Nutritional status evaluation in patients affected by Bethlem myopathy and Ullrich congenital muscular dystrophy

Silvia Toni¹, Riccardo Morandi¹, Marcello Busacchi¹, Lucia Tardini¹, Luciano Merlini², Nino Carlo Battistini¹ and Massimo Pellegrini¹*  
¹ Laboratory of Nutrition and Lifestyle, Department of Diagnostic, Clinical and Public Health Medicine, Modena, Italy  
² Laboratory of Musculoskeletal Cell Biology, Istituto Ortopedico Rizzoli, Bologna, Italy  
*Correspondence: Massimo Pellegrini, University of Modena and Reggio Emilia, Italy  
e-mail: massimo.pellegrini@unimore.it

INTRODUCTION
Mutations in the genes COL6A1, COL6A2, and COL6A3, coding for three α chains of collagen type VI, cause COL6-related myopathies (COL6-RM), including the severe Ullrich congenital muscular dystrophy (UCMD), the milder Bethlem myopathy (BM) (Bertini and Pepe, 2002; Allamand et al., 2010), and the Myosclerosis Myopathy in a single family (Merlini et al., 2008a,b).

The prevalence of UCMD and BM has been calculated as 0.13 per 100,000 and 0.77 per 100,000, respectively (Norwood et al., 2002; Mercuri et al., 2005). BM (MIM #158810) (Merlini et al., 1994) is characterized by axial and proximal muscle wasting and weakness with finger flexion contractures. BM is usually mild, sometimes slowly progressive (Pepe et al., 2002). BM has both dominant and recessive inheritance (Gualandi et al., 2009). Immunohistochemistry shows normal or mildly reduced levels of ColVI in the endomysium of most BM patients (Allamand et al., 2010). UCMD (MIM #254090) (Mercuri et al., 2005) is a severe congenital muscular dystrophy, characterized by early onset, generalized and rapidly progressive muscle wasting and weakness, proximal joint contractures, and distal joint hypermobility. Walking ability is rarely achieved or preserved during adolescence, and the rapid progression of the clinical symptoms usually leads to early death, due to respiratory failure (Mercuri et al., 2005). UCMD is caused both by recessive and de novo dominant mutations (Mercuri et al., 2005). ColVI appears to be strongly reduced or absent in muscle biopsies from UCMD patients.

A dystrophic mouse model, where collagen VI synthesis was prevented by targeted inactivation of the Col6a1 gene, allowed the investigation of the pathogenesis, revealing the existence of a Ca(2+)-mediated dysfunction of the mitochondria and sarcoplasmic reticulum and defective autophagy (Bernardi and Bonaldo, 2013). Similar defects contribute to the disease pathogenesis in patients, irrespective of the genetic lesion causing the collagen VI defect (Irwin et al., 2003; Grumati et al., 2010). These studies indicate that permeability transition pore opening and defective autophagy represent key elements of skeletal muscle fiber death, and provide a rationale for the use of cyclosporin A (Merlini et al., 2008a,b) and of nutritional interventions to correct defective autophagy (Merlini et al., 2014) in patients affected by COL6-RM, a strategy that holds great promise for treatment.

In the last decade, several studies have demonstrated that nutritional status and body composition are strictly related to clinical
outcomes and that nutritional intervention can be effective in the prevention and treatment of many diseases related to metabolism, bioenergetics, and even cancer.

According to a three-compartment model of body composition (Moon et al., 2008), total body weight is the sum of lean mass (LM), fat mass (FM), and bone mineral content (BMC). The LM, which includes the mass of the internal organs and that of the muscles, together with the BMC, form the fat-free mass (FFM), which represents the more metabolically active component of the human body. A "pathological" ratio between FFM and FM and/or an abnormal distribution of these components in the body, mainly trunk versus appendages, is found in many pathological conditions and correlates with the severity of the metabolic or energy status alteration; moreover, modifications of body composition per se may represent an independent health risk factor (Wohlfahrt et al., 2014). Augmented FM leads to a higher cardiometabolic risk and to a higher incidence of hypertension, diabetes (Rohan et al., 2013), and cardiovascular diseases.

Muscular dystrophies (MD) are characterized by progressive deterioration of muscle mass, muscle strength, and function. Resting energy expenditure (REE), which comprises 70% of daily energy needs, is determined by the amount and composition of the metabolically active fat-free mass (FFM). The reduced muscle mass and muscular activity, characteristic of MDs, could result in a significant parallel decrease in REE. Surprisingly, it was found that patients with Emery-Dreifuss MD (Vaisman et al., 2004) and Duchenne and Becker MD (Zanardi et al., 2003a; Gonzalez-Bermejo et al., 2005; Hogan, 2008; Elliott et al., 2012) may have increased energy expenditure. If not met with increased caloric intake, this greater energy expenditure may partially contribute to further deterioration in their muscle mass and function.

It has already been shown that patients with COL6-RM have reduced muscle mass, muscle strength, and muscle function (Mischone et al., 2013). To date, no data are available on the energy expenditure in COL6-RM. The aim of this study is to investigate the relationship between REE, body composition, and muscle strength in COL6-RM.

MATERIALS AND METHODS

PATIENTS

We analyzed eight adult patients (five women, three men, mean age 31 ± 9 years) with COL6-RM: seven had BM and one UCMD. The UCMD patient was never able to walk and was on nocturnal non-invasive mechanical ventilation. This study was approved by the institutional ethical committee of the Istituto Ortopedico Rizzoli (ClinicalTrials.gov identifier: NCT01438788). All subjects were fully informed about the study and gave their written informed consent.

BODY COMPOSITION

Nutritional status was evaluated throughout the study by both non-invasive and invasive techniques (El Ghoch et al., 2014). Body composition was obtained by DXA (Hologic 4500 W; software version 11.2; Hologic, Inc., Waltham, MA, USA) software, which provides regional and whole-body estimation of LM, FM, and BMC, according to the three-compartment model of body composition. FFM was calculated as the sum of LM and BMC, and has been provided for each body part (i.e., trunk and limbs) (Scaglioni et al., 2013). From these whole-body measures, the following derivative values were calculated: FMI (FM/height2), LM/height2, appendicular lean mass/height2 (ALMI). Appendicular lean mass (ALM) was the sum of bone-free and fat-free tissue masses in the arms and legs. Sarcopenic obesity was defined, according to Baumbergarter et al. (2004), as ALM divided by stature squared (ALMI) less than 7.26 kg/m² in men and 5.45 kg/m² in women and percentage body fat, derived by DXA, greater than 28% in men and 40% in women (Baumbergarter et al., 2004).

Anthropometric measurements included: body weight (Wt), height (Ht), and circumferences (waist, hip, waist-hip ratio (WHR)). All measurements were determined by the same operator following the Anthropometric Standardization Reference Manual recommendations (Lohman et al., 1988). Body mass index (BMI) was calculated as Wt [Kg/Ht(m²)]. We used BMI to categorized participants as obese (BMI ≥30), overweight (25 ≤BMI < 30), normal weight (18.5 < BMI < 25), or underweight ≤18.5.

ENERGY AND NITROGEN BALANCE

Resting energy expenditure was estimated by indirect calorimetry using a metabolic measurement cart with a canopy hood (CareFusion V max Encore, San Diego, CA, USA). Subjects were instructed to fast for 12 h and abstain from exercise for 24 h before the test (Mifflin et al., 1990). Before measuring REE, all subjects were asked to rest quietly in the supine position for approximately 30–40 min in an isolated room, with a temperature between 21° and 24°C. The criterion for a valid REE was 15 min of steady state, determined as a <5% variation in respiratory quotient (RQ)/minute and oxygen consumption/minute. Oxygen consumption and carbon dioxide production were used to calculate REE, in accordance with the Weir equation (Turell and Alexander, 1964). REE were also calculated with the equations based on weight, height, age, and sex [Harris-Benedict and Schofield (Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation, 1985; Roza and Shizgal, 1984)] using the free fat mass (FFM)-based predictive equations of Mifflin and Katch and McArdle (Mc Ardle et al., 1986; Mifflin et al., 1990).

Food intake was evaluated by a 7-day food diary and a dietitian interview (O’Connor et al., 2014). Nitrogen balance, an important tool for estimating adequate protein intake (Tarnopolsky et al., 1988), was calculated as the difference between nitrogen input (24 h dietary protein intake) and nitrogen output (24 h urinary urea nitrogen).

General and nutritional blood and urine laboratory analyses (albumin: transferrin; creatinine; uric acid; glucose; triglycerides; total, HDL, and LDL cholesterol; urinary creatinine; and nitrogen) were taken to assess the metabolic status of the patients. The 24-h urinary creatinine excretion value was used as an index of protein nutrition; the creatinine height index (CHI) and lean body mass was estimated from this value.

MUSCLE STRENGTH

A composite score (megascore) was calculated by summing the maximal force of eight physical tests (Bryan et al., 2003; Merlini et al., 2003) using a hand-held dynamometer (Type CT 3001, Citec, C.I.T. Technics BV, Groningen, The Netherlands) (Van der Ploeg
et al., 1991; Beenakker et al., 2001). Four muscle groups were examined bilaterally: hand grip, elbow flexors, knee extensors, and knee flexors (Merlino et al., 2002, 2003, 2004). Each individual muscle group was tested for at least 3 s using a “make” test (Merlino et al., 2004). The maximum force from three attempts was used in the analysis.

**STATISTICAL ANALYSIS**

Pearson correlation coefficients were calculated to study the association between different parameters. Statistical significance was set at 0.05. All analyses were conducted using the STATA software package for Windows 13.1 (Stata Corp, College Station, TX, USA).

**RESULTS**

**ANTHROPOMETRIC EVALUATION**

Anthropometric analysis showed that UCMD and BM patients have average BMI values in the range of normality, comparable with a healthy population (Janssen et al., 2002). Body circumference measurements do not show any significant variations from normality (Janssen et al., 2002) (Table 1).

**BODY COMPOSITION ANALYSIS**

Body composition analysis showed quantitative changes in all the partitions of the body. All the patients had a loss of muscle mass, as shown by a marked reduction of FFM, FFMI, and ALMI, and augmented FM, as indicated by % FM and FMI.

In particular, all patients were sarcopenic, based on ALMI, and seven were sarcopenic-obese, based on ALMI and % FM (Tables 2 and 3).

Moreover, from the DXA data (trunk-to-limb FM ratio) and anthropometric parameters (waist circumference and waist/hip ratio index), we can deduce that, in the two compartments, the increased fat tissue was equally distributed (DXA) compensating for the loss of muscle mass (circumferences).

Bone mineral content, the other component of the FFM, is also strongly reduced in these patients, especially in men. The data are confirmed by T-score calculations in our male patients (Male T-score $\leq -1.87$). T-score is a diagnostic for osteopenia (Kanis et al., 2013) (Tables 2 and 3).

**BLOOD, URINE BIOCHEMICAL ANALYSIS, AND NITROGEN BALANCE**

Blood and urine analysis do not show any specific pathological modification. In particular, although seven out of eight patients were obese, according to % fat determined by DXA, none had high levels of blood triglycerides, total cholesterol, HDL, or LDL (Jukema and Simoons, 1999). Blood creatinine levels were normal (Janssen et al., 2002) ($< 94$) (N.V. $< 94$). Blood and urine analysis do not show any specific pathological modification. In particular, although seven out of eight patients were obese, according to % fat determined by DXA, none had high levels of blood triglycerides, total cholesterol, HDL, or LDL (Jukema and Simoons, 1999). Blood creatinine levels were normal (Janssen et al., 2002) ($< 94$) (N.V. $< 94$).
Table 3 | Body composition values obtained by anthropometric and DXA analyses, in BM and UCMD patients, compared to a healthy population

| ID  | Sex | Age (years) | BMI (Kg/m²) | Fat % | FFM% | BFMI (Kg/m²) | Trunk to limbs FM ratio | ALMI (Kg/m²) |
|-----|-----|-------------|-------------|-------|------|--------------|------------------------|--------------|
| Pt. 1 | W  | 48          | 20.9        | 49.90↑| 50.1↓| 10.10↓       | 0.88↓                  | 3.60↓        |
|      |     | N.V. (18.5–24.9) | N.V. (39.8–40.8) | N.V. (59.2–60.2) | N.V. (10.72–11.20) | N.V. (0.92–0.947) | N.V. (6.93–6.9)          |
| Pt. 2 | W  | 19          | 22.10       | 53.50↑| 46.5↓| 11.80↑       | 0.83↑                  | 3.48↓        |
|      |     | N.V. (18.5–24.9) | N.V. (35.1) | N.V. (64.8) | N.V. (8.48) | N.V. (0.745) | N.V. (6.81)               |
| Pt. 3 | W  | 42          | 18.19       | 41.30↑| 58.7↓| 7.53↓        | 0.80↓                  | 3.81↓        |
|      |     | N.V. (18.5–24.9) | N.V. (38.9–39.8) | N.V. (60.2–62.1) | N.V. (10.27–10.72) | N.V. (0.897–0.920) | N.V. (6.95–6.93)          |
| Pt. 4 | W  | 29          | 24.60       | 55.00↑| 45↓  | 13.50↑       | 0.86↑                  | 4.94↑        |
|      |     | N.V. (18.5–24.9) | N.V. (38–37) | N.V. (63–64) | N.V. (8.9–9.35) | N.V. (0.796–0.841) | N.V. (6.86–6.9)           |
| Pt. 5 | W  | 22          | 29.44       | 58.50↑| 41.5↓| 17.40↑       | 0.73↓                  | 4.93↓        |
|      |     | N.V. (18.5–24.9) | N.V. (35.1–36) | N.V. (64–64.9) | N.V. (8.48–8.9) | N.V. (0.745–0.796) | N.V. (6.81–6.86)          |
| Pt. 6 | M  | 36          | 23.29       | 44.20↑| 45.8↓| 10.30↑       | 1.14↑                  | 5.05↓        |
|      |     | N.V. (18.5–24.9) | N.V. (26.6–27.5) | N.V. (72.5–73.4) | N.V. (7.19–7.57) | N.V. (1.125–1.183) | N.V. (8.09–9.12)           |
| Pt. 7 | M  | 27          | 18.76       | 18.30↓| 81.7↑| 3.34↓        | 1.15↑                  | 6.35↓        |
|      |     | N.V. (18.5–24.9) | N.V. (24.60–25.70) | N.V. (74.3–75.4) | N.V. (6.37–6.78) | N.V. (0.995–1.063) | N.V. (8.94–9.02)            |
| Pt. 8 | M  | 28          | 26.13       | 41.40↑| 58.6↓| 10.90↑       | 1.08↑                  | 5.81↓        |
|      |     | N.V. (18.5–24.9) | N.V. (24.60–25.70) | N.V. (74.3–75.4) | N.V. (6.37–6.78) | N.V. (0.995–1.063) | N.V. (8.94–9.02)            |

The table shows the body composition values of our subjects (M, men and F, women) compared to healthy values reported in the literature (Kelly et al., 2009; Wang et al., 2010). We used: ↑ when the values were increased; ↓ when the values were low.

RESTING ENERGY EXPENDITURE AND RESPIRATORY QUOTIENT

Resting energy expenditure was measured by indirect calorimetry and estimated by specific predictive equations. The REEs estimated by the equations based on weight, height, age, and sex accurately predicted the same values measured with indirect calorimetry [Harris-Benedict and Schofield ("Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation," 1985; Roza and Shizgal, 1984)]. REE was instead severely underestimated using the FFM-based predictive equations of Mifflin and Katch and McArdle (McArdle et al., 1986; Mifflin et al., 1990). Hence, considering that these patients are characterized by a reduced FFM in kilograms, we can deduce that there is a relative hypermetabolic state (Müller, 2007) with a higher ratio of REE/FFM (kg) (Figure 1).

Measured RQs values are indicative of a mixed nutrient-based metabolism. However, there was a sex difference concerning the type of substrates utilized. While male patients had a higher carbohydrate-based metabolism (57.67 ± 14.57% carbohydrates), women showed a higher lipid-based metabolism (56.00 ± 16.39% lipids) (Table 5) (Figure 2). There was a strong positive correlation between the quantity of FFM in kg and the percentage of carbohydrates metabolized during REE. This correlation, however, was negative for the percentage of lipids metabolized. No correlation was found between the percentage of proteins used as a metabolic substrate and FFM.

All in all, patients are characterized by an augmented REE per kilogram of FFM; additionally, subjects with higher FFM values metabolize more carbohydrates and less lipids than the ones with minor FFM levels (Figure 3).

MUSCLE STRENGTH

Megascores expressed as the sum of the muscle strength of eight different tests, were 1093.33 (±306.37) Newton in men and 572.60 (±233.11) in women. These values were markedly reduced, as muscle strength was low in all muscle groups, compared with the normative values (Van der Ploeg et al., 1991;Beenakker et al., 2001).

There was a strong correlation between muscle strength, expressed as Megascore, and the various indices of muscle mass, but no correlation with the indices of body fat. A strong correlation was found between Megascore and blood creatinine. This correlation was even stronger with urinary creatinine and the metabolic substrate and FFM.

This trend is maintained in the correlation between the Megascore and both trunk and limbs FFM. However, the linear correlation coefficient was higher between Megascore and appendicular FFM than Megascore and Trunk FFM (Figure 5). FFM% also correlated with Megascore, but the correlation was weaker.

Table 3 | Body composition values obtained by anthropometric and DXA analyses, in BM and UCMD patients, compared to a healthy population.
No correlation was found any between the Megascore and the indices of fat mas, FM in kilograms, FM percentage, or REE/FM ratio.
FIGURE 2 | UCMD and BM patients’ metabolism. Percentage of Carbohydrates (CHO), Lipids (L) and Proteins (P) utilized during indirect calorimetry examination. These values were calculated from respiratory quotients and urine nitrogen measurement [Livesey and Elia, 1988]. Mean values in men (left) and women (right) are reported.

FIGURE 3 | Collagen VI myopathies: FFM metabolism. (Left) Linear correlation between FFM in kg and percentage of carbohydrates used as metabolic substrate (correlation coefficient $R = 0.89$ and $P < 0.01$), and (right) correlation between FFM and percentage of lipids used as metabolic substrate (correlation coefficient $R = -0.87$ and $P = 0.01$). White dots represent men and black dots represent women. The percentage of substrate utilized was calculated using the respiratory quotient and urinary urea nitrogen [Livesey and Elia, 1988]. Data from one BM were missing.

FIGURE 4 | Muscle strength and functional body composition. As expected there was no correlation between Megascore and REE/FM, but instead a strong correlation between Megascore and the REE/FFM ratio ($R = -0.94$, $P < 0.001$) (right).

with the new concept of functional body composition [Müller et al., 2009].

COL6-RM patients showed a REE, analyzed by indirect calorimetry, in the range of normality, despite the severe reduction of the FFM. Predictive formulas estimate REE values in line with what we actually have found by indirect calorimetry. Applying the concept of functional body composition and relating FFM to energy expenditure, we found a clear deviation from normality.
FIGURE 5 | Muscle strength and body composition. Correlation between appendicular LM (A) and Trunk LM (B) with the Megascore (respectively for limbs and trunk, $R = 0.87 \ (P < 0.01)$ and $R = 0.80 \ (P < 0.05)$). (C) Correlation between total FFM in kg and Megascore ($R = 0.84, \ P < 0.01$).

FIGURE 6 | Muscle strength and creatinine. (A) Correlation between Megascore and blood creatinine ($R = 0.94, \ P = 0.001$), or (B) urine creatinine (UC) ($R = 0.96, \ P < 0.001$). (C) Correlation between Megascore and CHI (Rosenfalck et al., 1994) ($R = 0.93, \ P = 0.001$).

(metabolic disequilibrium) with a considerably augmented REE per kilogram of FFM. Our assumptions are supported by the fact that REE, estimated through FFM-based equations, clearly underestimate the effective metabolism of these patients by 25–30% (Figure 1).

If we compare the metabolism predicted by FFM-based-formulas with the values of REE, adjusted for body composition, we find that the FFM of these patients is overworking or in a hypermetabolic state (Müller, 2007). A similar metabolic alteration has been reported by Zanardi et al. in Duchenne patients (Zanardi et al., 2003b). It has been considered an enduring enigma: why is the ratio of REE to metabolically active tissue mass, expressed as the REE/FFM ratio, greater in magnitude in subjects with a small FFM than in subjects with a large FFM? (Heymsfield et al., 2002).

In COL6-RM patients, FFM requires more energy than in healthy subjects, and because FFM is made up of muscle and internal organs, we can only speculate as to which of these two components presents a hypermetabolic state. In particular, the augmented REE/FFM ratio could be due to a non-physiological increase in visceral organ metabolism, or to an altered energy expenditure of muscle cells. However, even if our study cannot
completely answer this question, all evidence suggests that the second hypothesis is the right one.

If we consider the pathological alteration of muscle structures, underlined in mouse model studies (De Palma et al., 2013), with the loss of muscular protein stability leads to a loss of efficiency, and additional energy is necessary to maintain muscle cell homeostasis. Even if this theory has been demonstrated in other studies no one had ever pointed out that this behavior is strictly correlated with the severity of the disease. Our hypothesis is that, in a dystrophic environment, the energy that the muscle has to spend in order to maintain its function is inversely proportional to the total muscular mass. Moreover, if the augmented REE/FFM were due to an increased metabolism of the visceral organs, we should have found some alterations in bio-humoral parameters (Gelfand et al., 1987; Izamis et al., 2012). On the contrary COL6-RM subjects do not show any important variation in blood or urine biochemical assays.

Additionally, there was no correlation between the REE/FFM and the clinical status portrayed by Megascore analysis (Figure 4). Therefore, we can exclude increased adipocytokines energy expenditure as a source of the hypermetabolic state. If we consider the pathological alteration of muscle structures, underlined in mouse model studies (De Palma et al., 2013), with the loss of muscular protein stability leads to a loss of efficiency, and additional energy is necessary to maintain muscle cell homeostasis. Even if this theory has been demonstrated in other studies no one had ever pointed out that this behavior is strictly correlated with the severity of the disease. Our hypothesis is that, in a dystrophic environment, the energy that the muscle has to spend in order to maintain its function is inversely proportional to the total muscular mass. Moreover, if the augmented REE/FFM were due to an increased metabolism of the visceral organs, we should have found some alterations in bio-humoral parameters (Gelfand et al., 1987; Izamis et al., 2012). On the contrary COL6-RM subjects do not show any important variation in blood or urine biochemical assays.

Finally, recent discoveries about mitochondrial metabolism (Bernardi and Bonaldo, 2008; Shaham et al., 2010) offer a possible biochemical explanation of the increased REE/FFM ratio. In particular, Bernardi et al. have found important alterations in mitochondrial membrane permeability due to the CoA6 mutation. The lack of Collagen VI causes increased transient openings of the Permeability Transition Pore ion channel in the mitochondrial inner membrane, with consequent mitochondrial depolarization and energy dissipation. This leads to a switch of the ATP synthase into an ATP hydrolase with a progressive impairment of respiration which may be responsible for the augmented REE spent by the muscular mass. This pathophysiology mechanism may explain our findings.

We also discovered that the metabolic substrates consumed by these patients are strictly related to their FFM (Figures 2 and 3). In particular, patients with higher FFM have a carbohydrate-based metabolism, while the ones with lower FFM prevalently use fatty acids as metabolic substrates. These findings suggest that patients with a relatively greater FFM have more muscular mass, and consequently an increased glycogen reserve to be used, compared to patients with lower FFM (Tsujino et al., 2000). On the other hand, the fat-based metabolism is explained by the increasing fat infiltration with disease progression; the depletion of muscular mass and the correlated decrement in glycogen storage lead to a metabolic shift toward burning fatty acids, whose reserves increase and infiltrate muscular tissue (Tagliavini et al., 2014). These evidences suggest that the worsening of the pathology is closely correlated to important changes in muscle metabolism.

Another significant result is the correlation between FFM and muscle strength, summarized by the Megascore (Figure 5). Other studies have previously demonstrated that Megascore is a good indicator of patients’ muscular efficiency, paralleling muscle function (Merlini et al., 2002, 2004). Hence, the correlation between muscle Megascore and FFM is more a portrait of the muscular mass and its efficiency, rather than of visceral organ activity. The strong correlations between Megascore and appendicular LM, where the non-muscle component of FFM is minimal, support this hypothesis.

This is a perfect expression of the concept of functional body composition: in COL6-RM, a pure body composition parameter like appendicular LM is directly correlated to muscular strength (Megascore) and contributes to the diagnosis of sarcopenia, a condition in which the loss of skeletal muscle mass is associated with lower muscle strength and function (Abellán Baumgartner et al., 1998; van Kan et al., 2012).

Creatinine is derived from the metabolism of creatine, which is transformed into phosphocreatine and used by muscles as an energetic substrate (Hoagland et al., 1945). A higher production of creatinine is linked to a higher use of phosphocreatine and, consequently, to greater muscular efficiency. In order to confirm this hypothesis, and to exclude any artifact due to individual body composition, we have analyzed the CHI, which evaluates urinary creatinine levels by normalizing the individual differences among subjects (Rosenfalk et al., 1994). Even in this case, the CHI scores perfectly correlate to the muscle strength, as evaluated by Megascore (Figure 6).

Hence, even if Franciotta et al. (2003) declare that urinary creatinine is not a good predictive indicator of skeletal muscular mass in Duchenne dystrophy; our results suggest that it is a good indicator of muscular performance.

Another important correlation is the one between blood creatinine and Megascore. Even if we have to consider renal filtration, blood creatinine gives an instant picture of the muscle metabolism (Baxmann et al., 2008). The probability of finding circulating creatinine is directly proportional to the quantity of phosphocreatine produced and used by muscle cells. Considering our results about urinary creatinine and CHI, this finding confirms the previous ones.

All in all, in this study, we have pointed out the importance of a nutritional approach to genetically based pathologies, such as UCMD and BM diseases. Additionally, we have underlined the necessity of a functional body composition analysis, which could be a powerful clinical tool for patients’ follow-up and prognosis.

The main limit of our study is represented by the scant number of recruited patients, caused by the rarity of these pathologies; hence, our conclusions should be confirmed by the analysis of a wider sample of subjects.

Our results confirm and complete what has been reported in the literature about collagen VI myopathies, further supporting the rationale for nutritional interventions aimed at correcting the metabolic imbalance and maintaining the patient’s muscular mass.

**AUTHOR CONTRIBUTIONS**

Silvia Toni made substantial contributions to acquisition of the data, carried out the nutritional evaluation, and performed the statistical analysis. Riccardo Morandi and Marcello Busacchi substantially contributed by discussing the data, writing the manuscript, and performing statistical analysis. Lucia Tardini contributed to the acquisition of data and carried out the anthropometric evaluation. Luciano Merlini conceived the study and participated in its design and coordination. Nino Carlo Battistini evaluated the
body composition data. Massimo Pellegrini participated in the design of the study, contributed to the statistical analysis, and has contributed substantially in the interpretation of data.

ACKNOWLEDGMENTS

This study was supported by Grant no. GUP11007 (Luciano Merlini) from Telethon, Italy.

REFERENCES

Allamand, V., Merlini, L., Bushby, K., and Consortium for Collagen VI-Related Myopathies. (2010). 16thth ENMC international workshop on collagen type VI-related myopathies, 22–24 May 2009, naarden, The Netherlands. Neuromuscul. Disord. 20, 346–354. doi:10.1016/j.nmd.2010.02.012

Ballesteros, M., Cortes, A., Arleaga, C. I., Puerto, R., and Bojac, B. (1994). [The usefulness of serum creatinine levels in identifying hospital malnutrition]. Nutr. Hosp. 9, 186–196.

Baumgartner, R. N., Koeblar, K. M., Gallagher, D., Romero, L., Heymsfield, S. B., Ross, R. R., et al. (1998). Epidemiology of sarcopenia among the elderly in New Mexico. Am. J. Epidemiol. 147, 755–763. doi:10.1093/oxfordjournals.aje.a009520

Baumgartner, R. N., Wayne, S. J., Waters, D. L., Janssen, I., Gallagher, D., and Morley, J. E. (2004). Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly. Obes. Res. 12, 1995–2004.

Bazan, A. C., Ahmed, M. S., Menoz, N. C., Menoz, V. B., Pereira, A. B., Kirsztajn, G. M., et al. (2003). Can we eliminate placebo in ALS clinical trials? Eur. J. Neurol. 10, 346–354. doi:10.1016/j.ejnn.2002.10.012

Janssen, I., Katzmarzyk, P. T., and Ross, R. (2002). Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines. Arch. Intern. Med. 162, 2074–2079. doi:10.1001/archinte.162.18.2074

Jarrya, M., Quijano-Roy, S., Monnier, N., Béhin, A., Avila-Smirnov, D., Romero, N. B., et al. (2012). Whole-body muscle MRI in a series of patients with congenital myopathy related to TPM2 gene mutations. Neuromuscul. Disord. 22(Suppl. 2), S137–S147. doi:10.1016/j.nmd.2012.06.037

Jekena, J. W., and Simoons, M. L. (1999). Treatment and prevention of coronary heart disease by lowering serum cholesterol levels; from the pioneer work of C.D. de Langen to the third “Dutch consensus on cholesterol.” Acta Cardiol. 54, 163–168.

Karlis, J. A., McCloskey, E. V., Johansson, H., Cooprer, C., Rizzioli, R., and Regnister, J. Y. (2013). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos. Int. 24, 23–57. doi:10.1007/s00198-012-2074-y

Kenny, T. L., Wilson, K. E., and Heymsfield, S. B. (2009). Dual energy X-ray absorptiometry body composition reference values from NHANES. PLoS ONE 4:e56716. doi:10.1371/journal.pone.0056716

El Ghoch, M., Milanese, C., Calugi, S., Pellegrini, M., Battistini, N. C., and Dalle Grumati, P., et al. (2010). Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myotubal degeneration. Nat. Med. 16, 1313–1320. doi:10.1038/nm.2247

Gualandi, F., Urciuolo, A., Martoni, E., Sabatelli, P., Squarzoni, S., Bovolenta, M., et al. (2009). Autosomal recessive Bethlem myopathy. Neurology 73, 1883–1889. doi:10.1212/wnl.0b013e3181c3f2a

Heymsfield, S. B., Gallagher, D., Kotler, D. P., Wang, Z., Allison, D. B., and Heshka, S. (2002). Body-size dependence of resting energy expenditure can be attributed to nonenergetic homogeneity of fat-free mass. Am. J. Physiol. Endocrinol. Metab. 282, E132–E138.

Hoagland, C. L., Gilder, H., and Shank, R. E. (1945). The synthesis, storage, and excretion of creatine, creatinine, and glycosamine in progressive muscular dystrophy and the effects of certain hormones on these processes. J. Exp. Med. 81, 423–438. doi:10.1084/jem.81.5.423

Hogan, S. E. (2008). Body composition and resting energy expenditure of individuals with Duchenne and Becker muscular dystrophy. Can. J. Diet. Pract. Res. 69, 208–212. doi:10.3148/69.4.2008.208

Irwin, W. A., Bergamin, N., Sabatelli, P., Reggiani, C., Meghiaian, A., Merlini, L., et al. (2013). Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. Nat. Genet. 35, 367–371. doi:10.1038/ng.270

Izamis, M. L., Uygur, K., Sharma, N. S., Uygur, B., Yarmsh, M. L., and Berthiaume, F. (2012). Development of metabolic indicators of burn injury: very low density lipoprotein (vldl) and acetoacetate are highly correlated to severity of burn injury in rats. Metabolites 2, 458–478. doi:10.3390/metabolites2030048

Jukema, J. W., and Simoons, M. L. (1999). Treatment and prevention of coronary heart disease by lowering serum cholesterol levels; from the pioneer work of C.D. de Langen to the third “Dutch consensus on cholesterol.” Acta Cardiol. 54, 163–168.

Kanis, J. A., McCloskey, E. V., Johansson, H., Cooper, C., Rizzioli, R., and Regnster, J. Y. (2013). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos. Int. 24, 23–57. doi:10.1007/s00198-012-2074-y

Kelly, T. L., Wilson, K. E., and Heymsfield, S. B. (2009). Dual energy X-ray absorptiometry body composition reference values from NHANES. PLoS ONE 4:e7038. doi:10.1371/journal.pone.0007038

Livesey, G., and Elia, M. (1998). Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. Am. J. Clin. Nutr. 47, 608–628.

Lohman, T. G., Martorell, R., and Roche, A. E. (1988). Anthropometric Standardization Reference Manual. Chicago II: Human Kinetics Books.

McArdle, W. D., Katch, F. L., and Katch, V. L. (1986). Exercise Physiology Energy, Nutrition, and Human Performance. Philadelphia: Lea & Febiger.

Mercuri, E., Lampe, A., Allsop, J., Knight, R., Pane, M., Kinashi, M., et al. (2005). Muscle MRI in Ulrrich congenital muscular dystrophy and Bethlem myopathy. Neuromuscul. Disord. 15, 303–310. doi:10.1016/j.nmd.2005.01.004

Merlini, L., Angelin, L., Topolo, E., Braghetta, P., Sabatelli, P., Zamparelli, A., et al. (2008a). Cyclinoprin A corrects mitochondrial dysfunction and muscle apopto- sis in patients with collagen VI myopathies. Proc. Natl. Acad. Sci. U. S. A. 105, 5225–5229. doi:10.1073/pnas.0800962105

Merlini, L., Martoni, E., Grumati, P., Sabatelli, P., Squarzoni, S., Urciuolo, A., et al. (2008b). Autosomal recessive myositis ossificans is a collagen VI disorder. Neurology 71, 1245–1253. doi:10.1212/wnl.0b013e3287610168

Merlini, L., Bertini, E., Minetti, C., Mongini, T., Morandi, L., Angelini, C., et al. (2004). Motor function-muscle strength relationship in spinal muscular atrophy. Muscle Nerve 29, 548–552. doi:10.1016/j.muscu.20018

Merlini, L., Mazzone, E. S., Solari, A., and Morandi, L. (2002). Reliability of hand-held dynamometry in spinal muscular atrophy. Muscle Nerve 26, 64–70. doi:10.1016/sus.261066
Toni et al. Collagen VI myopathies: nutritional assessment

Merlini, M., Morandi, L., Granata, C., and Ballestrazzi, A. (1994). Bethlem myopathy: early-onset benign autosomal dominant myopathy with contractures. Description of two new families. Neuromusc. Disord. 4, 503–511. doi:10.1016/0960-8966(94)90091-4

Merlini, L., Nishino, I., and Consortium for Autophagy in Muscular Dystrophies. (2014). 201st ENMC international workshop: autophagy in muscular dystrophies – translational approach, 1-3 november 2013, Bussum, The Netherlands. Neuromusc. Disord. 24, 546–561. doi:10.1016/j.nmd.2014.03.009

Merlini, L., Solari, A., Vija, G., Bertini, E., Minetti, C., Mongini, T., et al. (2003). Role of a vegetarian diet in patients with Bethlem myopathy and Ulrich congenital muscular dystrophy. Sci. World J. 3, 281–286. doi:10.1100/1476-5918.2003.0002

Mifflin, M. D., St Jeor, S. T., Hill, L. A., Scott, B. I., Daugherty, S. A., and Koh, Y. O. (1990). A new predictive equation for resting energy expenditure in healthy individuals. Am. J. Clin. Nutr. 51, 241–247.

Mischone, M. T., Bruno, F., Ripamonti, C., Nerviti, G., Orsini, R., Faldini, C., et al. (2013). Body composition, muscle strength, and physical function of patients with Bethlem myopathy and Ulrich congenital muscular dystrophy. Scientific World Journal 13:25684. doi:10.1100/1476-5918.2013.25684

Moon, J. R., Tobkin, S. E., Smith, A. E., Roberts, M. D., Ryan, E. D., Dalbo, V. J., et al. (2008). Percent body fat estimations in college men using field and laboratory methods: a three-compartment model approach. Dyn. Med. 7, 7. doi:10.1186/1476-5918-7-7

Müller, M. F. (2007). Malnutrition and hypermetabolism in patients with liver cirrhosis. Am. J. Clin. Nutr. 85, 1167–1168.

Muller, M. F., Bosy-Westphal, A., Later, W., Haas, V., and Heller, M. (2009). Functional body composition: insights into the regulation of energy metabolism and some clinical applications. Eur. J. Clin. Nutr. 63, 1045–1056. doi:10.1038/ejcn.2009.55

Norwood, F. L. M., Harling, C., Chinnery, P. F., Eagle, M., Bushby, K., and Straub, V. (2009). Prevalence of genetic muscle disease in Northern England: in-depth analysis of a muscle clinic population. Brain 132, 3175–3186. doi:10.1093/brain/awn331

O’Connor, L. M., Lentjes, M. A. H., Luben, R. N., Khaw, K.-T., Wareham, N. J., and Oosterhuis, H. J. (1991). Hand-held myometry: in-depth analysis of a muscle clinic population. Brain 132, 3175–3186. doi:10.1093/brain/awn331

Patton, C. O., and Kim, C. (1976). Graphic method for assessing body fat from skinfold thicknesses. J. Appl. Physiol. 41, 754–758.

Pepe, G., de Visser, M., Bertini, E., Bushby, K., Vanegas, O. C., Chu, M. L., et al. (2002). Ultrastructural changes in muscle cells of patients with collagen VI-related myopathies. Muscles Ligaments Tendons J. 3, 281–286. doi:10.1016/j.mlt.2009.03.008

Tagliavini, F., Sardone, F., Squarzoni, S., Maraldi, N. M., Merlini, L., Faldini, C., et al. (2014). Ultrastructural changes in muscle cells of patients with collagen VI-related myopathies. Muscle & Nerve 50, 631–640. doi:10.1002/mus.24004

Tarnopolsky, M. A., MacDougall, J. D., and Atkinson, S. A. (1988). Influence of protein intake and training status on nitrogen balance and lean body mass. J. Appl. Physiol. 65, 187–193.

Tsuji, S., Nonaka, I., and DiMauro, S. (2000). Glycogen storage myopathies. Neurol. Clin. 18, 125–150. doi:10.1016/S0733-8619(05)70181-X

Tuffery-Giraud, S., Saquet, C., Chambert, S., Echenne, B., Marie Cuisois, J., Rivet, F., et al. (2004). The role of muscle biopsy in analysis of the dystrophin gene in Duchenne muscular dystrophy: experience of a national referral centre. Neuromuscul. Disord. 14, 650–658. doi:10.1016/j.nmd.2004.05.002

Turell, D. J., and Alexander, J. K. (1964). Experimental evaluation of weiss’s formula for estimating metabolic rate in man. J. Appl. Physiol. 19, 946–948.

Van den Ploeg, R. J., Fidler, D., and Oosterhuis, H. J. (1991). Hand-held myometry: reference values. J. Neurol. Neuropsych. Psychiatry 54, 244–247. doi:10.1136/jnnp.54.3.244

Van Kan, G., Houles, M., and Vellas, B. (2012). Identifying sarcopenia. Curr. Opin. Clin. Nutr. Metab. Care 15, 436–441. doi:10.1097/MCO.0b013e3283b85bb4

Wang, Z., Heymsfield, S. B., Chen, Z., Zhu, S., and Pierson, R. N. (2010). Estimation of percentage body fat by dual-energy x-ray absorptiometry: evaluation by in vivo human elemental composition. Phys. Med. Biol. 55, 2619–2635. doi:10.1088/0031-9155/55/9/003

Wills, T. A., Hollingsworth, K. G., Coombs, A., Sweeney, M. L., Andersen, S., Stojkovic, T., et al. (2014). Quantitative magnetic resonance imaging in limb-girdle muscular dystrophy 2I: a multinational cross-sectional study. PLoS ONE 9:e90377. doi:10.1371/journal.pone.0090377

Wößner, P., Somers, V. K., Sochor, O., Kullo, I., Jean, N., and Lopez-Jimenez, F. (2010). Influence of body fatness distribution and total lean mass on aortic stiffness in nonobese individuals. Am. J. Hypertens. doi:10.1093/ajh/hip153

Study. J. Clin. Nutr. 57, 241–247. doi:10.1038/ejcn.2014.03.1012

Zanardi, M. C., Tagliabue, A., Orcesi, S., Berardinielli, A., Uggetti, C., and Pichiecchio, A. (2003a). Body composition and energy expenditure in Duchenne muscular dystrophy. Eur. J. Clin. Nutr. 57, 273–278. doi:10.1038/sj.ejcn.1601524

Zanardi, M. C., Tagliabue, A., Orcesi, S., Berardinielli, A., Uggetti, C., and Pichiecchio, A. (2003b). Body composition and energy expenditure in Duchenne muscular dystrophy. Eur. J. Clin. Nutr. 57, 273–278. doi:10.1038/sj.ejcn.1601524

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 June 2014; accepted: 26 October 2014; published online: 17 November 2014.

Citation: Toni S, Morandi R, Busachi M, Tardini I, Merlini L, Battistini NC and Pellegrini M (2014) Nutritional status evaluation in patients affected by Bethlem myopathy and Ulrich congenital muscular dystrophy. Front. Aging Neurosci. 6:315. doi:10.3389/fnagi.2014.00315

This article was submitted to the journal Frontiers in Aging Neuroscience.

Copyright © 2014 Toni, Morandi, Busachi, Tardini, Merlini, Battistini and Pellegrini. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.