscle preparations and experimental conditions

The non-perfused, intact muscle is a convenient tissue-cell preparation provided muscle shape, size, specific metabolic rate and environmental O_2 availability (therefore temperature) have been adequately selected to ward off alterations in the core of soleus muscles incubated at 37 °C (Van Breda et al., 1990). The experiments were thus performed at 30 °C (Chinet et al., 1992) on muscles maintained at approximately 130 % of their relaxed length by means of stainless-steel frames to which proximal and distal tendons of a pair of muscles were attached. Both soleus

Abbreviations used: GUP, glucose uptake; TCA, tricarboxylic acid; FFA, free fatty acid; 2DG, 2-deoxy-D-glucose; EDL, extensor digitorum longus.

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and extensor digitorum longus (EDL) muscle pairs were dissected out intact immediately after decapitation of the animal (male or female *mdx* or control C57Bl/10 mouse). Each mounted pair was then placed in the test chambers of twin microcalorimeters where it was continuously superfused with a Krebs-Ringer standard solution (mM: NaCl, 116.8; $NaHCO_3$, 25; KCl, 5.9; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $CaCl_2$, 1.27; glucose, 5) equilibrated at pH 7.4 under continuous gassing with a 95% O_2/5% CO_2 mixture (solution called Krebs–Ringer throughout the text). Practically complete equilibration of the perifusing medium with the gas phase was obtained with thin-layer tonometers. That the above experimental conditions allow for long-term maintenance of metabolic activity and physiological integrity of the preparations was judged from: (1) the typical decrease in basal heat production rate that is less than 0.3% per hour in muscles incubated in a purely electrolytic medium (Chinet et al., 1977); (2) the fact that following a coloration of muscles with periodic acid–Schiff reagent after 6 h incubation in the calorimeters, neither *mdx* nor control muscles exhibited an anoxic core (Decrouy, 1992); and (3) the fact that glucose uptake was strongly increased in response to a physiological concentration of insulin (1.7 nM) in the perifusing medium (Le Marchand-Brustel et al., 1978).

Measurements of heat production rate

Before each experiment, preparations were superfused for up to 4 h, i.e. as long as necessary for complete thermostatic equilibration of the microcalorimeters. Heat production rate (E) was computed from the voltage difference, induced by the muscles' heat production in the test chamber, between two series of six thermal gradient layers surrounding test and control chambers, minus a blank difference recorded before introduction of the preparation into the test chamber (for details see Chinet et al., 1977). The experimental tests were performed by modifying the composition of the Krebs-Ringer continuously pumped through the calorimeter chambers (addition/removal of glucose; increase of KCl to 20.9 mM; addition of 1 g/l 2DG; addition of insulin, etc.) during a test period of 40 min. The change of the rate of heat production induced by any given test was quantified during the new steady state observed over the last 20 min of the 40 min period.

Measurement of 2-deoxy-D-glucose (2DG) uptake

2DG uptake was measured in muscles incubated under the same conditions as those prevailing in the parallel calorimetric experiments. In particular, the preparations were first superfused at 30 °C with the Krebs-Ringer solution before the experiments themselves (the 3–4 h necessary for thermostatic equilibration of the microcalorimeters). The muscles of each pair were then separated, one muscle being incubated for an additional 20 min in 5 ml of the test medium (either Krebs-Ringer with 5 mM glucose or a potassium-enriched solution, i.e. extracellular K^+ raised to 20.9 mM at constant osmolarity and $[K^+][Cl^-]$ product, with 5 mM glucose), the other muscle being incubated in the same medium, to which insulin (1.7 nM) and BSA (2 g/l) had been added. This procedure allowed us to quantify the effect of insulin on 2DG uptake relative to the 2DG uptake measured in the contralateral muscle incubated in the absence of insulin. Interindividual variability of the non-insulin-dependent 2DG uptake was therefore corrected, which improved the assessment of the insulin-dependent component of 2DG uptake.

2DG uptake was quantified during a second 20 min period during which the muscles were transferred to a 2 ml chamber in which 2 μCi of tritiated 2DG ($^{1-3}$H-2DG, 18.3 Ci/mmol, Sigma) had been added to the glucose-containing medium. This second 20 min period coincided with the second 20 min half of the 40 min periods during which heat-production rates were measured in the calorimetric studies. Preliminary tests allowed us to verify that, under these experimental conditions, 2DG uptake was linear from 5 to 40 min.

Glucose uptake was thus assessed from the uptake of trace amounts of radiolabelled 2DG in a medium containing 5 mM glucose. Although several reports indicate that there is no discrimination between glucose and 2DG as far as skeletal-muscle glucose transporters are concerned (Ferré et al., 1985; Rennie et al., 1983), this procedure may still be discussed on the grounds of a difference of affinity of the carriers between glucose and 2DG. In practice, however, our parallel calorimetric measurements clearly indicated that replacement of glucose with 2DG very rapidly and intensely affected the energy metabolism of the incubated muscles (see Results section), so that with such a technique only very short incubation times (not compatible with the parallel microcalorimetric experiments) would have been possible. We therefore considered that, given our experimental conditions, the addition of traces of triated 2DG to a glucose-containing medium was the best compromise to assess glucose uptake.

At the end of the final incubation period, the muscles were rinsed 5 times in 5 ml of ice-cold saline in order to remove radiolabelling from the extracellular space, then freed from their tendons, blotted dry, weighed and dissolved in 0.5 ml of tissue solubilizer (BTS450, Beckman) overnight at room temperature. After complete solubilization, 10 ml of a liquid-scintillation mixture (Ready Organic, Beckman) including 0.7% glacial acetic acid was added. The radioactivity was then counted in a liquid-scintillation counter (Beckman, SL 7500) and corrected for quenching. Glucose uptake was quantified using as a reference the radioactivity measured in samples of freshly prepared dilutions of the incubation medium used in the experiments.

Statistical analysis

Since skeletal muscles of adult *mdx* mice, contrary to those of Duchenne muscular dystrophy patients and with the exception of the diaphragm (Stedman et al., 1991), exhibit only minimal infiltration (Tanabe et al., 1986; Anderson et al., 1987; Torres and Duchen, 1987; Coulton et al., 1988), all values are reported as means ± SEM normalized per g wet weight as measured at the end of the experiment. Heat-production rates were normalized in mW/g and glucose uptakes in mmol/g per 30 min. Means were compared using the Mann and Witney U-test for unpaired samples [calorimetric measurements and measurements of basal and KCl-stimulated glucose uptake (GUP)], glucose uptake and the sign test for paired samples (effects of insulin on basal and KCl-stimulated GUP) (Statgraphics Software).

RESULTS

Experiments in which heat production and glucose uptake were measured under comparable experimental conditions

It is known that mammalian striated muscles lose a significant part of their glycogen store during dissection at room temperature after the animal has been killed, or more generally during any period of energy dissipation in excess with respect to oxidative energy regeneration, and then recover it almost completely under superfusion in a glucose-containing medium (Fréminet et al., 1980; Chinet et al., 1977). In part of the present experiments (Table 1, E0), muscles had been incubated in the absence of exogenous glucose from the beginning of the experiment, that is, they had not been allowed to recover their complete glycogen

Table 1 Measurement of heat-production rates in *mdx*- and control-muscles

(A) Steady-state rates of specific heat production in *mdx*- and control-muscles during: (a) Incubation without glucose (E0); (b) Incubation with 5 mM glucose (E5); (c) Incubation with 5 mM glucose and stimulation of heat production by use of a 20.9 mM KCl concentration in the perifusing medium (EKCl) (see text). (B) Changes induced on heat production rates by addition of insulin (1.7 nM) in the perfusing medium: (a) in muscles incubated with 5 mM glucose ($dE5_{ins}$); (b) in muscles incubated with 5 mM glucose plus 20.9 mM KCl concentration in the perfusing medium ($dEKCl_{ins}$). All values (mean ± S.E.M., with the number of experiments in parentheses) are normalized in mW/g wet muscle weight. NS, no statistically significant difference

	Soleus		EDL	
	mdx	Control	*mdx*	Control
(A) Steady-state rates				
(a) Incubation without glucose (E0)	2.59 ± 0.08 (15)	2.99 ± 0.10 (19) $P < 0.01$	2.45 ± 0.13 (8)	2.18 ± 0.12 (8) NS
(b) Incubation with glucose (E5)	2.70 ± 0.13 (9)	4.27 ± 0.15 (16) $P < 0.01$	2.58 ± 0.18 (8)	2.62 ± 0.10 (11) NS
(c) Incubation with glucose (EKCl)	5.91 ± 0.27 (6)	10.66 ± 0.48 (7) $P < 0.01$	6.16 ± 0.30 (6)	9.47 ± 0.41 (7) $P < 0.01$
(B) Changes induced				
(a) Incubation with glucose ($dE5_{ins.}$)	0.18 ± 0.01 (5)	0.06 ± 0.02 (6) $P < 0.01$	0.09 ± 0.02 (5)	0.06 ± 0.03 (7) NS
(b) Incubation with glucose ($dEKCl_{ins.}$)	−0.02 ± 0.04 (5)	−0.56 ± 0.10 (7) $P < 0.01$	+0.14 ± 0.03 (5)	−1.19 ± 0.15 (7) $P < 0.01$

Table 2 Basal and KCl-stimulated rates of GUP in the presence or absence of insulin

Muscles were incubated with 5 mM glucose under all conditions. Mean values ± S.E.M. are in mmol/g per 30 min, with the number of experiments in parentheses. None of the eight comparisons of normalized GUP values between *mdx* and control revealed statistically significant differences.

	Soleus		EDL	
	mdx	Control	*mdx*	Control
Basal GUP	2.51 ± 0.23 (5)	1.97 ± 0.32 (5)	1.26 ± 0.25 (4)	1.13 ± 0.29 (5)
Basal GUP + insulin	6.54 ± 0.68 (5)	5.01 ± 0.46 (5)	2.48 ± 0.24 (4)	2.94 ± 0.27 (5)
KCl-stimulated GUP	6.01 ± 0.72 (5)	6.83 ± 0.69 (5)	4.43 ± 0.39 (5)	4.98 ± 0.37 (5)
KCl-stimulated GUP + insulin	7.22 ± 1.03 (5)	8.82 ± 0.94 (5)	5.55 ± 0.85 (5)	6.12 ± 0.43 (5)

store. The values obtained were compared with basal heat-production measured in muscles incubated with Krebs-Ringer containing 5 mM glucose from immediately after dissection (Table 1, E5), that is, in muscles that had been allowed to recover their complete glycogen store. E0 and E5 were both significantly larger in soleus, but not in EDL, muscles of control mice. In addition, E5 was significantly larger than E0 ($P < 0.01$) in control muscles only. This result showed that, contrary to what happens in control-mouse muscles, rates of energy production in the *mdx*-mouse were not dependent on the availability of extracellular glucose. This phenomenon was probably not due to an impaired intake of glucose by the muscles. Indeed, the measurements of glucose uptake under similar experimental conditions showed that glucose uptake was as large in *mdx* as in control muscles (Table 2, basal GUP). In particular, the amount of chemical energy (potential energy) provided by exogenous glucose in *mdx*-mouse muscles was larger than the actual rate of energy dissipation in the soleus (for which basal heat-production was most affected), and in the EDL it amounted to as high a proportion of basal heat-production as in control muscles (75 %) [(Table 3, row (c)].

It is known that insulin can have effects on metabolism other than those related to its action on glucose transport. For example, it can stimulate glucose conversion to 3-carbon compounds (DeFronzo et al., 1981) and increase the activity of the pyruvate dehydrogenase enzyme complex (Larner et al., 1979). To test whether insulin, either through such an effect or via the significant increase of intracellular glucose availability it induces, can affect energy expenditure differently in *mdx* than in control muscles, we measured the effects of insulin on heat production in the glucose-containing perfusing medium.

Addition of a small, physiological dose of insulin (1.7 nM) under basal conditions strongly increased glucose uptake in all muscles ($P < 0.01$) (Table 2, basal GUP + insulin), but in no case consistently affected heat-production rates (Table 1, $dE5_{ins.}$), suggesting that in all muscles the main fate of the additional glucose taken up by insulin stimulation was glycogen synthesis or lactate production. One should notice, however, that even if the effects of insulin on heat-production rates were of small amplitude, they were very reproducible and increased heat production significantly more in *mdx*- than in control-mouse soleus muscles. In the presence of insulin, glucose uptake provided a larger amount of chemical energy than was actually dissipated by heat production [Table 3, row (f)].

Sarcoreticular Ca^{2+}-release by KCl-induced sarcolemmal depolarization was used on muscles incubated with glucose as a means of increasing the steady-state rate of muscle-fibre energy utilization. The new steady states of heat production after KCl-

Table 3 Comparison of heat production rates (E) and insulin-induced changes in heat production rates (dE) (same values as in Table 1) with GUP and with insulin-induced changes in glucose uptake (same values as in Table 2 but glucose uptake is expressed here as an amount of chemical energy, i.e. the potential energy brought into the cells by glucose, which is 3.75 kcal/g)

In all cases, the muscles were incubated with 5 mM glucose and values are in mW/g wet muscle weight. The comparisons between heat-production and glucose-uptake rates are assessed in lines (c), (f), (i) and (l) as the difference between the amount of energy (potential energy) provided by glucose uptake and the energy actually dissipated by the muscles during the same time. This value is positive when the energy from glucose uptake is larger than total energy expenditure measured by direct calorimetry.

			Soleus		EDL	
			mdx	Control	*mdx*	Control
Basal conditions	(a)	E	2.7	4.3	2.6	2.6
	(b)	GUP	3.8	2.8	1.9	1.7
	(c)	(b−a)	1.0	−1.5	−0.7	−0.9
Basal conditions + insulin	(d)	dE	0.18	0.06	0.09	0.06
	(e)	dGUP	5.91	4.46	1.92	2.64
	(f)	(b+e)−(a+d)	6.83	2.9	1.13	1.68
KCl-stimulated conditions	(g)	E	5.9	10.7	6.2	9.5
	(h)	GUP	8.8	10.0	6.5	7.3
	(i)	(h−g)	2.9	−0.7	0.3	−2.2
KCl-stimulated conditions + insulin	(j)	dE	−0.02	−0.56	+0.14	−1.19
	(k)	dGUP	1.77	2.92	1.64	1.66
	(l)	(h+k)−(g+j)	4.69	2.78	1.80	0.65

induced sarcolemma depolarization (Table 1, E KCl) essentially reflected increased energy dissipation by intracellular Ca^{2+} homeostatic processes and cross-bridge cycling (a moderate contracture under 20.9 mM extracellular K^+ accounts for only a small part of the energy dissipated) (Chinet et al., 1992). The increase in heat production induced by KCl-stimulation of calcium recirculation was significantly smaller in *mdx* than in control muscles. This further increased the difference in heat production between *mdx* and control muscles (Table 1, EKCl versus E5). The KCl-induced rise in heat production was accompanied in all muscles by an increase in glucose uptake (Table 2, KCl-stimulated GUP). This increase was particularly marked in the case of the *mdx* soleus, whose rate of chemical energy uptake potentially covered 148 % of its energy dissipation rate (Table 3, row (i)]. Also in the *mdx*-mouse EDL muscles the increase in glucose-uptake was larger than the increase in the energy requirements since the negative balance between glucose uptake and heat production observed under basal conditions became positive under KCl stimulation [Table 3, row (i) versus row (c)].

Addition of insulin while heat production and glucose uptake were already increased by extracellular KCl further increased glucose uptake in all muscles (Table 2, KCl-stimulated GUP+insulin) but only poorly modified heat production in *mdx*-mouse muscles and slightly but significantly reduced heat production in control muscles (Table 1, $dEKCl_{ins.}$). Therefore, in the presence of insulin, the balance between the potential energy provided by the uptake of exogenous glucose and the rate of heat production became positive in all muscles [Table 3, row (l)].

Experiments in which only calorimetric measurements were performed.

Given that glucose uptake was at least as large in *mdx* as in control muscles, the above results strongly suggest that the activity of glycolysis and/or the TCA cycle are impaired in the *mdx*-mouse muscles. To study this possibility further, we measured the changes in heat-production rates induced by experimental manipulations likely to affect the activity of glycolysis and/or the TCA cycle.

Addition of 2DG in the perifusing medium

2DG is normally taken up by the cell and phosphorylated into 2DG-6-phosphate, and then enters glycolysis. However, 2DG-6-phosphate cannot be converted into 2-deoxyfructose-6-phosphate, which leads to inhibition of the phosphogluco-isomerase with consequent reduction of glycolysis (Brown, 1962). Using 2DG at a high concentration (5 mM) in the perifusing medium to strongly inhibit glycolysis, we verified the extent to which, under our experimental conditions, glycolysis participates in the maintenance of basal heat-production in muscles. This study showed that 2DG inhibited heat-production rates about four times more in control and in *mdx* muscles (Table 4, dE 2DG). Since our studies on glucose uptake (where 2DG was used only as a tracer) revealed that 2DG is indeed taken up by *mdx* as well as control muscles, the results suggest that heat-production rate is less dependent on glycolysis in *mdx* than in control muscles.

Addition of octanoate

To see whether the apparent impairment of basal glucose oxidation in *mdx*-mouse muscles was accompanied by some impairment of FFA oxidation or, on the contrary, could be partly compensated for by an increased oxidation of fatty acids, we tested the thermogenic response of the muscles to the addition to the incubation medium of the sodium salt of a short-chain fatty acid, octanoate (2 mM), a substrate whose catabolism is both independent of the anaerobic glycolytic pathway and little influenced by carbohydrate availability, at least in normal muscles (Fritz and Kaplan, 1960; Lossow and Chaikoff, 1955). As with glucose, the thermogenic response to exogenous octanoate was significantly reduced in *mdx*-mouse muscles, and this reduction

Table 4 Assessment of the contributions of glucose and octanoate (2 mM) utilizations to the rate of energy dissipation in *mdx* and control muscles

The assessment of glucose oxidation was done using the decrease of heat production induced by inhibition of glycolysis with 2DG. The inhibitory effect of 2DG (1 g/l) on E (dE2DG) was not reversed by the removal of 2DG. The capacity of the muscles to oxidize FFA was assessed by adding octanoate to the perifusate of muscles previously incubated either with 5 mM glucose or without glucose. The stimulatory effect of octanoate (2 mM) was rapidly reversed by the removal of octanoate under both conditions. Mean values ± S.E.M. (with the number of experiments in parentheses) are in mW/g wet muscle weight.

	Soleus		EDL	
	mdx	Control	*mdx*	Control
dE2DG	−0.13 ± 0.01 (4)	−0.47 ± 0.05 (4) $P < 0.01$	−0.06 ± 0.04 (4)	−0.24 ± 0.05 (4) $P < 0.05$
dEoctanoate	1.14 ± 0.10 (6)	1.75 ± 0.02 (5) $P < 0.01$	0.50 ± 0.05 (6)	0.69 ± 0.05 (5) $P < 0.05$
dEoctanoate (glucose-deprived)	1.23 ± 0.04 (11)	1.66 ± 0.14 (11) $P < 0.01$	0.53 ± 0.03 (11)	0.68 ± 0.04 (9) $P < 0.01$

Table 5 Effect of 5 mM pantothenate (vitamin B5) on the thermogenic response of muscles previously incubated in the absence of glucose to addition of 5 mM glucose in the perifusing medium

	Soleus		EDL	
	− Pantothenate	+ Pantothenate	− Pantothenate	+ Pantothenate
dEcontrol	0.713 ± 0.293	0.903 ± 0.385 NS	0.312 ± 0.073	0.496 ± 0.219 NS
dE*mdx*	0.216 ± 0.058	0.550 ± 0.321 $P < 0.05$	0.075 ± 0.057	0.272 ± 0.212 $P < 0.05$

did not depend on intracellular glucose availability (Table 4, dE octanoate).

Addition of pantothenate

The previous results lead us to attempt to improve the supply to the TCA cycle with the end products of glycolysis and lipolysis by simply stimulating with pantothenate (vitamin B5) (5 mM) the cytoplasmic synthesis of CoA and, indirectly, the mitochondrial concentration of CoA (see Tahiliani et al., 1992) and therefore the production of CoAHS. This experiment allowed us to increase glucose-induced heat production in all muscles, but this increase was significantly larger in *mdx* than in control muscles (Table 5). As a result, the response of *mdx* muscles was improved; from 30 % of the response of control muscles it increased to 60 % in the soleus, and from 25 % it increased to 50 % in the EDL.

DISCUSSION

No visible damage, nor any functional alteration of the calcium uptake processes of the sarcoplasmic reticulum in the *mdx* mouse have ever been confirmed (Turner et al., 1988), and we have shown in a previous article that only 45 % of the energetic defect under KCl stimulation could possibly be explained by a reduced rate of calcium recirculation (Decrouy et al., 1993). Therefore, when we first observed that basal heat-production rate was decreased in *mdx*-mouse muscles incubated in the presence of glucose as the only energy source (Decrouy et al., 1993), an attractive hypothesis was that a defective number, translocation process and/or conformation of the glucose carriers in *mdx*-mouse muscle fibres entailed a reduction of glucose uptake, which ultimately resulted in an adaptative decreased rate of energy production. However, recent reports by MacLennan et al. (1991) and Olichon-Berthe et al. (1993), together with the present results, converge to indicate that the rate of glucose uptake is normal in *mdx*-mouse muscles. Since in this study glucose uptake was normalized in the same way as heat production, the present results exclude the possibility that the lower specific heat-production rate in *mdx*-mouse muscles is the result of the normalization procedure. Therefore, the present results indicate that it is not a rate-limiting uptake of glucose but rather a primary energetic defect that underlies the reduction of both basal and KCl-induced heat production in *mdx*-mouse muscles.

On the other hand, McLennan et al. (1991) reported that rates of glucose uptake as well as lactate production by *mdx*-mouse muscles were not significantly different from those observed in control muscles. In contrast, the ratio of lactate produced to glucose taken up suggested that about 10 % of the glucose was fully oxidized in control muscles, while, in *mdx* muscles, the energetic equivalent of the lactate released matched the glucose taken up, suggesting that none of the glucose taken up was fully oxidized. In the present study, investigation of the energy-flow passing through glycolysis by inhibiting it with 2DG revealed that heat-production rate was inhibited by 11 % and 9 % respectively in the soleus and EDL muscles of control mice. These values were reduced to 4.8 and 2.3 % in the corresponding *mdx* muscles, suggesting, in agreement with the study of

McLennan et al, that glucose flow through glycolysis was strongly reduced in *mdx* muscles.

The reduced thermogenic effect of octanoate revealed that FFA oxidation was also impaired in *mdx*-mouse muscles. This result further indicated that the energetic defect was not specifically related to glucose utilization through the Embden–Meyerhof pathway, but resulted from metabolic processes shared by carbohydrate and FFA oxidation. In this respect, our observation that the increased availability of CoAHS induced by pantothenic acid stimulated heat production more effectively in *mdx* than in control muscles suggested that some enzyme complex of the TCA cycle or CoA transport to mitochondria could be impaired in *mdx*-mouse muscles. In addition, the difference in the thermogenic response to insulin between *mdx* and control muscles (despite an equal sensitivity of glucose transport), under basal as well as under KCl-stimulated conditions, further suggested that insulin-dependent mechanisms could be involved.

It is also interesting to notice that some important symptoms of the *mdx*-mouse muscles resemble those observed in muscles of subjects suffering severe food restriction or starvation. Indeed, glucose oxidation and ion pumping are decreased in these subjects' skeletal muscles (Henrikson, 1990), which entails an increase of intracellular Ca^{2+} (Russel et al., 1984). Weakening of muscular contraction following transformation of white fibres into red fibres, as observed in *mdx* EDL muscles, is also favoured. Finally protein breakdown is also stimulated (see Henrikson, 1990). These characteristics common to *mdx*-mouse muscles on one hand, and to normal muscles suffering a reduction of substrate supply on the other, are compatible with the general idea that a defect of energy production might progressively entail morphological changes and the increase of sarcoplasmic free Ca^{2+} in *mdx*-muscle fibres and, ultimately, fibre degeneration.

Finally, the question may be raised as to whether the absence of dystrophin in the membrane of the *mdx*-mouse muscles can lead to metabolic phenomena comparable to those observed in mitochondrial myopathies (Lucas-Heron et al., 1990), with even more severe consequences, since the resulting metabolic defect of energy metabolism may be suspected of participating in cell degeneration, a phenomenon also reported in neuronal disease associated with metabolic malfunction (Blass et al., 1988; Beal, 1992). According to us (see Chinet et al., 1994), the response is probably 'yes'. Indeed, many observations suggest that the efficiency of the TCA cycle critically depends on the complex organization of mitochondrial enzymic systems (e.g., Förster, 1988; Letellier et al., 1993), and various pieces of evidence can be interpreted to suggest that this organization might depend on that of the cytoskeleton. Not only mitochondrial but also glycolytic enzymes, which have usually been considered typical cytosoluble enzymes, are now recognized as being partly associated with cytoskeletal structures (Pagliaro, 1993). In conclusion, we believe that the present results point to the possible consequences of the lack of dystrophin in intracellular cytoskeletal organization of enzymic complexes, and, thereby, on the control of energy metabolism in the muscle cell.

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REFERENCES

Anderson, J. E., Ovalle, W. K. and Bressler, B. H. (1987) Anat. Rec. **219**, 243–257
Beal, M. F. (1992) Ann. Neurol. **31**, 119–130
Blass, J. P., Sheu, R. K.-F. and Cedarbaum, J. M. (1988) Rev. Neurol. **114**, 453–563
Brown, J. (1962) Metabolism **11**, 1098–1112
Chinet, A., Clausen, T. and Girardier, L. (1977) J. Physiol. (London) **265**, 43–61
Chinet, A., Decrouy, A. and Even, P. C. (1992) J. Physiol. (London) **455**, 663–678
Chinet, A., Even, P. C. and Decrouy, A. (1994) Experientia, **50**, 602–605
Coulton, G. R., Morgan, J. E., Partridge, T. A. and Sloper, J. C. (1988) Neuropathol. Appl. Neurobiol. **14**, 53–70
Decrouy, A. (1992) Thèse d'Université en Physiologie, Université de Poitier, France, pp. 143
Decrouy, A., Even, P. C. and Chinet, A (1993) Experientia **49**, 843–849
DeFronzo, R. A., Jacot, E., Jequier, E., Maeder, E., Warren, J. and Felder, J. P. (1981) Diabetes **30**, 1000–1007
Ferré, P., Leturque, A., Burnol, A-F., Penicaud, L. and Girard, J. (1985) Biochem. J. **228**, 103–110
Förster, M. E. (1988) J. Theor. Biol. **133**, 1–11
Fréminet, A., Leclerc, L., Pogart, C., Huel, C. and Gentil, M. (1980) J. Physiol. (Paris) **76**, 113–117
Fritz, I. B. and Kaplan, E. (1960) Am. J. Physiol. **198**, 39–44
Head, S. (1993) J. Physiol. (London) **469**, 11–19
Henrikson, J. (1990) Eur. J. Clin. Nutr. **44**(suppl.1), 55–64
Larner, K., Galasko, G., Cheng, K., De Paoli-Roach, A. A., Huang, L., Daggy, P. and Kellogg, J. (1979) Science **206**, 1408–1410
Le Marchand-Brustel, Y., Jeanrenaud, B. and Freychet, P. (1978) Am. J. Physiol. **3**, E348–E358
Letellier, T., Malgat, M. and Mazat, J-P. (1993) Biochem. Biophys. Acta **1141**, 58–64
Lossow, W. J. and Chaikoff, J. L. (1955) Arch. Biochem. Biophys. **57**, 23–40
Lucas-Heron, B., Schmidt, N. and Ollivier, B. (1990) J. Neurol. Sci. **95**, 327–334
MacLennan, P., McArdle, A. and Edwards, R. H. T. (1991) Biochem. J. **275**, 477–483
Olichon-Berthe, C., Gautier, N., Van Obberghen, E. and Le Marchand-Brustel, Y. (1993) Biochem. J. **291**, 257–261
Pagliaro L. (1993) NIPS **8**, 219–223
Rennie, M. J., Idström, J-P., Mann, G. E., Scherstén, T. and Bylund-Fellenius, A-C. (1983) Biochem. J. **214**, 737–743
Russell, D. McR., Atwood, H. L., Whittaker, J. S., Itakura, T., Walker, P. M., Mickle, D. A. G. and Jeejeebhoy, K. N. (1984) Clin. Sci. **67**, 185–194
Stedman, H. H., Sweeney, H. L., Shrager, J. B., Maguire H. C., Panattieri, N. A., Petrof B., Narusawa M., Leferovich J. M., Sladky, Y. and Kelly A. M. (1991) Nature (London) **352**, 536
Tahiliani, A. G., Keene, T. and Kaplan, R. S. (1992) J. Bioenerg. Biomembr. **24**, 635–640
Takagi, A., Kojima, S., Ida, M. and Araki, M. (1992) J. Neurol. Sci. **110**, 160–164
Tanabe, Y., Esaki, K. and Nomura, T. (1986) Acta Neuropathol. **69**, 91–95
Torres, L. F. B. and Duchen, L. W. (1987) Brain **110**, 269–299
Turner, P. R., Westwood, T., Regen, C. M. and Steinhardt, R. A. (1988) Nature (London) **335**, 735–738
Turner, P. R., Fong, P., Denetclaw, W. F. and Steinhardt, R. A. (1991) J. Cell. Biol. **115**, 1701–1712
Van Breda, E., Keizer, H. A., Glatz, J. F. C. and Geurten, P. (1990) Biochem. J. **267**, 257–260

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