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# Body composition analysis as an indirect marker of skeletal muscle mass in Huntington's disease

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ABSTRACT

*Background:* Skeletal muscle wasting is likely to play an important role in the Huntington's disease (HD) pathogenesis. Our aim was to analyze the body composition, and specifically fat-free mass (FFM), as an indirect marker of skeletal muscle in patients with HD, and its association with HD severity and energy balance.
*Methods:* Cross-sectional, case–control study. Body composition was analyzed using bioelectrical impedance. Information was collected as regards of the anthropometrics, disease severity [Unified Huntington Disease Rating (UHDRS) and Total functional capacity (TFC) scores], CAG repeats, protein catabolism, energy intake and energy expenditure.
*Results:* Twenty two patients with HD [mean age 50.3 ± 15.6, mean UHDRS of 27.9 ± 23.7, median TFC of 11 (IQR: 7; 13); median body mass index 23.6 (IQR: 26.8; 22.5)], and 18 controls were included. Both groups were similar in terms of age, gender, body mass index, body composition, physical activity level, and protein catabolism. FFM was correlated with energy intake ($r = 0.73$, $p < 0.001$), resting energy expenditure ($r = 0.64$, $p = 0.001$) and physical activity ($r = 0.54$, $p = 0.003$), but not with CAG repeats, or HD severity.
*Conclusions:* Our results do not support the presence of significant muscle wasting in patients with early-moderate Huntington's disease. However, to prevent muscle wasting in HD, dietary strategies, in addition to physical exercise, should be further investigated.



Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of polyglutamine repeats of the mutated Huntington (*mHtt*) protein. Ubiquitous *mHtt* expression causes lesions, not only in specific brain areas, such as the striatal nuclei, basal ganglia, and cerebral cortex, but also in peripheral areas tissues including, skeletal and cardiac muscle, and pancreatic B cells. Weight loss in HD patients and animal models is associated with global muscle wasting, independent of locomotor activity [16]. In the R6/2 mouse model of HD, overall catabolic phenotype and severe muscle wasting have been found secondary to activation of the apoptotic and autophagic pathways [19]. At molecular level, mitochondrial dysfunctions, peroxisome proliferator-activated receptor alpha signaling, and heat shock transcription factor 1 activation were identified as major players in the muscle HD-related pathology [17,24]. However, many aspects of HD neuromuscular transmission and muscle physiology remain unanswered and need to be studied more extensively.

Skeletal muscle mass can be reduced despite normal body mass index (BMI). Skeletal muscle loss has been associated with declining physical performance, with a negative prognostic effect on falls, disability, and mortality risk [14,18]. It has been hypothesized that by improving muscle function in HD, the progression of disease onset could be delayed and the lifespan extended [24]. Therefore, based on previous evidence, skeletal muscles might be an attractive target for future therapies.

There are no systematic studies looking at skeletal muscle wasting in patients with HD. Overall, muscle mass can be quantified at different levels of body composition. Some methods, such as MRI and CT, have high validity but are complex and costly. Dual energy X-ray absorptiometry has intermediate cost and complexity with good reproducibility, and is more reliable for the research setting. Other methods, such as administration of tritium (D3)-marked creatine and measurement of urinary D3-creatinine, are still in a preclinical phase of development [14]. Instead, bioelectrical impedance analysis (BIA) is inexpensive and easy to perform in most settings, being the preferred method for clinical practice.

Fat mass (FM) and fat-free mass (FFM) are the two compartments into which body composition is classically divided by using BIA. FFM consists of all non-fat tissues, and is commonly used as an indirect

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marker of skeletal muscle mass. Since there is preliminary evidence that skeletal muscle wasting might play an important role in the HD pathogenesis [13,24], the aims of this study were to analyze the body composition, and specifically FFM, and its relation with energy intake, total energy expenditure, catabolism, CAG repeats, physical activity, use of antidopaminergic drugs, and HD severity.

## 1. Materials and methods

This study was a single-center, case–control, cross-sectional, observational study, and was part of a study designed to evaluate the energy expenditure in patients with HD (submitted for publication). We included a consecutive sample of ambulatory HD mutation carriers and healthy controls, after signing the informed consent. Controls were usually recruited from family members to minimize the effects of environmental conditions. Patients diagnosed with diabetes mellitus, thyroid disease, other neurodegenerative, heart, pulmonary, or skeletomuscular disease, pregnancy or breastfeeding, active cancer, and medication known to affect metabolism/endocrine function, were excluded. This study was approved by Ethics Committee of the Complejo Universitario Burgos-Soria.

### 1.1. Assessments

Sociodemographic information and HD severity assessed by the Unified HD Rating scale (UHDRS) and Total Functional capacity (TFC) [6], were collected. Anthropometrics, such as the subscapular skinfold and BMI were collected as nutritional status estimators. In accordance with the World Health Organization international standards [25], a BMI ranging from 18.5 to 24.9 kg/m$^2$ was considered nutritionally normal, whereas a BMI <18.5 kg/m$^2$ was associated with a deficient nutritional status, and BMI >25 kg/m$^2$ was associated with overweight and obesity. Psychiatric symptoms severity was assessed using the Problems Behavior Assessment scale (short form), (PBA-S), with higher scores indicating higher severity [10]. Comorbidity information was collected using the Cumulative Illness Rating Scale-Geriatric (CIRS-G), with higher scores indicating higher comorbidity [20].

Body composition was measured by using a body composition analyzer BC 418 MA (Tanita UK®, Yiewley, UK). Total energy expenditure (TEE) was computed as the sum of resting energy expenditure (REE), physical activity (PA), and an estimated percentage of 10% DIT (energy required for the digestion, absorption, usage and storage of nutrients after food intake). REE was measured by indirect calorimetry in the morning after an overnight fast and not smoking. The MasterScreen™ CPX of Jaeger™ from Bioenergy facility was used for the REE measurement. After resting in bed in a quiet room and stabilization of respiratory quotient values for at least five minutes, REE was measured twice during 30 min. Data were analyzed by using the LabManager and IntelliSupport application, and adjusted for blood urea nitrogen.

PA energy expenditure was measured using triaxial accelerometer (ActiGraph GT3X). The device was affixed securely to the waist and data recorded for 10 h. Patients were free to do the activities they usually practiced. The patients did not know when the devices were on. Data was analyzed by using the ActiLife 6 software by using Freedson combination algorithm. Physical activity level was measured by using the Questionnaire Physical Activity quotient ([8], WHO) developed by the WHO, which comprises 19 questions, and physical activity is computed in terms of high, moderate, and low level of physical activity.

Dietary calorie intake was measured by using 24-h dietary recalls, validated for the Spanish population [1]. Kilocalories and macronutrients data were obtained using the software, Alimentación y Salud 2.0. For operational reasons, the dietary calorie intake was classified into low consumption according to age and gender Spanish Dietary Recommended Intakes (SDRI) approved by the Spanish Nutritional Association (<67% of recommended intake) vs. normal consumption (≥67%) [21]. Blood for the analysis of creatine phosphokinase (CPK) values, and protein catabolism (24-urinary nitrogen excretion) was also collected. Blood urea nitrogen (BUN) was calculated by the following equation: [urinary urea (gr / day) ∗ 0.56 (hypercatabolic state)] [15].

### 1.2. Analysis

Differences between groups were assessed using parametric and non-parametric tests based on the normal distribution of the data [Student *t* test (mean, standard deviation), Mann–Whitney U test (median, interquartile range), respectively]. Qualitative data comparison was performed using the Chi-squared test. Association between FFM and quantitative variables were calculated using Pearson or Spearman correlation coefficients, as appropriate. A linear regression analysis was conducted using the FFM as the dependent variable, and energy intake and total energy expenditure as the dependent variables. Significance was determined as an alpha value set at 0.05. The IBM-SPSS version 19 statistical software was used for data analysis.

## 2. Results

Twenty two patients with HD [mean age 50.3 ± 15.6, mean UHDRS of 27.9 ± 23.7, median TFC of 11 (IQR: 7; 13); median body mass index of 23.6 (IQR: 26.8; 22.5)], and 18 controls were included (Table 1). Both groups (cases and controls) were homogeneous in terms of age, gender, and BMI. In our HD sample, FFM was within the 50 percentile values for population-based data from the UK Biobank (healthy-white-ethnic men and women) (Table 2).

When both groups were compared, no significant differences were found in terms of FMM, FM, dietary caloric intake, CPK, comorbidities, physical activity level, and protein catabolism (Table 1). In HD patients, body compositions (FM, and FFM) were similar when patients were on antidopaminergics, compared to those not receiving them ($p = 0.28$).

In the group of patients with HD, FFM was correlated with REE ($r = 0.64$, $p = 0.001$) and PA ($r = 0.54$, $p = 0.003$), but FFM and FM were not correlated with age, HD duration, CAG repeats, UHDRS motor, cognitive, PBA-S or TFC scores. In terms of anthropometrics, in both groups of participants, the BMI was associated with FM [$r = 0.68$, $p < 0.0001$ (controls); $r = 0.82$, $p < 0.0001$ (patients)] but not with FMM. FFM was inversely correlated with subscapular skinfold in the HD group ($r = -0.53$, $p = 0.01$). FFM was highly correlated with energy caloric intake [$r = 0.73$, $p < 0.001$ (patients), $r = 0.50$, $p = 0.03$ (controls)], TTE [$r = 0.60$, $p = 0.002$ (patients); $r = 0.77$, $p < 0.001$ (controls)], but not with FM. According to the SDRI, carbohydrates, fat and fiber intake were below the SDRI in both groups. Macronutrients intake was correlated with FFM, but not with FM. Particularly, in the group of HD patients, FFM was correlated with the intake of proteins ($r = 0.57$, $p = 0.005$), carbohydrates ($r = 0.61$, $p = 0.002$), and fat ($r = 0.43$, $p = 0.44$). In the linear regression analysis, FFM was only associated with TEE ($\beta = 0.55$, $p = 0.001$) in the control group. This model was insufficient for the HD group.

## 3. Discussion

Based on the results of our study, compared to healthy controls, patients with mild-moderate HD have similar body composition and protein catabolism. In our HD sample, FFM was within the 50 percentile values for population-based data from the UK Biobank [7]. Thus, our results do not support the presence of significant muscle wasting in patients with mild-moderate HD. But, why did we not find any significant muscle wasting in our patients with HD? One possibility is the fact that, in terms of functionality, our HD patients had a similar physical activity level compared to controls, and a subsequently lower risk for muscle wasting. Another possibility is that animal HD models might develop more severe peripheral ubiquitous *mHtt* expression, or at least this expression is more likely to be age-dependent, or in advanced stages of the disease [16]. A third possibility is the lack of BIA precision, since

**Table 1**
Demographics and clinical characteristics comparison.

| | Patients n = 22 | Controls n = 18 | p value |
|---|---|---|---|
| Women (%) | 14 (63) | 9 (50) | 0.36 |
| Age (years) | | | |
| Mean ± (SD) | 50.3 ± 15.6 | 47.4 ± 13.8 | 0.55 |
| Use of antidopaminergics (%) | 13 (59) | – | – |
| HD duration | | | |
| Median (IQR) | 9.0 (5.0; 9.0) | – | – |
| CAG repeats | | | |
| Mean ± (SD) | 43.0 ± 6.0 | – | – |
| UHDRS motor score | | | |
| Mean ± (SD) | 27.9 ± 23.7 | – | – |
| TFC | | | |
| Median (IQR) | 11.0 (13.0; 7.0) | – | – |
| UHDRS cognitive score | | | |
| Median (IQR) | 167.0 (242.5; 100.2) | – | – |
| PBA-S score | | | |
| Median (IQR) | 10 (7; 20.5) | – | – |
| Comorbidity | | | |
| Median (IQR) | 0 (0; 2.2) | 0 (0;2.0) | 0.78 |
| BMI (kg/m$^2$) | | | |
| Median (IQR) | 23.6 (26.8; 22.5) | 25.4 (26.3–24.0) | 0.11 |
| FFM (kg) median (IQR) | | | |
| Total | 46.0 (58.5; 40.6) | 49.5 (62.7–42.9) | 0.27 |
| Male | 59.0 (64.1; 58.3) | 62.7 (77.5–52.3) | 0.72 |
| Female | 43.0 (45.7; 39.1) | 43.2 (48.6–40.9) | 0.36 |
| FM (kg) median (IQR) | | | |
| Total | 14.4 (18.5; 10.1) | 19.5 (25.4–11.1) | 0.15 |
| Male | 8.9 (12.5; 6.7) | 12.0 (23.1–8.8) | 0.10 |
| Female | 18.1 (20.6; 14.3) | 23.3 (25.4–18.8) | 0.07 |
| BUN (mg/dl) | | | |
| Mean ± (SD) | 9.3 ± 3.7 | 11.1 ± 4.1 | 0.23 |
| Calorie intake (kcal/day) | | | |
| Mean ± (SD) | 1946.4 ± 756.7 | 2056.5 ± 548.8 | 0.98 |
| <67% dietary allowances (%) | 14 (65) | 9 (50) | 0.52 |
| Macronutrients intake (gr/day) | | | |
| Median (IQR) | | | |
| Proteins | 79.98 (40.99; 179.04) | 87.67 (58.34; 127.71) | 0.39 |
| Carbohydrates | 209.08 (116.48; 419.93) | 220.19 (108.12; 375.27) | 0.96 |
| Fat | 65.62 (28.36; 163.53) | 95.65 (33.13–160.06) | 0.15 |
| Fatty acids (PUFA/SFA) | 0.50 (0.10; 1.40) | 0.50 (0.40–1.40) | 0.86 |
| Fiber | 17.36 (6.61; 36.70) | 20.11 (8.10–47.51) | 0.26 |
| CPK UI/L | | | |
| Median (IQR) | 76.0 (64.2; 113.0) | 92.0 (66.0–131.0) | 0.62 |
| Physical activity level (%) | | | |
| Low | 2 (11.1) | 6 (27.3) | 0.27 |
| Medium | 8 (44.4) | 11 (50.0) | |
| High | 8 (44.4) | 4 (18.2) | |

UHDRS = Unified Huntington Disease Rating Scale; TFC = Total Functional Capacity; Problems Behavior Assessment scale (short form) = PBA-S, BMI = Body Mass Index; FFM = Fat Free Mass; FM = Fat Mass; BUN = blood urea nitrogen; CPK = creatine phosphokinase; PUFA = polyunsaturated fats; SFA = saturated fats.

the assessment of a FFM less than 1–5-2 kg is limited. A fourth possibility could be a selection bias, since we have included patients and controls with normal BMI. Finally, since the dietary intake has been found to be correlated with FFM [22], the lack of FFM differences can be due to a similar intake of macronutrients among both groups of participants.

As expected, in both groups, we found that physical activity and REE were correlated with higher FFM. However, although in the REE, under normal physiological conditions, skeletal muscle can account for up to 20% [23], it is unknown in HD, and deserves further investigation.

**Table 2**
Fat-free mass in HD patients vs. reference values for a healthy white-ethnic population.

| Gender | FFM HD subjects | FFM reference values |
|---|---|---|
| Men | | |
| Percentile 50 | 59.0 | 59.0 |
| Women | | |
| Percentile 50 | 43.0 | 42.2 |

Reference values were obtained for the group of participants within the same age (45–59 years old), and BMI (18.50–24.99) [7].

Based on the WHO standards [25], our sample was normoweighted, in accordance with our previous cross-sectional, Spanish, multicentre study aimed to analyze the association between nutritional status with HD severity in 224 subjects, 60.2% with mild-moderate HD (TFC score≥7), and 39.8% severe HD (TFC score <7) [5]. On the contrary, other studies have found a higher frequency of low BMI in HD patients [2,3]. At this time, we do not have compelling explanation for these findings. The most likely explanations would be different characteristics of the sample, lifestyle (adherence to the Mediterranean diet, physical activity), environmental conditions, the presence of family caregivers, or rapid changes in the prevalence of obesity in the general population [12], which might counteract the HD mutation, at least partially.

However, body composition, more than BMI, seems to be a determinant of health and prognosis [11], and low FFM is one of the diagnostic criteria for cachexia. Although, BMI has been classically used as a nutritional state marker [25], it is well recognized that body weight and BMI are inadequate markers of underlying alterations in body composition in the elderly and diseased populations [11]. In fact, in our study, BMI was only highly associated with FM, and not with FMM. Of note, FFM was inversely correlated with subscapular skinfold in the HD group,

suggesting its possible use as an anthropometric marker of muscle wasting in HD. However, longitudinal studies have shown that skinfold thicknesses cannot be used to assess changes in body composition, because of age-related fat redistribution, with a central distribution of fat over time [9].

For the first time, we have analyzed the association of body composition with the intake of antidopaminergic drugs in HD. In contrast to other studies, where the use of antipsychotic drugs has been associated with elevated metabolic parameters, and FM [4], in our HD sample, the use of antidopaminergic drugs was not associated with any significant change in body composition. Different methodology and sample characteristics should be taken into account in order to understand these discrepancies.

Our study has several limitations, including the small sample size and the lack of randomization. In addition, we have to recognize that the use of BIA in HD has not been validated. By using FFM, we assume that all non-fat and non-bone tissue are muscle mass, but a small percentage in organs, tendons and cartilage is also included [9]. We have included participants with normal BMI, and therefore our results cannot be extrapolated to patients with significant weight loss. Nevertheless, on the other hand, we have shown that the assessment of body composition can be useful as an indirect biomarker of muscle wasting. We have also extensively studied the principal parameters associated with FFM in patients with HD and its association with antidopaminergic drugs. In fact, in order to extrapolate our results to the general HD population, there is no doubt that muscle wasting longitudinal data including patients with low BMI, would be more precise and sensitive than cross-sectional data.

In conclusion, body composition is easy to assess by BIA, and can be used for assessment of nutritional state and muscle wasting, in a research and clinical practice setting. Body composition can be therefore used to plan nutritional strategies to improve prognosis of the disease. In order to prevent muscle wasting in HD, dietary strategies, in addition to physical exercise should be further investigated.

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## Disclosure of conflict of interest

The authors have no conflict of interest to report.

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