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# Skeletal Muscle Phenotype in Patients Undergoing Long-Term Hemodialysis Awaiting Kidney Transplantation

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## Abstract

**Background and objectives** Age and comorbidity-related sarcopenia represent a main cause of muscle dysfunction in patients on long-term hemodialysis. However, recent findings suggest muscle abnormalities that are not associated with sarcopenia. The aim of this study was to isolate functional and cellular muscle abnormalities independently of other major confounding factors, including malnutrition, age, comorbidity, or sedentary lifestyle, which are common in patients on maintenance hemodialysis. To overcome these confounding factors, alterations in skeletal muscle were analyzed in highly selected patients on long-term hemodialysis undergoing kidney transplantation.

**Design, setting, participants, & measurements** In total, 22 patients on long-term hemodialysis scheduled for kidney transplantation with few comorbidities, but with a long-term uremic milieu exposure, and 22 age, sex, and physical activity level frequency-matched control participants were recruited. We compared biochemical, functional, and molecular characteristics of the skeletal muscle using maximal voluntary force and endurance of the quadriceps, 6-minute walking test, and muscle biopsy of *vastus lateralis*. For statistical analysis, mean comparison and multiple regression tests were used.

**Results** In patients on long-term hemodialysis, muscle endurance was lower, whereas maximal voluntary force was not significantly different. We observed a transition from type I (oxidative) to type II (glycolytic) muscle fibers, and an alteration of mitochondrial structure (swelling) without changes in DNA content, genome replication (peroxisome proliferator activator receptor  $\gamma$  coactivator-1 $\alpha$  and mitochondrial transcription factor A), regulation of fusion (mitofusin and optic atrophy 1), or fission (dynamin-related protein 1). Notably, there were autophagosome structures containing glycogen along with mitochondrial debris, with a higher expression of light chain 3 (LC3) protein, indicating phagophore formation. This was associated with a greater conversion of LC3-I to LC3-II and the expression of *Gabralp1* and *Bnip3l* genes involved in mitophagy.

**Conclusions** In this highly selected long-term hemodialysis population, a low oxidative phenotype could be defined by a poor endurance, a fiber-type switch, and an alteration of mitochondria structure, without evidence of sarcopenia. This phenotype could be related to uremia through the activation of autophagy/mitophagy.

**Clinical Trial registration numbers:** NCT02794142 and NCT02040363.

## Introduction

Muscle weakness and muscle atrophy are common features in patients on long-term hemodialysis (HD) and appear as an emerging risk factor contributing to the loss of functional capacity, frailty, and, ultimately, premature death (1,2).

In contrast with initial studies in patients on HD showing similarities with age-related sarcopenia (3) (fiber atrophy, activation of catabolic pathway), the type of muscle remodeling and associated proteome seem specific to the disease (4). In particular, a fiber transition from a slow to fast type has been reported

in these patients (5). In addition, a “mitochondrial-acquired myopathy” has also been observed, resulting in reductions in muscle oxidative capacity (6) and energy supply (7), which may further activate the muscle autophagy pathway (8). Such energy deficiency should logically reduce muscle endurance rather than muscle strength. Although muscle dysfunction and signaling in patients on long-term HD remain to be fully characterized, the presence of factors including anemia, comorbidity, protein energy wasting, inflammation, or sedentary lifestyle could lead to different muscle phenotypes (9). In particular,

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patients awaiting kidney transplantation—who have few comorbidities and low levels of protein energy-wasting syndrome—could display a specific muscle phenotype, providing an opportunity to characterize the specific involvement of kidney dysfunction on skeletal muscle.

In addition, muscle phenotype in patients before transplantation could explain a lack of muscle recovery after transplantation. In spite of microenvironment improvement after transplantation (uremic milieu, inflammation, sedentariness), a frequent blunted muscle function recovery post-transplantation constitutes a prognosis factor (10). The pre-existence of subtle muscle abnormalities generally not detected in patients on long-term HD without sarcopenia may be a worsening factor (11). In such patients, a low energy supply related to low muscle endurance and mitochondrial impairment could counteract the improvement that could be expected after ruling out the consequences of kidney dysfunction.

Therefore, the objective of this study was to characterize structural muscle and mitochondrial abnormalities associated with muscle functional impairment in a subpopulation of highly selected patients on long-term HD undergoing kidney transplantation, without major confounding factors such as advanced age, malnutrition, comorbidities, or sedentary lifestyle.

## Materials and Methods

Additional information can be found in the Supplemental Material.

### Participants

All participants were recruited from January 2015 to May 2018. Patients on long-term HD for >1 year and referred for kidney transplantation were enrolled from the University Hospital of Montpellier, France, on the day of transplantation. The inclusion criterion was an age between 40 and 80 years. Exclusion criteria were diabetes, protein energy wasting (12), clinical infection, or cardiovascular event during the 3 months before inclusion, active cancer, liver disease, or HIV infection at the time of evaluation. In addition, 21 control participants were recruited prospectively on the following criteria: age between 40 and 80 years, no evidence of chronic illnesses, normal kidney function (GFR  $87 \pm 10$  ml/min per  $1.73 \text{ m}^2$ ), no history of kidney disease or hypertension, and low physical activity level. Physical activity during the last 3 months before evaluation was assessed using Voorrips questionnaire, which was validated in patients on long-term HD (13,14). A Voorrips score <9.4 defined a low physical activity (13).

The study was conducted according to the principles of the Declaration of Helsinki and approved by the institutional ethics committee of Marseille in January 2015 (2014-A01965–42, ClinicalTrials.gov identifiers: NCT02794142 and NCT02040363).

### Study Design

After inclusion, clinical examination, functional assessments, and biologic parameters were performed during the waiting time for a crossmatch result. The procedure for assessments and muscle biopsy was similar for controls.

Medical history allowed the determination of Charlson comorbidity index. All participants completed the Voorrips physical activity questionnaire. A muscle biopsy was obtained from the *vastus lateralis* of the nondominant leg with a classic needle method (15) before kidney transplantation and after the assessment of muscle outcomes, for immunohistochemistry, transmission electron microscopy, RNA, and protein analyses. All data were recorded using electronic case report forms.

### Outcomes

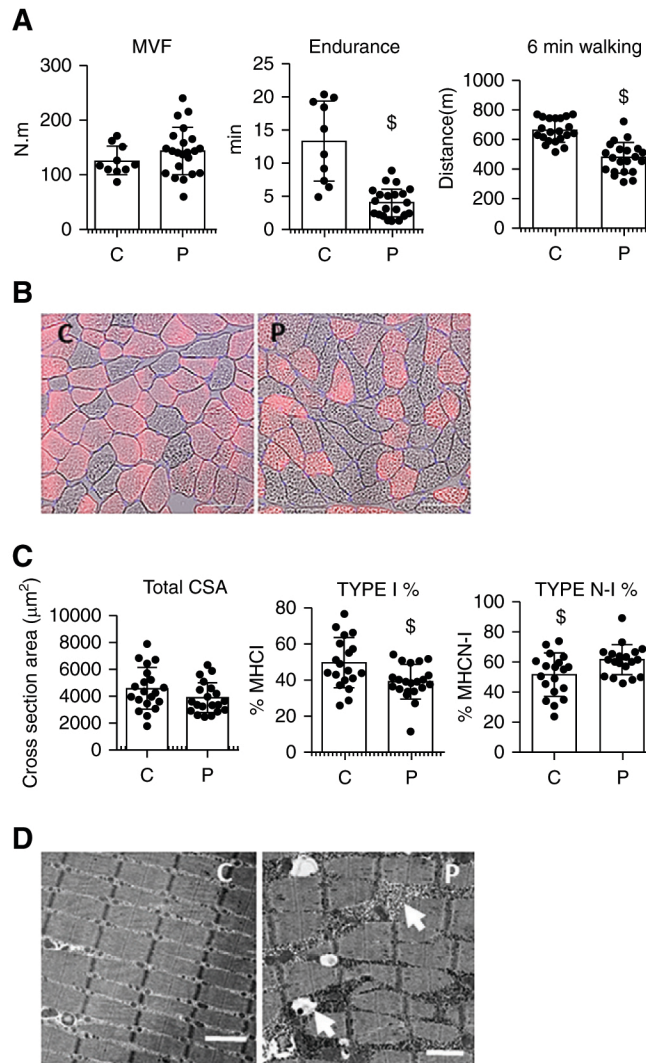
Exercise capacity was assessed using the international recommendations with a 6-minute walking test (16). Muscle mass was assessed by the creatinine index. A cutoff point of 18.82 mg/kg per day is indicative of a clinical muscle atrophy and has shown significant associations with mortality (17). Maximal voluntary force and endurance of the quadriceps were assessed on an exercise bench (18). Maximal voluntary force was assessed on a 1-second plateau and values of the contractions had to be reproducible (less than 5% of variability between values). Maximal voluntary force was defined as the highest peak torque (N.m) of three valid and reproducible maximal contractions of the dominant leg. Maximal voluntary force values were compared with the French adult normative database. Quadriceps endurance time was then assessed on the dominant leg and consisted of repeated knee extensions against weights corresponding to 40% of the maximal voluntary force at a pace of 12 movements/minute until exhaustion (18). A reduction of maximal voluntary force >10% at 1, 2, and 3 minutes after the end of the test was mandatory to validate the quadriceps endurance time. Protein energy wasting was defined according to the international criteria of the International Society of Renal Nutrition and Metabolism (12). According to the revised European Consensus on Definition and Diagnosis (EWGSOP2) (16), the diagnosis criteria for sarcopenia were low muscle strength, low muscle quantity, or quality (assessed here by creatinine index). The cutoff points were 88 N.m for maximal voluntary force and 18.82 mg/kg per day for the creatinine index (19).

### Exploratory End Points

Exploratory end points included immunohistochemistry and transmission electron microscopy, immunoblotting, real-time quantitative PCR analyses, and mitochondrial DNA analysis as detailed in the Supplemental Material.

### Statistical Analyses

Qualitative data were compared with a chi-squared test. Quantitative data were compared between groups either with *t* tests, or Welch tests when variances were unequal with normal distributions (Figure 2 [Murf1 mRNA]; Supplemental Figure 1 [Caspase 3]; Supplemental Figure 4 [Myostatin mRNA]), or nonparametric Mann–Whitney tests when distributions were not normal (Figure 1 [Endurance]; Figure 2 [Igf1 mRNA, Atrogin mRNA, Foxo3 mRNA]; Figure 3 [mitochondrial size]; Figure 4 [Gabaralp1 mRNA]; Supplemental Figure 3 [mi-DNA/n-DNA, Mitofusin]; ultrasensitive C-reactive protein [mentioned in the text]). The Shapiro–Wilk test was used to assess the normality of the data and a Fisher test for the comparison of



**Figure 1. | Endurance and 6-minute walking parameters are changed in patients associated with a transition from type I (oxidative) to type II (glycolytic) fibers.** (A) (Left panel) Maximal voluntary force of the quadriceps (C,  $n=10$ ), (middle) endurance test (C,  $n=10$ ), (right) 6-minute walk test (C,  $n=21$ ). (C, control; P, patient;  $n=22$ ). Data are presented as mean $\pm$ SD (\$,  $P<0.05$ ). (B) Representative pictures of transverse cryosections of *vastus lateralis* from a healthy participant (C) or a patient (P), showing the superposition of the bright field picture with the myosin heavy chain 1 (red) and Hoechst 33258 (blue) staining pictures. Scale bar: 100  $\mu$ m. (C) Myosin percentage and cross-section area (CSA) from control participants (C) or patients (P): type I graph shows the percentage of type I myofibers stained with an anti-myosin I (MHC I). Type N-I graph shows the myofibers not stained by the anti-myosin I antibody (indicated as MHC N-1). ( $n=21$ ). Data are presented as mean $\pm$ SD (\$,  $P<0.05$ ). (D) Electron microscopy of *vastus lateralis*. Scale bar: 1  $\mu$ m, arrows show the zones of proteolysis and zones of glycogen or lipid accumulation. MVF, maximal voluntary force; N-I, no fiber type 1.

variances. Spearman correlation was used to assess the relationship between quantitative variables. Multiple linear regressions were used to adjust  $P$  values from control/patients on sex and age. A test was considered significant if  $P\leq 0.05$ . Statistical analysis was performed using Statview and XLSTAT.

## Results

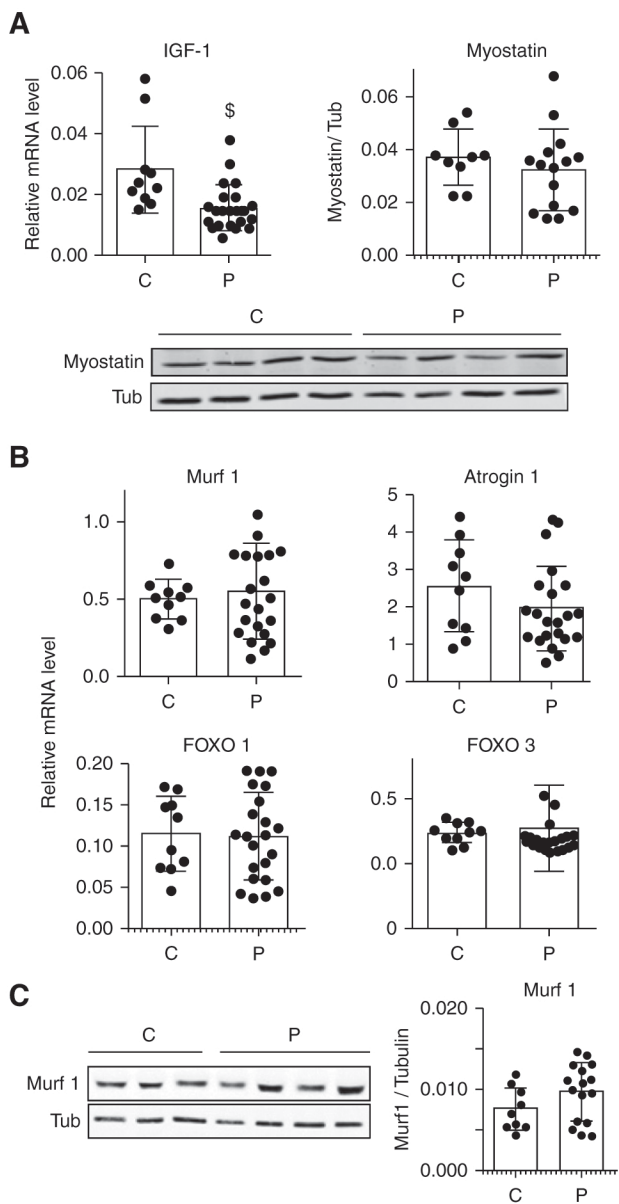
### Characteristics of the Studied Population

Characteristics of the studied populations are summarized in Table 1. Patients do not significantly differ ( $P>0.05$ ) from controls for age (58, 95% confidence interval [95% CI], 54 to 62 years for patients and 59, 95% CI, 56 to 62 years for controls), sex (14 out of 22 men in patients and 10 out of 21 men

in controls), physical activity level (Voorrips score) (6.1, 95% CI, 4.1 to 8.1 for patients and 6.2, 95% CI, 4.3 to 8.1 for controls), body mass index (25.7, 95% CI, 23.8 to 27.6  $\text{kg}/\text{m}^2$  for patients and 24.7, 95% CI, 23.1 to 26.3  $\text{kg}/\text{m}^2$  for controls), and serum albumin (4.3, 95% CI, 4.0 to 4.5 g/dl for patients and 4.6, 95% CI, 4.4 to 4.8 g/dl for controls). Creatinine index ( $20.72\pm 2.45$  mg/kg per day) confirmed the absence of protein energy wasting, whereas low levels of inflammation could be evidenced with ultrasensitive C-reactive protein (6.7, 95% CI, 2.3 to 11.2 mg/L for patients and 1.3, 95% CI, 0.5 to 2.1 mg/L for controls;  $P=0.02$ ).

### Muscle Functionality and Morphology

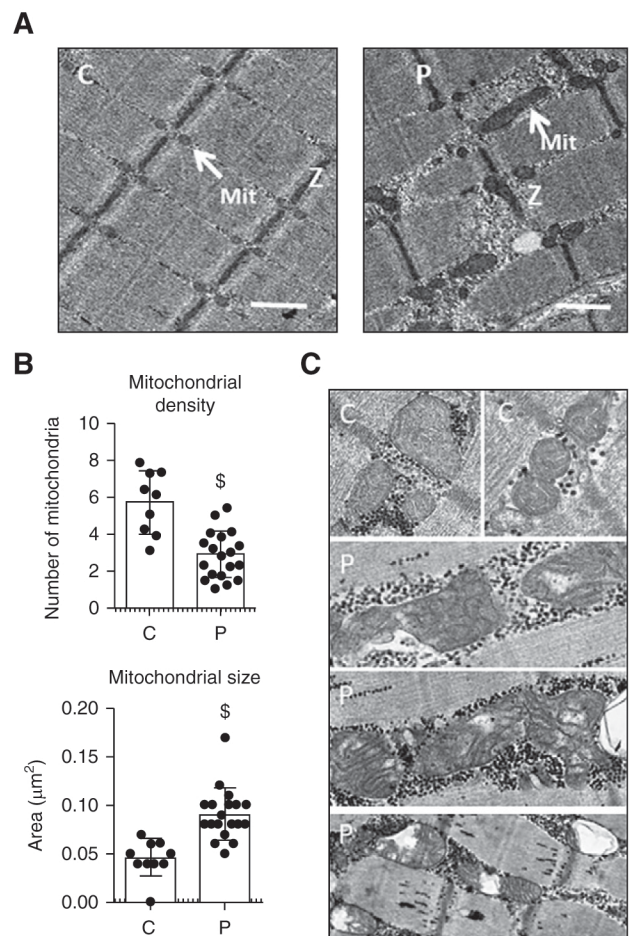
Maximal voluntary force of the dominant leg was not significantly different between groups (Figure 1A). No



**Figure 2. | The ubiquitin-proteasome is not involved in muscle wasting in patients who are hemodialysis selected.** Real-time quantitative PCR analysis of *IGF-1* (A, left), *Murf 1*, *Atrogin 1*, *Foxo1*, *Foxo3* expression. (B) Western blot analysis of myostatin (A, right) and *Murf 1* protein expression (C) on muscle biopsies. (C, control,  $n=10$ ; P, patient,  $n=22$ ). Data are presented as mean $\pm$ SD (\$,  $P<0.05$ ).

patient met the criteria for sarcopenia. All patients had weaker endurance capacity (quadriceps endurance time:  $13.4\pm 6.1$  minutes in controls versus  $4.1\pm 2.2$  minutes in patients,  $P<0.001$ , and 6-minute walking distance ( $666\pm 79$  m in controls versus  $478\pm 102$  m in patients,  $P<0.001$ ) (Figure 1A).

Histologic analysis of total cross-section area (Figure 1B), type I fiber cross-section area (type I), or cross-section area for other types of fibers (type N-I) showed no significant difference between groups (Table 2). After adjustment for age, sex, and age+sex, a significant difference was observed between groups (Table 3). Because sex appeared



**Figure 3. | The ultrastructure of the mitochondria is altered in patients.** (A) Zoom of muscle electron microscopic views, which show sarcomeric disorder and degradation of patients *vastus lateralis* muscles (Mit, mitochondries; Z, Z line; scale bar: 500 nm). (B) Quantification of mitochondrial density and size. (C, control,  $n=10$ ; P, patient,  $n=22$ ). Data are presented as mean $\pm$ SD (\$,  $P<0.01$ ). (C) Example of mitochondria analysis on control (C) or patient (P); electron microscopic views of large swollen or damaged mitochondria are shown.

to be a confounding factor for cross-section area, we separately analyzed total cross-section area in men and women (Table 2). The difference in total cross-section area was significant in men but not in women, indicating subclinical atrophy appeared in some patients that could not be evidenced with our clinical investigations.

Fiber type quantifications showed in patients (1) lower MHC type I fiber % (39%, 95% CI, 35 to 44 for patients and 49, 95% CI, 43% to 56 for controls;  $P=0.01$ ) and (2) higher MHC other type fibers % (termed N-I fibers) (61%, 95% CI, 56 to 65 for patients and 51%, 95% CI, 44 to 58 for controls;  $P=0.01$ ) (Figure 1C).

In the muscle ultrastructure, analyzed by transmission electron microscopy, we observed sarcomeric disorganization in all patients (Figure 1D) with zones of proteolysis and zones of glycogen or lipid accumulation. Length of sarcomeres was not modified ( $1.94\pm 0.05$   $\mu\text{m}$  for controls and  $1.82\pm 0.09$   $\mu\text{m}$  for patients).

### Signaling Pathways Involved in Muscle Wasting in Patients

At the mRNA level, a weaker expression in *Igf1* was observed, whereas no difference in Forkhead box O1 and 3, transcription factors activated by the IGFI pathway, was reported (Figure 2, A and B).

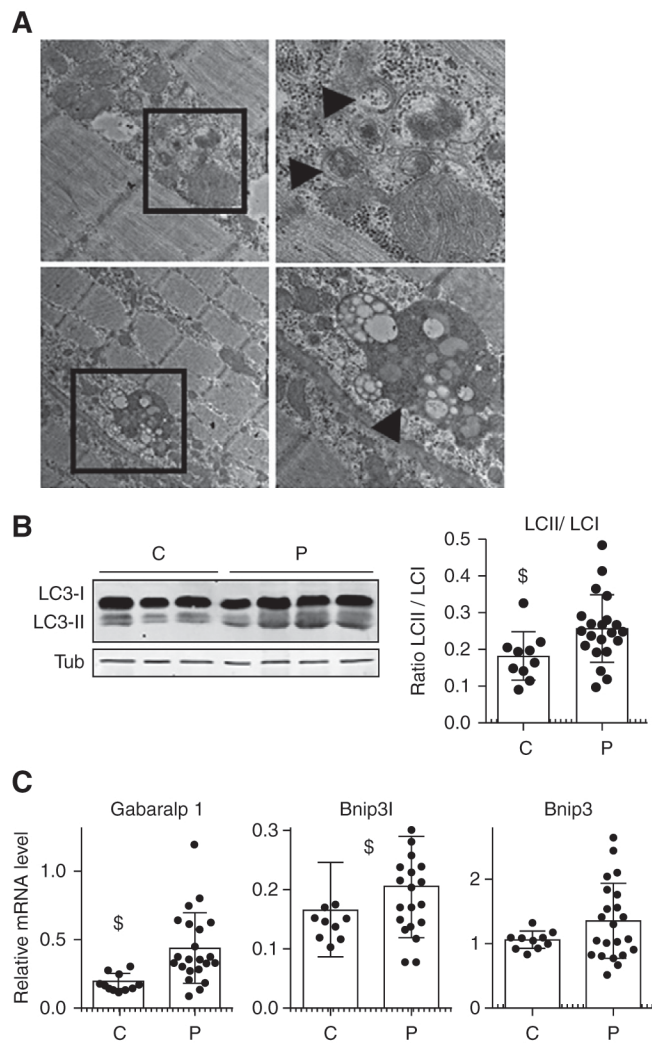
By studying catabolism pathways evaluated by western blotting analysis, neither difference in myostatin at the transcriptional (Supplemental Figure 4) nor protein expression levels (Figure 2A) were observed. Moreover, neither protein level of muscle ring finger-1 nor mRNA level of Atrogin1 (atrophy F-box) were found statistically different between patients and controls (Figure 2, B and C), suggesting the ubiquitin-proteasome pathway was not involved in the process of muscle wasting. Last, western blotting analysis did not reveal any calpain 1 or caspase 3 activation because no detection of autolyzed fragment of calpain 1 (which usually shows calpain activation) was observed (Supplemental Figure 1).

### Mitochondrial Impairment in Patients on Long-Term Hemodialysis

Because of the role of the mitochondria in the production and use of cell energy, we conducted a study on mitochondrial morphology and density. A lower repartition of mitochondria in patient muscle coupled with a bigger mitochondrial size were evidenced (Figure 3A). A negative correlation was also observed between 6-minute walking distance and mitochondria area (Supplemental Figure 2). Quantification of mitochondrial density and analysis of mitochondrial size by stereology confirmed the larger mitochondrial size and indicated a lower number of mitochondria (Figure 3B). In addition, the mitochondria of patients presented a swelling phenotype and/or disrupted internal structures (Figure 3C). The amount of mitochondrial DNA was similar in patients compared with controls (Supplemental Figure 3A). In accordance with this result, the expression levels of mitochondrial transcription factors peroxisome proliferator activator receptor  $\gamma$  coactivator-1 $\alpha$  and mitochondrial transcription factor A involved in mitochondrial genome replication were not modified. Analysis of protein expression involved in mitochondria fusion (mitofusin and optic atrophy 1) or fission (dynamin-related protein 1) did not show any significant changes (Supplemental Figure 3, B and C).

### Autophagy

Transmission electron microscopy confirmed the presence of autophagic build-up in patients. Indeed, autophagosome structures containing glycogen along with mitochondrial debris or other cytoplasmic elements were observed (Figure 4A). We also detected a higher conversion level of microtubule-associated protein 1A/1B-light chain 3 LC3-I to LC3-II in patients, which is consistent with the formation of autophagosomes (Figure 4B). Last, a greater *Gabralp1* gene expression was also reported (Figure 4C), and, in agreement with the mitochondrial phenotype (bigger size and swelling), we found a stronger (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like) *Bnip3l* gene expression whose corresponding protein is involved in the process of mitophagy.



**Figure 4. | Selected patients who are on long-term hemodialysis present autophagy system activation.** (A) Representative example of electron microscopy image detected on muscle biopsies in patients (the images on the right were an enlargement of black square). Arrowhead shows autophagosome structure, (B) western blot analysis of LC3 protein activation. The ratio LC3-II/LC3-I, which reflects LC3 activation, was quantified ( $\$, P < 0.05$ ). (C) Real-time quantitative PCR analysis of *Gabralp 1*, *Bnip 3l*, and *Bnip 3*. (C, control,  $n = 10$ ; P, patient,  $n = 22$ ). Data are presented on the histograms as mean  $\pm$  SD ( $\$, P < 0.05$ ).

### Discussion

This study reported a weaker endurance, a slow to fast fiber-type switch, and mitochondrial abnormalities in the skeletal muscle of patients on long-term HD. Conversely, muscle weakness, lower clinical mass, and activation of the ubiquitin-proteasome system could not be evidenced.

Patients on long-term HD before kidney transplantation constitute a highly selected population. Among patients on HD, those placed on the waiting list for transplantation are healthier with a better long-term survival (20). When compared with our healthy controls, our patients showed a remarkably low burden of muscle deleterious factors, an adequate nutritional status, a preserved creatinine index, and a low grade inflammation despite a  $4.2 \pm 2.6$  year-dialysis vintage. Thus, it is not surprising to find a better exercise

| Parameters                        | Patients on Long-Term Hemodialysis, n=22 | Controls, n=21 |
|-----------------------------------|--|----------------|
| Age, y                            | 58±9                                     | 59±7           |
| Sex, male, n (%)                  | 14 (64)                                  | 10 (48)        |
| Charlson score                    | 3.6±1.4                                  | —              |
| Duration of CKD, y                | 16.0±11.6                                | —              |
| Dialysis vintage, y               | 4.2±2.6                                  | —              |
| BMI, kg/m <sup>2</sup>            | 25.7±4.3                                 | 24.7±3.3       |
| Voorrips score                    | 6.1±4.3                                  | 6.2±4.1        |
| Maximal voluntary force, N.m      | 144.4±43.7                               | 127.1±26.6     |
| Creatinine index, mg/kg per d     | 20.7±2.5                                 | —              |
| Quadriiceps endurance time, min   | 4.1±2.2                                  | 13.4±6.1       |
| 6-minute walking distance, m      | 478±102                                  | 666±79         |
| <b>Initial nephropathy, n (%)</b> |  |                |
| Glomerulonephritis                | 7 (32)                                   | —              |
| Polycystic kidney disease         | 6 (27)                                   | —              |
| Vesico-ureteral reflux            | 4 (18)                                   | —              |
| IgA nephropathy                   | 2 (9)                                    | —              |
| Hypertensive nephrosclerosis      | 2 (9)                                    | —              |
| Diabetes mellitus                 | 1 (5)                                    | —              |
| Serum creatinine, mg/dl           | 9.3±2.8                                  | 0.8±0.2        |
| Serum albumin, g/dl               | 4.3±0.6                                  | 4.6±0.2        |
| hs-CRP, mg/L                      | 6.7±10.0                                 | 1.3±1.1        |
| nPCR, g/kg/d                      | 1.0±0.2                                  | —              |
| Hb, g/dL                          | 11.9±1.3                                 | 14.4±1.8       |
| eqKT/V                            | 1.6±0.8                                  | —              |

BMI, body mass index; hs-CRP, ultrasensitive C-reactive protein; nPCR, normalized protein catabolic rate, Hb, hemoglobin; eqKT/V, equilibrated KT/V.

capacity (6-minute walking distance) in our kidney transplant recipients before transplantation than in the general population of patients on HD (20). Unlike previous studies that focused on patients on dialysis or in patients receiving a kidney transplant, our study aimed for the first time to characterize muscle dysfunction in patients on dialysis on the waiting list, on the day of kidney transplantation (10,14,20).

Lower endurance without weakness is the main characteristic of patients on long-term HD before kidney transplantation. Quadriiceps endurance and 6-minute walking distance were lower without any weakness by contrast with previous studies (21). Similar findings have been observed in rodent models of kidney failure (22). Although the 6-minute walking test has only a moderate correlation

to maximum oxygen uptake as measured by a bicycle ergometer test, it is nevertheless a good measure of exercise capacity in patients on maintenance HD. Moreover, it depends on the muscle function and appears to be more closely related with daily life physical activities and quality of life (23). This weaker endurance is consistent with the histologic analysis showing a transition from less fatigable slow to fast fiber in patients (24) and the impaired muscle mitochondria (25). Because the Voorrips score was not lower in patients than in controls, weaker muscle endurance may not be the consequence of a disuse and cannot be attributed to the patient's physical activity reduction.

The muscle phenotype in patients on long-term HD referred for kidney transplantation is not driven by

| Parameters, μm <sup>2</sup>        | Patients on Long-Term Hemodialysis, n=21 | Control Participants, n=21 | P Value            |
|------------------------------------|--|----------------------------|--------------------|
| <b>Total cross-section area</b>    |  |                            |                    |
| All                                | 3795±119                                 | 4507±1577                  | 0.10               |
| Men                                | 4246±1205                                | 5747±1316                  | 0.02 <sup>a</sup>  |
| Women                              | 3061±306                                 | 3491±905                   | 0.22               |
| <b>Type I cross-section area</b>   |  |                            |                    |
| All                                | 4350±1203                                | 5313±2162                  | 0.06               |
| Men                                | 4676±1320                                | 6654±1678                  | 0.006 <sup>a</sup> |
| Women                              | 3821±795                                 | 4283±1751                  | 0.50               |
| <b>Type N-I cross-section area</b> |  |                            |                    |
| All                                | 3300±1121                                | 4124±1836                  | 0.09               |
| Men                                | 3804±1147                                | 5366±1326                  | 0.008 <sup>a</sup> |
| Women                              | 2480±306                                 | 3108±1574                  | 0.28               |

Type N-I, other types of fibers.

<sup>a</sup>Significant difference between groups if P<0.05 by using t test.

**Table 3. Influence of age and sex on maximum voluntary force, endurance, 6-minute walking test, and muscle structure by using a multiple linear regression model**

| Dependent Variables     | Independent Variables | $\beta$ g Difference (95% Confidence Interval) | P Value             |
|-------------------------|-----------------------|--|---------------------|
| Maximal voluntary force | Group                 | 0.26 <sup>a</sup>                              |                     |
|                         | Group+age             | -0.17 (-0.54 to 0.20)                          | 0.36                |
|                         | Group+sex             | -0.11 (-0.38 to 0.15)                          | 0.38                |
|                         | Group+age+sex         | -0.15 (-0.40 to 0.10)                          | 0.24                |
| Endurance               | Group                 | 0.76 (0.51 to 1.00)                            | <0.001 <sup>b</sup> |
|                         | Group+age             | 0.79 (0.54 to 1.03)                            | <0.001 <sup>b</sup> |
|                         | Group+sex             | 0.79 (0.57 to 1.02)                            | <0.001 <sup>b</sup> |
|                         | Group+age+sex         | 0.79 (0.56 to 1.03)                            | <0.001 <sup>b</sup> |
| 6-min walk test         | Group                 | 0.72 (0.50 to 0.94)                            | <0.001 <sup>b</sup> |
|                         | Group+age             | 0.73 (0.51 to 0.94)                            | <0.001 <sup>b</sup> |
|                         | Group+sex             | 0.77 (0.56 to 0.98)                            | <0.001 <sup>b</sup> |
|                         | Group+age+sex         | 0.82 (0.64 to 1.00)                            | <0.001 <sup>b</sup> |
| Type I fibers, %        | Group                 | 0.40 (0.10 to 0.70)                            | 0.01 <sup>b</sup>   |
|                         | Group+age             | 0.39 (0.09 to 0.68)                            | 0.01 <sup>b</sup>   |
|                         | Group+sex             | 0.45 (0.15 to 0.76)                            | 0.005 <sup>b</sup>  |
|                         | Group+age+sex         | 0.43 (0.12 to 0.73)                            | 0.008 <sup>b</sup>  |
| Type NI fibers, %       | Group                 | -0.40 (-0.70 to -0.10)                         | 0.01 <sup>b</sup>   |
|                         | Group+age             | -0.39 (-0.68 to -0.09)                         | 0.01 <sup>b</sup>   |
|                         | Group+sex             | -0.45 (-0.75 to -0.15)                         | 0.005 <sup>b</sup>  |
|                         | Group+age+sex         | -0.43 (-0.73 to -0.12)                         | 0.008 <sup>b</sup>  |
| Type I CSA              | Group                 | 0.29 (-0.02 to 0.60)                           | 0.06                |
|                         | Group+age             | 0.28 (-0.03 to 0.59)                           | 0.08                |
|                         | Group+sex             | 0.37 (0.093 to 0.65)                           | 0.01 <sup>b</sup>   |
|                         | Group+age+sex         | 0.37 (0.08 to 0.66)                            | 0.01 <sup>b</sup>   |
| Type N-I CSA            | Group                 | 0.27 (-0.04 to 0.58)                           | 0.09                |
|                         | Group+age             | 0.26 (-0.06 to 0.57)                           | 0.10                |
|                         | Group+sex             | 0.37 (0.11 to 0.63)                            | 0.006 <sup>b</sup>  |
|                         | Group+age+sex         | 0.39 (0.12 to 0.65)                            | 0.004 <sup>b</sup>  |
| Total CSA               | Group                 | 0.26 (-0.05 to 0.57)                           | 0.10                |
|                         | Group+age             | 0.24 (-0.07 to 0.55)                           | 0.12                |
|                         | Group+sex             | 0.36 (0.12 to 0.61)                            | 0.005 <sup>b</sup>  |
|                         | Group+age+sex         | 0.38 (0.13 to 0.63)                            | 0.003 <sup>b</sup>  |
| Mitochondrial density   | Group                 | 0.70 (0.41 to 0.99)                            | <0.001 <sup>b</sup> |
|                         | Group+age             | 0.67 (0.39 to 0.96)                            | <0.001 <sup>b</sup> |
|                         | Group+sex             | 0.68 (0.39 to 0.96)                            | <0.001 <sup>b</sup> |
|                         | Group+age+sex         | 0.67 (0.38 to 0.96)                            | <0.001 <sup>b</sup> |
| Mitochondrial area      | Group                 | -0.64 (-0.95 to -0.33)                         | <0.001 <sup>b</sup> |
|                         | Group+age             | -0.63 (-0.94 to -0.31)                         | <0.001 <sup>b</sup> |
|                         | Group+sex             | -0.61 (-0.90 to -0.31)                         | <0.001 <sup>b</sup> |
|                         | Group+age+sex         | -0.61 (-0.92 to -0.31)                         | <0.001 <sup>b</sup> |

NI, no type 1 fibers; CSA, cross-section area.

<sup>a</sup>P value corresponding to the independent variable "Group (control/patient)" in the model when adjusted (or not) on age, sex, or age+sex. For each multiple regression, the difference (95% confidence interval) of the standardized group coefficient ( $\beta$ g) was indicated.

<sup>b</sup>P<0.05.

sarcopenia. In our study, no patient presented a diagnosis of sarcopenia, according to maximal voluntary force and creatinine index measurements. Indeed, after adjustment for age and sex, maximal voluntary force was not statistically different from controls frequency-matched for age, sex, and physical activity level, and almost twice the previously reported level in general patients on long-term HD (19). Creatinine index was above the clinical limit of 18.82 mg/kg per day described for the diagnosis of uremia-related muscle atrophy (17) in all patients.

Histologic analysis suggests a nonsignificant difference in muscle fiber cross-section area between patients and controls. However, after adjustment for sex and age, a significant reduction in fiber size was obtained mainly in men (Tables 2 and 3). As a result, the smaller myotube size in men would then suggest the existence of a potential sub-clinical atrophy, which could be related to a weak disequilibrium between synthesis and degradation pathways. In

line with this hypothesis, a weaker muscle *Igf-1* expression, without activation of Forkhead box O1 and 3, was observed.

In contrast with previous studies showing an exacerbation of protein catabolism mainly due to ubiquitin-proteasome system, or myostatin increase (26) in patients on HD, here in patients who are pretransplant, we did not find any activation of the system or other catabolic pathways (ubiquitin-proteasome myostatin, calpain 1 and caspase 3) in patient muscle in men or in women (Figure 2 and Supplemental Figure 1). The lack of an ubiquitin proteasome system-driven atrophy in our highly selected patients on long-term HD before kidney transplantation supports the hypothesis of a moderate chronic disequilibrium between mass regulation pathways.

Fiber switch and mitochondrial dysfunction muscle energetics lead to the muscle phenotype. Histologic analysis showed a transition from slow to fast fiber in patients, in

agreement with previous observations (5) and in concordance with the weaker endurance. There is accumulating evidence in patients with CKD of an “acquired mitochondrial myopathy” (6,9), with reduction of mitochondrial mass (27) and respiratory function (28). In this context, our study is the first to show evidence of this acquired mitochondrial myopathy in patients who are pretransplant. Indeed, by contrast to controls, mitochondria were found to be sparse and swollen in muscle fibers of patients (Figure 3, A–C), as previously reported. Mitochondrial function is acknowledged to depend on the organelle structure (29). Thus, the mitochondrial abnormalities observed in this study could be related to mitochondria dysfunction suggesting a decoupling of oxidative phosphorylation (30). However, no difference in mitochondrial DNA content was observed, in agreement with a recent study (31). Also, factors involved in mitochondria biogenesis, such as peroxisome proliferator activator receptor  $\gamma$  coactivator-1 $\alpha$  and mitochondrial transcription factor A, and mitochondrial dynamic balance factors between fission and fusion were not altered in our patients waiting for kidney transplantation (32). Our results are also in line with an early onset of mitochondrial abnormalities during CKD time course (30,33).

The mechanisms of pretransplantation muscle phenotype are autophagy and mitophagy. Moreover, we have observed an activation of autophagy pathway in the muscle of patients before transplantation (34). Autophagosomes accompanied by a higher LC3-II/LC3-I ratio have been observed on muscle biopsy (35). In accordance with this result, stronger expressions of both *Gabralp1gene* and *Bnip3l* (implicated in mitophagy) were observed in our patients. An activation of the autophagy has previously been reported in the muscle of patients with CKD (4), and was induced by serum of uremic mice (8). Mitophagy features in our patients on long-term HD before transplantation suggest a muscle adaptive response to improve or restore the altered mitochondrial function in the muscle (36). However, Su *et al.* reported a deleterious effect of autophagy on mitochondrial function in myotubes cultured in uremic condition (8). The discrepancy between these *in vitro* and *in vivo* studies limits the interpretation of mitophagy features observed in our patients.

The loss of “muscle oxidative” phenotype in patients on long-term HD could be related to the muscle microenvironment. The uremic milieu could be responsible for such early “acquired mitochondrial myopathy.” Culture of myocytes with uremic toxin revealed a mitochondrial dysfunction and ATP shortage (37). In addition, uremic toxin indoxyl sulfate, which did not alter muscle mass (37), impaired endurance and mitochondrial function in CKD mice. Similarly, mice muscle exposure to uremic toxins decreases oxidative phosphorylation and increases reactive oxygen species (38). Last, the negative correlation between the 6-minute walking distance and dialysis vintage ( $r = -0.66$ ;  $P < 0.001$ ) is in line with the role of uremic milieu on the skeletal muscle of our patients on long-term HD (Supplemental Figure 2 and Supplemental Table 1).

In the muscle of patients with CKD, a smaller muscle capillarization may lead to muscle hypoxia (39). However, the muscle O<sub>2</sub> kinetics during exercise may indicate reduced mitochondria capacity (40), in line with the

mitochondria swelling found in our study and the lower mitochondrial density and function found previously (41). Although there is a tight coupling between muscle capillary supply and O<sub>2</sub> mitochondrial utilization (42,43), the link between the role of disturbed microcirculation and hypoxia on mitochondrial impairment remains to be assessed in patients on HD. Recently, it has been shown that uremic toxins impair skeletal muscle regeneration (44).

Perspective: skeletal muscle phenotype in pretransplant patients on long-term HD and muscle recovery after kidney transplantation.

The capacity of the skeletal muscle to recover—after kidney transplantation in particular—is a major issue with a heterogeneous response. Some studies have shown a lack of muscle function/mass recovery after kidney transplantation (45), or the lack of response to nutritional and exercise intervention in kidney transplant candidates. Conversely, Lim *et al.* have shown significant improvements in the maximal oxygen uptake in patients 12 months after kidney transplantation (46). These indirect muscle parameters indicate an improvement in muscle function after transplantation (46). Interestingly, Nanmoku *et al.* have shown that muscle mass worsening after transplantation was linked with muscle mass reduction before transplantation, suggesting a role of muscle dysfunction phenotype that influences the response to kidney transplantation (47).

In patients on long-term HD awaiting transplantation, our study shows, for the first time, a poor muscle endurance without weakness or proteasome activation. These observations were obtained in a relatively small population of highly selected patients on HD with few comorbidities; therefore, such potential generalization should be taken with caution. However, given the unique characteristics of our selected population, the evidence of a “poorly enduring, low oxidative” muscle phenotype appears specific of uremia, independently of age, sex, and potential comorbidity. This muscle dysfunction may be explained by both the transition from type I to type II muscle fibers and an alteration of mitochondria structure through the activation of autophagy and mitophagy. The relative role of uremic toxins, altered microcirculatory oxygen transport, and delivery deserve further studies to understand the recovery capacity post-transplantation.

#### Disclosures

J.-P. Cristol reports receiving research funding from Siemens; receiving honoraria from Fresenius Medical Care, Radiometer, and Servier; and other interests/relationships with the Dialysis center AIDER santé. M. Le Quintrec reports receiving honoraria from Alexion. M. Hayot reports serving as a scientific advisor or member of the Research committee of Korian Group, France. All remaining authors have nothing to disclose.

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