

Duchenne muscular dystrophy: a model for studying the contribution of muscle to energy and protein metabolism

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Abstract – Duchenne muscular dystrophy (DMD) is associated with a dramatic muscle mass loss. We hypothesized that DMD would be associated with significant changes in both energy and protein metabolism. We studied the resting energy expenditure (REE) in DMD and control children using indirect calorimetry, and their protein metabolism using an intravenous infusion of leucine and glutamine labeled with stable isotopes. In spite of a 75 % muscle mass loss in the DMD children, the REE only decreased by 10 %. DMD was associated with increased leucine oxidation but neither protein degradation nor protein synthesis were different from that of the controls. In contrast, whole body turnover of glutamine, an amino acid mainly synthesized in the muscle, was significantly decreased. These studies emphasized the quantitatively poor contribution of muscle to energy and protein metabolism in children. The qualitative impact of muscle mass loss on amino acid metabolism (glutamine) offers a fascinating field of research for the next few years and has therapeutic potential. © Inra/Elsevier, Paris

Duchenne muscular dystrophy / resting energy expenditure / protein metabolism / glutamine / muscle

Résumé – **Dystrophie musculaire de Duchenne : un modèle pour l'étude de la contribution du muscle au métabolisme énergétique et protéique.** La myopathie de Duchenne de Boulogne (MDB) entraîne une fonte musculaire profonde. Comme le muscle représente 50 % de la masse métabolique active et la principale réserve protéique de l'organisme, la MDB devrait être associée à une baisse de la dépense énergétique de repos (DER) et du métabolisme protéique. Nous avons mesuré chez des enfants atteints de MDB et des enfants témoins la DER par calorimétrie indirecte et le métabolisme protéique à l'aide d'une perfusion intraveineuse d'acides aminés (leucine et glutamine) marqués par des isotopes stables. Malgré une perte de masse musculaire de 75 %, la DER n'est diminuée que de 10 % dans la MDB. Chez ces mêmes enfants l'oxydation de la leucine est augmentée mais ni la dégradation ni la synthèse protéique ne diffèrent des résultats obtenus chez les témoins. En revanche, le renouvellement plasmatique de glutamine, un

acide aminé principalement synthétisé dans le muscle, apparaît significativement diminué. Ces observations suggèrent que la participation du muscle dans le métabolisme énergétique et protéique global de l'enfant est modérée. Le métabolisme de certains acides aminés est modifié par la fonte musculaire et ouvre des voies de recherche avec des implications thérapeutiques potentielles. © Inra/Elsevier, Paris

myopathie de Duchenne de Boulogne / dépense énergétique de repos / métabolisme protéique / glutamine / muscle

1. INTRODUCTION

Muscle is a key organ in nutritional studies. In adults, muscle contributes up to 40 % of total body weight and 50 % of the metabolically active organs. Body proteins are mainly located in muscle. Nutritional diseases have a great impact on muscle mass and function. However, the way muscle mass loss may affect whole body energy and protein metabolism is poorly understood. In this paper we studied Duchenne muscular dystrophy (DMD) to help answer these questions, focusing on children.

DMD is the most frequent congenital muscle dystrophy and affects 1 in 3 500 male births. This X-linked disease is associated with a defect in dystrophin expression in the submembrane that leads to a dramatic muscle mass loss [17]. At the age of 10, DMD boys have only 25 % of the control children's muscle mass. The functional burden for the weakened muscles is major. Most of the time the affected chil-

dren lose ambulation near 10 years old and become dependent on a wheel-chair. DMD is also associated with nutritional problems. Obesity occurs in 50 % of cases when these boys lose ambulation, whereas multifactorial denutrition occurs later (decreased energy intake, recurrent sepsis, surgery). We studied energy and protein metabolism in DMD and control children.

2. RESTING ENERGY EXPENDITURE IN DMD

Our hypothesis was that muscle mass loss would decrease resting energy expenditure (REE) early in the time course of the disease. This might contribute to the onset of obesity that increases the burden of the disease.

REE was measured in 13 9–13-year-old DMD boys and 9 age-matched controls. The subjects' characteristics and estimated body composition are given in *table 1*. REE was significantly decreased

Table I. Children's characteristics in REE experiment [11].

	<i>n</i>	Weight (kg)	Height (cm)	W/A (%)	Muscle (kg)	FFM (kg)
Controls	9	30.2 ± 1.5	137 ± 3	96 ± 3	11.9 ± 1.5	25.3 ± 1.4
DMD	13	32.2 ± 2.7	133 ± 3	113 ± 6	3.3 ± 0.3	18.9 ± 2.2
<i>P</i>		NS	NS	NS	< 0.01	< 0.01

Results are means ± SEM; DMD: Duchenne muscular dystrophy; W/A: weight for age ratio according to Edwards' charts [9]; FFM: fat-free mass estimates should be analysed with caution since no method has been validated for body composition in DMD; P: significance of observed differences using non-parametric Mann-Whitney test.

in DMD children (*figure 1*); however, the actual magnitude of the decrease was surprisingly low in comparison to the dramatic muscle mass loss. Expressed per kg of fat-free mass, REE tended to be higher in the DMD group. Results should be analysed in relation to the relative contribution of the metabolically active organs to REE [18]. In fact, although muscle accounts for 35 % of total body weight in healthy children of that age, it represents only 15 % of REE. This contribution is small when compared to that of the brain which accounts for less than 5 % of total body weight but 35–40 % of REE [1]. Our data and those available in the literature suggest that muscle is a poor predictor of

REE in children. In adults, the muscle contribution to REE is higher (20–25 %); however, the relatively greater proportion of visceral to skeletal muscle mass, which is seen in malnutrition might also explain the apparently greater REE expressed per kg of fat-free mass [24].

3. WHOLE BODY PROTEIN TURNOVER IN DMD

Since most of the body protein is located in muscle, we expected a decreased rate of leucine turnover, an index of whole body protein turnover, in DMD children. We compared whole body

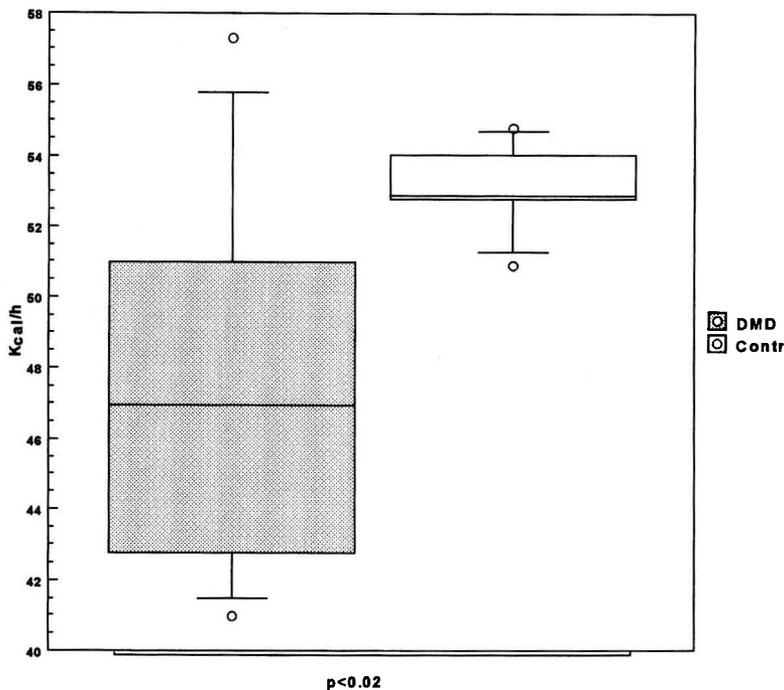


Figure 1. Resting energy expenditure in DMD [11]. DMD: Duchenne muscular dystrophy (grey bars); controls (open bars); box and whiskers plot, upper and lower limits of the box indicate, respectively, the 75th and the 25th percentile, upper and lower limits of the whiskers indicate, respectively, the 90th and the 10th percentile, extreme values appear out of these bounds, line indicates median value; significance of observed differences tested using non-parametric Mann-Whitney test.

leucine turnover in six 10–12-year-old DMD boys and in five sex, weight and height matched short stature controls. Leucine kinetics was determined from a primed, continuous infusion of leucine labelled with stable, non-radioactive, isotope (L-[1-¹³C]leucine) as previously described [21]. The patients' characteristics are given in *table II*.

It appears from *figure 2* that only irreversible leucine oxidation was higher in the DMD group; however, neither the whole body leucine turnover rate nor the non-oxidative leucine disposal, an index of whole body protein synthesis, were different between groups [13]. Once again these results might appear paradoxical with respect to the dramatic muscle mass loss. In contrast to its mass which is 40 % of the total body weight in adults and less in children, muscle protein turnover is low (1–2 % per day) [8]. In spite of their low contribution to total body weight, other organs such as the gut and the liver have a high protein turnover rate (35 and 20 %, respectively) [8]. As for REE, these data suggest that muscle plays a minor role in predicting whole body protein turnover, particularly in children.

The qualitative impact of muscle mass loss on whole body protein turnover rate should also be considered in addition to the quantitative point of view. Although considered as a non-essential amino acid, glutamine becomes a 'conditionally essen-

tial' amino acid in several pathologic conditions [20]. Glutamine is the most abundant free amino acid in the body [7] and is mainly synthesized in the muscle. It is a major gluconeogenic precursor [14, 23], and a major 'fuel' for tissues with a high cell turnover rate (gut, immune system) [6]. It also plays a central role in the regulation of protein turnover [19]. In fact, we have previously shown that a doubling of the plasma glutamine concentration was associated with a significant increase in protein synthesis estimates [10, 12]. Conversely, when plasma glutamine concentration is lowered following treatments with phenyl-butyrate, a glutamine chelator used in urea cycle defects, estimates of protein synthesis decrease significantly [3].

We studied glutamine exchange in the plasma of DMD boys and controls using glutamine labelled with a stable isotope (L-[2-¹⁵N]glutamine) using a previously described method [4, 5]. Glutamine exchange in the plasma appears lower in DMD when compared to controls (321 ± 22 versus $425 \pm 37 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, $P < 0.05$) [13]. The same results were obtained for alanine, another amino acid mainly synthesized in the muscle and a major gluconeogenic precursor [10]. The impact of such observations on protein metabolism is still poorly understood. DMD boys do not suffer from hypoglycemia [16]. This might be partly explained by their increased levels of ketone bodies and free

Table II. Children's characteristics in whole body protein turnover experiment [13].

	<i>n</i>	Age (years)	Weight (kg)	Height (cm)
Controls	5	12.7 ± 0.8	36.8 ± 6.0	136.0 ± 5.8
DMD	6	9.4 ± 0.7	27.8 ± 3.1	125.0 ± 2.6
<i>P</i>		0.02	NS	NS

Results are means ± SEM; DMD: Duchenne muscular dystrophy; *P*: significance of observed differences using non-parametric Mann-Whitney test.

fatty acid [22], and highlights the major protein sparing effect of fat [2].

In conclusion, muscle is a poor determinant of REE and whole body protein turnover in children. The qualitative impact of muscle mass loss on protein metabolism remains to be further explored. The long-term metabolic effect of decreased glutamine, alanine and other amino acids as well as the therapeutic potential [10] offer a fascinating field of research for the next few years.

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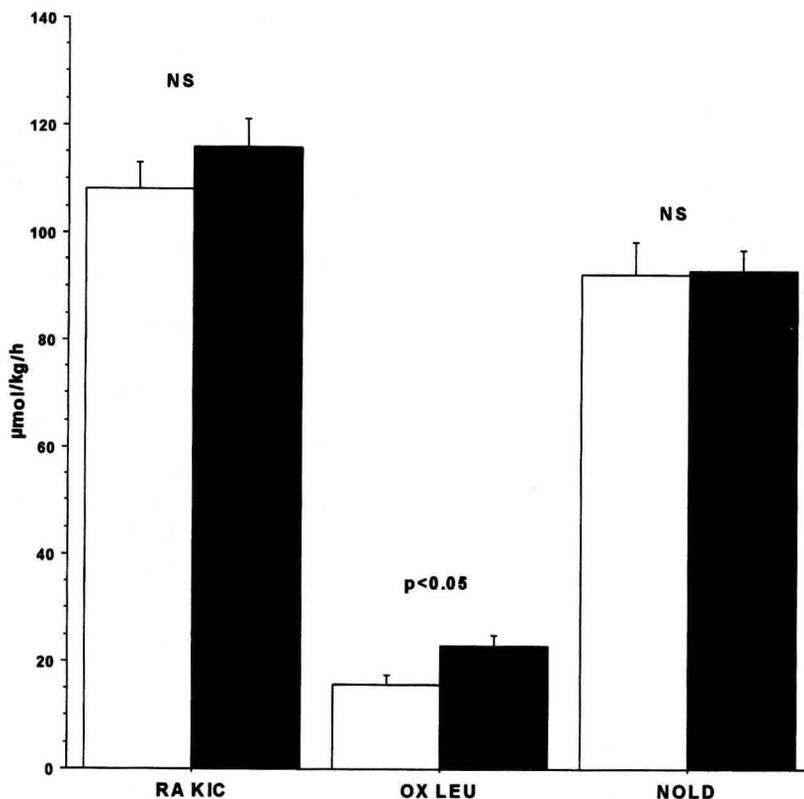


Figure 2. Whole body leucine kinetics in DMD [13]. Results are means \pm SEM; DMD: Duchenne muscular dystrophy; open bars (controls), solid bars (DMD); Ra,leu: whole body leucine turnover rate calculated from α -ketoisocaproic acid (KIC); Oxleu: irreversible leucine oxidation rate; NOLD: non-oxidative leucine disposal; significance of observed differences using non-parametric Mann-Whitney test.

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