In order to challenge in vivo muscle Ca\(^{2+}\) homeostasis and analyze consequences on mitochondrial H\(_2\)O\(_2\) release (MHR) and sarcopenia, we injected Ca\(^{2+}\) ionophore A23187 (200 µg/kg, ip) in adult and old rats and measured gastrocnemius mass and mitochondrial Ca\(^{2+}\) content (MCC) using radioactive Ca\(^{48}\) 48 h after injection. In a second experiment performed in old rats, we measured isocitrate dehydrogenase (ICDH) activity as an index of MCC, MHR, mitochondrial respiration, citrate synthase, COX and antioxidant enzyme activities 24 h after a 150 µg/kg injection. In adult rats, muscle mass and MCC were unchanged by A23187. In old rats, MCC increased 24 h after injection as reflected by a significant increase in ICDH activity; measured MCC tended to increase at 48 h. MHR and Mn-SOD activity were significantly increased at 24 h, and GPX activity was reduced. Muscle mass was unchanged but was negatively correlated with MCC in control and treated old rats. In conclusion, in old rats, A23187 probably induced a mitochondrial Ca\(^{2+}\) overload responsible for the observed increase in MHR without leading to muscle atrophy on a short term basis.

**Keywords:** aging, calcium, skeletal muscle, free radicals, mitochondria, A23187, SOD, GPX.

**INTRODUCTION**

The loss of skeletal muscle mass and function during aging (sarcopenia) causes loss of autonomy and increases morbidity in the elderly (1). Changes in the rates of muscle protein synthesis and degradation, but also of mitochondrial function are contributing factors for the development of sarcopenia [2,3,4]). In particular, age-related muscle protein loss could be related to protein oxidation since it has been
shown that oxidized proteins are prone to degradation [5]). Oxidation of muscle proteins during aging could occur due to increased generation of reactive oxygen species (ROS). ROS are generated by several cellular or exogenous pathways, but a large proportion is produced by the mitochondrial respiratory chain [6]) at the level of complexes I and III [7]). Calcium (Ca\(^{2+}\)) could play a role in mitochondrial ROS generation during aging. Indeed, accumulation of Ca\(^{2+}\) in mitochondria increases ROS production [8,9]). In addition, mitochondria are known to buffer cytosolic Ca\(^{2+}\) during excitation-contraction coupling [10]) and pathophysiological circumstances [11]). It was shown that Ca\(^{2+}\) uptake by sarcoplasmic reticulum is reduced during aging [12]). This could in turn increase intracellular and mitochondrial Ca\(^{2+}\) content and thus trigger ROS production, muscle protein oxidation, necrosis [13]) and apoptosis by outer membrane permeabilization and release of apoptotic factors (for review see [14]). Oxidative stress and Ca\(^{2+}\) homeostasis are closely related and could be involved in skeletal muscle aging, but to our knowledge, the link between Ca\(^{2+}\) and mitochondrial ROS generation in skeletal muscle during aging has not been studied. The aim of this work was to determine if excess mitochondrial Ca\(^{2+}\) induced by in vivo administration of the Ca\(^{2+}\) ionophore A23187 [15]) could increase mitochondrial ROS generation (estimated by mitochondrial H\(_2\)O\(_2\) release - MHR), and thus potentially lead to a loss of muscle mass.

MATERIAL AND METHODS

Animals and experimental design

All procedures were performed according to current legislation on animal experimentation in France. We used 6 (adult) and 21-23 (old) -month old male Wistar rats obtained from Elevage Janvier (Le Genest-St-Isle, France). They were maintained in standard conditions with temperature controlled at 22°C, 12 h:12 h light-dark cycles, and with water and standard laboratory pellets ad libitum. In a first experiment, we analyzed the effect of calcium ionophore A23187 on mitochondrial Ca\(^{2+}\) concentration (MCC). Adult (n=7) and old (n=6) treated animals received an intraperitoneal injection of A23187 (200 µg/kg) dissolved in ethanol (0.67 ml/kg) and age-matched control animals (n = 6 per age) received ethanol alone (0.67 ml/kg). Animals were sacrificed 48 h after injection. The results of this experiment led us to analyze the effect of A23187 on MHR in a second experiment performed only in old animals (21 mo) at an earlier time (24 h) after A23187 injection, comparing A23187 injected (n=8) and vehicle injected (n=7) animals. We also slightly reduced the amount of ionophore injected (150 µg/kg) to avoid any mortality. In all cases, general anesthesia was induced by intraperitoneal injection and gastrocnemius muscle from each limb was quickly excised, stripped of all non-muscular tissues and weighed. Muscles used for Ca\(^{2+}\) measurement and enzymatic assays were stored immediately after excision in liquid nitrogen before storage at -80°C while mitochondria isolation procedures were performed immediately on the fresh tissue.

Experiment 1: measurement of in vivo mitochondrial Ca\(^{2+}\) content after ionophore injection

On the day of sacrifice, rats were anesthetized using a mixture of ketamine (120 mg/kg) and xylazine (6 mg/kg) solubilized in NaCl (0.9%). Radioactive calcium (\(^{45}\)Ca, 0.925 MBq dissolved in
0.5 ml of 0.9% NaCl - Amersham Biosciences, Orsay, France) was injected in the left saphenous vein. In 2 old rats, this injection could not be performed, and only 5 animals per group were used in old rats. Anesthesia was maintained for 45 min to allow $^{45}$Ca to diffuse in the organism so that specific activity of the tracer became uniform in every cell compartment. Gastrocnemius of both legs were harvested and weighed. Muscle mitochondria were prepared according to Pepe et al. [16]). Extraction medium (pH 7.4) contained 250 mM sucrose, 5 mM HEPES, 2 mM EDTA, and also 30 µM diltiazem and 3.2 µM ruthenium red to prevent Ca$^{2+}$ entering the mitochondria through the Ca$^{2+}$ uniport and escaping from the mitochondrial matrix through Na/Ca exchange. Ruthenium red was prepared according to Luft [17]). Mitochondrial Ca$^{2+}$ was extracted with perchloric acid (0.6 M, final concentration). After homogenization, the extract was centrifuged (10,000 g, 5 minutes) and the supernatant put in a scintillation vial containing 10 ml of Ultima Gold (Perkin Elmer, Courtaboeuf, France). $^{45}$Ca radioactivity was measured (2000CA, Packard) for one hour and corrected for quenching and chemoluminescence. Ca$^{2+}$ specific activity was determined in the plasma by evaluating Ca$^{2+}$ radioactivity as mentioned above. The amount of Ca$^{2+}$ in the plasma was determined by atomic absorption photometry (Perkin Elmer 3300, Courtaboeuf, France - absorption wavelength set at 423 nm) after perchloric acid extraction and addition of lanthane chloride (0.1%) in the extract. The specific activity of Ca$^{2+}$ was considered to be similar in the plasma and mitochondrial compartments after 45 minutes of diffusion in the organism [18]). MCC was calculated as the ratio of mitochondrial radioactivity to plasma specific activity and expressed in nmol Ca$^{2+}$/mg mitochondrial protein.

Experiment 2: effect of ionophore injection on mitochondrial metabolism

a) Mitochondria isolation

Mitochondria were isolated immediately after gastrocnemius muscle sampling as described previously [19]), using subtilisin as proteolytic enzyme. The final pellet of mitochondria was suspended in 0.3 M sucrose at 4-6 mg/ml approximate protein concentration.

b) Respiratory measurements

Mitochondrial oxygen consumption (nmol oxygen/min/mg mitochondrial protein) was monitored at 30°C with a Clark-type oxygen electrode (Hansatech Norfolk, UK) in 0.3 M sucrose, 10 mM KH$_2$PO$_4$, 20 mM Tris, 2 mM EDTA, pH 7.4. The mitochondrial suspension (0.15 mg protein) was added first. Then the substrate (5 mM glutamate + 5 mM malate or 24 µM palmitoyl carnitine + 2 mM malate) and 360 µM ADP were rapidly added one after the other to measure state 3 and state 4 respiration rates.

c) Mitochondrial H$_2$O$_2$ release

MHR was measured as described previously [19]) with some modifications. The principle of this method is to incubate mitochondria and to measure H$_2$O$_2$ produced after removal of mitochondria by centrifugation, avoiding any interference with fluorescent components of the respiratory chain. In addition, the fluorescent probe is added only after mitochondria removal and can be oxidized only by H$_2$O$_2$ that was produced before, during incubation. Briefly, isolated mitochondria (0.2 mg/ml of mitochondrial proteins) were suspended in an incubation buffer (0.3 M sucrose, 20 mM Tris, 10 mM KH$_2$PO$_4$, 2 mM EDTA, pH 7.4) supplemented or not with 10 µM antimycin A under continuous magnetic stirring at 37°C. A sample was taken to serve as control and stored on ice until further analysis. The mitochondria were incubated 1 minute after addition of 5 mM glutamate and 5 mM malate into the respiration medium. A second sample was taken and stored on ice. Samples were centrifuged (9,000 g, 5 min, 4°C) to remove mitochondria and supernatants were meticulously collected. H$_2$O$_2$ measurement was performed in each supernatant by fluorimetric analysis after addition of 2',7'-dichlorofluorescin (DCFH - 125 µM and 12.5 µM for assays with and without antimycin A, respectively) prepared just before use from
diacetyldichlorofluorescein (DCFDA) according to Black & Brandt [20]). Oxidation of DCFH by \( \text{H}_2\text{O}_2 \) to the fluorescent 2',7'-dichlorofluorescein (DCF) in the presence of horseradish peroxidase (12.5 units/ml) was evaluated on a FLx800 fluorimeter (Bio-Tek, Saint Quentin, France) with excitation and emission wavelengths set to 485 and 528 nm, respectively. Calibration in the same experimental conditions was performed with known amounts of \( \text{H}_2\text{O}_2 \). Comparison of \( \text{H}_2\text{O}_2 \) amounts in control and after incubation reflected MHR. Values were expressed in nmol \( \text{H}_2\text{O}_2/\text{min/mg mitochondrial protein}. \\

d) Enzymatic assays
Muscles were finely pulverized in liquid nitrogen, homogenized in cold buffer containing 20 mM Tris-HCl, 250 mM sucrose, 40 mM KCl, 2 mM EDTA, pH 7.2 (1:10 w/v) and centrifuged at 1,000 g for 10 minutes at 4°C. Supernatants were collected and used for measurement of catalase activity as described by Aebi [21]), or centrifuged again at 10,000 g for glutathione peroxidase (GPX) and superoxide dismutase (SOD) assays. GPX activity was assessed as reported previously [22]). SOD activity was assayed with the RANSOD kit from Randox laboratories (Montpellier, France). Inhibition of Cu-Zn SOD by addition of 1 mM sodium cyanide to the reaction buffer allowed measurement of mitochondrial manganese-dependent SOD (Mn-SOD) activity. Citrate synthase (CS) activity was evaluated in muscle homogenate prepared for mitochondria isolation as described previously [23]). Isocitrate dehydrogenase (ICDH) activity was measured on isolated mitochondria. They were treated with Triton X-100 10% (9:1 vol/vol) according to Robinson et al [23]). Cytochrome c oxidase (COX) activity was determined by monitoring the oxidation of reduced cytochrome c at 550 nm in isolated mitochondria from gastrocnemius muscle.

e) Mitochondrial protein oxidative damage

The carbonyl content of mitochondrial fractions (100 µg of proteins) was determined in triplicate as described previously [24]) by reaction with \(^3\text{H} \) sodium borohydride (9.5 mCi/mmol - Amersham Bioscience, Orsay, France). Radioactivity was measured with a liquid scintillation analyzer (Packard). The carbonyl content was expressed in \( 10^3 \) DPM/mg mitochondrial protein.

Statistical analysis

Results are presented as mean ± SE and were analyzed using one-way (or two-way when specified) variance analysis and t test. A paired t test was used to compare \( \text{H}_2\text{O}_2 \) release with or without antimycin A for the same mitochondria preparation. Simple regression analysis was performed to study the link between MCC and muscle weight. Variance-covariance analysis was used to analyze the effect of A23187 treatment on MCC using muscle weight as a covariate. Differences were considered significant at P lower than 0.05.

RESULTS

Influence of A23187 on muscle weight and mitochondrial Ca\(^{2+}\) content

1) First experiment: Gastrocnemius weight was significantly reduced during aging but was unchanged 48 h after A23187 injection whatever the age (in % of body weight: 0.52 ± 0.01; 0.53 ± 0.02; 0.41 ± 0.08*; 0.38 ± 0.02*, for adult control, adult A23187, old control and old A23187 rats respectively (n = 6 per group; *: P < 0.05 from corresponding adult group). Mitochondrial Ca\(^{2+}\) content (MCC) was evaluated after mitochondrial extraction under conditions such that influx of the divalent cation into and its efflux from the organelles were
In old control rats, we obtained a very high value for MCC in one animal, and it is questionable whether this rat should be included or not in the analysis. In old rats, MCC was inversely correlated with muscle weight whatever the group (Figure 1A, P < 0.05) and whether the "extreme" old control rat was included or not. In adult animals, no linear correlation was found between these parameters (Figure 1A). Treatment had no significant effect on MCC 48 h after A23187 administration in adult rats (Mean ± SE in nmol / mg mitochondrial protein: 1.98 ± 0.22 in adult control rats with n=7; 1.91 ± 0.15 in adult treated rats with n=6; see Figure 1B). In old rats, there was no significant effect when including the "extreme" old control rat. When excluding it, mean (± SE) values for MCC were 1.67 ± 0.31 nmol / mg mitochondrial protein (n=4) in control old rats and were 2.24 ± 0.61 (n=5) in treated old rats. This difference was significant when using a variance-covariance analysis with muscle weight as a covariate (Figure 1B). MCC was unchanged with age.

2) In the second experiment performed only in old rats (injection of 150 µg/kg, sacrifice after 24 h), no effect of A23187 was observed on muscle weight (0.42 ± 0.02 vs 0.43 ± 0.02 % of body weight for control (n = 7) and A23187 (n = 8) rats respectively). In this experiment, our main objective was to measure MHR. Thus, we could not measure MCC using radioactive Ca²⁺ (radioactive contamination, amount of isolated mitochondria which was necessary). We used ICDH activity as a marker of Ca²⁺ concentration, since it has been well described that an increase in mitochondrial Ca²⁺ concentration stimulates this activity [11]). A23187 injection caused a significant rise in mitochondrial ICDH activity (Figure 2), suggesting an increase in MCC. Interestingly, ICDH activity was also negatively correlated with muscle weight, but it was significant only in the control group (data not shown).

Effect of A23187 on mitochondrial metabolism (second experiment)

1) Respiration: Oxygen consumption during state 3 and 4 was unaffected by A23187 treatment when palmitoyl carnitine/malate or glutamate/malate were used as substrate (Table 1). Respiratory control ratio (RCR) was also unchanged by treatment whatever the substrates (Table 1). RCR values were consistent with a good functionality of isolated mitochondria. ATP synthesis seemed also unchanged because ADP/O ratio (in the presence of glutamate and malate as substrates) was unaffected by treatment (2.6 ± 0.2 vs 2.5 ± 0.1 in control and A23187 groups respectively). Finally, whatever the treatment, state 3 respiration rates were lower with palmitoyl carnitine/malate as substrates than with glutamate/malate.

2) Mitochondrial H₂O₂ release: The effect of A23187 on MHR was analyzed in the presence of glutamate and malate as substrates. A two-fold increase in MHR was observed in the A23187 group as compared to control animals (Figure 3A). When the electron transport chain was fully reduced by antimycin A, MHR increased 7-fold and 4-fold in the control and the A23187 group, respectively.
Figure 1: Mitochondrial Ca\(^{2+}\) content was negatively correlated with muscle weight in old rats and not in adult rats (1A); Effect of A23187 injection (1B). Mitochondria were isolated from 6 or 23 month-old control and A23187 treated rat gastrocnemius muscles and mitochondrial Ca\(^{2+}\) content (MCC) was evaluated as described in materials and methods. Muscle weights and MCC values are expressed in gram and in nmol/mg mitochondrial protein respectively. 1A: One control old rat had a very high value for MCC ("extreme" old control rat) and it is questionable whether it should be included in the analysis. There was a significant correlation between MCC and muscle weight in old control and A23187 rats (whether the "extreme" old control rat was included or not), but not in adult rats. Mean values (± SE) are given for adult rats (n=7 and 6 in control and treated group respectively), and individual values are given for old rats, as well as regression values (correlation coefficient R\(^2\) and P). 1B: Effect of A23187 injection on MCC in adult and old rats. There was no significant effect of A23187 on MCC in adult rats. *: in old rats, it was only when the "extreme" old control rat was excluded, and when the variability induced by the correlation between muscle weight and MCC was taken into account by variance-covariance analysis, than a significant difference (P = 0.05) was found between control and treated old rats.
Figure 3B. In that condition, MHR remained significantly higher in the A23187 group than in the control group.

3) Protein oxidative damage: Mitochondrial protein carbonyl content was not significantly different between A23187 and control groups (Figure 4).

4) Mitochondrial and antioxidant enzyme activities: CS activity was significantly decreased after A23187 injection (240 ± 47 vs 120 ± 29* mU/mg muscle protein, in control and A23187 groups respectively, *: P < 0.05 from control). COX activity remained similar between control and treated rats (7.6 ± 1.5 vs 9.9 ± 1.4 µmol/min/mg mitochondrial protein, respectively). Catalase and Cu-Zn SOD activities were not affected by treatment, but Mn-SOD activity was significantly higher in the A23187 group as compared to the control group (Table 2). On the contrary, after A23187 injection, GPX activity was significantly downregulated (Table 2).

Table 1: Effect of in vivo A23187 injection on mitochondrial respiration in old rat muscle

<table>
<thead>
<tr>
<th>Groups</th>
<th>G/M</th>
<th>P/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (4)</td>
<td>200.6 ± 5.3</td>
<td>18.0 ± 3.7</td>
</tr>
<tr>
<td>A23187 (7)</td>
<td>215.6 ± 15.8</td>
<td>15.7 ± 2.5</td>
</tr>
<tr>
<td>State III</td>
<td>45.2 ± 9.4 *</td>
<td>13.7 ± 3.7</td>
</tr>
<tr>
<td>A23187 (7)</td>
<td>50.9 ± 7.2 *</td>
<td></td>
</tr>
<tr>
<td>State IV</td>
<td>16.7 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>RCR</td>
<td>12.2 ± 1.7</td>
<td>16.1 ± 2.8</td>
</tr>
<tr>
<td>A23187 (7)</td>
<td>4.5 ± 1.6 *</td>
<td></td>
</tr>
</tbody>
</table>

21 month-old rats were injected intraperitoneally with Ca²⁺ ionophore A23187. Twenty four hours after injection, mitochondria were isolated from gastrocnemius muscle and isocitrate dehydrogenase activity was measured as described in materials and methods. Values shown are means ± S.E. (n = 7) and are expressed in nmol/min/mg mitochondrial protein. *: significantly different from control group (P < 0.05).
21 month-old rats were injected intraperitoneally with Ca\(^{2+}\) ionophore A23187. Twenty four hours after injection, mitochondria were isolated from gastrocnemius muscle, incubated with glutamate/malate as substrates, and H\(_2\)O\(_2\) release (MHR) was measured as described in materials and methods. Values shown are means ± S.E (n = 5/8) and are expressed in nmol H\(_2\)O\(_2\)/min/mg mitochondrial protein. MHR was evaluated in the absence of (A) or in the presence of 10 µM antimycin A (B). *: significantly different from control group (P < 0.05), #: significantly different from incubation without antimycin A (P < 0.05).

21 month-old rats were injected intraperitoneally with Ca\(^{2+}\) ionophore A23187. Twenty four hours after injection, mitochondria were isolated from gastrocnemius muscle and protein carbonyl content was evaluated as described in materials and methods. Values shown are means ± S.E. (n = 7/8) and are expressed in 10\(^3\) DPM/mg mitochondrial protein.
DISCUSSION

Our aim was to analyze the interaction between skeletal muscle mitochondrial calcium content (MCC), mitochondrial H$_2$O$_2$ release (MHR) and muscle atrophy during aging. The reduction of Ca$^{2+}$ uptake by sarcoplasmic reticulum during aging [12] could result in an increase in average MCC and in turn stimulate MHR [8,25,26,27]). Thus, alteration of Ca$^{2+}$ homeostasis could contribute to the development of oxidative stress and apoptosis in muscle and/or to the loss of muscle fibers during the aging process through an increase in degradation of oxidized proteins. We demonstrated that in vivo mitochondrial Ca$^{2+}$ overload increases oxidative stress in skeletal muscle.

In order to challenge muscle Ca$^{2+}$ homeostasis in vivo, we injected old rats with Ca$^{2+}$ ionophore A23187 which is known to increase membrane permeability to the Ca$^{2+}$ ion and thus should induce a re-equilibration of Ca$^{2+}$ gradients between mitochondria, cytosol and the extracellular compartment [15,28]). The consequences of this treatment were analyzed in adult and old rat gastrocnemius muscles. Forty-eight hours after A23187 injection, MCC was unchanged in adult rats. It tended to increase in old rats (+40%), but the results were not clear-cut due to the variability introduced by the significant relation observed between MCC and muscle weight in old rats. Furthermore, an extreme MCC value was observed in the control group of aged animals. The value was out of the Normal law and we decided to outclude it. Under these conditions, A23187 treatment significantly increased MCC. This was testified by ICDH activity that was significantly increased 24 h after injection [29]).

A23187 injection significantly increased glutamate/malate supported MHR. This effect is likely to be due to the increase in MCC. Indeed, in vitro addition of Ca$^{2+}$ in isolated heart [8]) and liver [25,26,27]) mitochondria significantly increased MHR. It was proposed that Ca$^{2+}$ could increase mitochondrial superoxide production through a molecular rearrangement of the mitochondrial inner membrane [30]). This is consistent with the fact that the A23187 mediated increase in MHR was still significant when the electron transport chain was fully

| Table 2: Effect of in vivo A23187 injection on muscle antioxidant enzyme activities in old rats |
|---------------------------------|-----------------|-----------------|
| Cu/Zn SOD                       | 243 ± 14        | 253 ± 13        |
| Mn-SOD                          | 6.9 ± 0.2       | 7.7 ± 0.2 *     |
| GPX                             | 6.3 ± 0.4       | 5.4 ± 0.3 *     |
| Catalase                        | 277 ± 32        | 276 ± 46        |

21 month-old rats were injected intraperitoneally with Ca$^{2+}$ ionophore A23187. Cu/Zn and Mn-SOD, GPX and catalase activities were measured in gastrocnemius muscle as described in materials and methods. Values shown are means ± S.E. (n = 6/7) and are expressed in U/min/g tissue. *: significantly different from control animals (P ≤ 0.05).
reduced by antimycin A: calcium seems to be able to induce a permanent modification in mitochondrial inner membrane which increases MHR in the basal state and in the antimycin A stimulated state. In contrast, we showed previously (19) that the increase in glutamate/malate supported MHR observed in soleus muscle during normal aging disappeared after antimycin A treatment. In that case, variations in MHR during aging depended only on the reduction state of the electron transport chain. Thus, finally, our present results are consistent with a stimulation of muscle MHR through an increase in MCC by A23187 injection.

This observed increase in MHR also depends on variations of Mn-SOD and GPX activities. Mn-SOD activity was increased in A23187 injected animals (+12%), and GPX activity was reduced (-14%). Both factors could contribute to the observed increase in net MHR. However, given the magnitude of increase in MHR after A23187 treatment (+120%), it is likely that it was essentially due to an increase in superoxide production. The observed increase in Mn-SOD activity reflects probably a protective adaptative response. The observed decrease in GPX activity contributed to the A23187 induced oxidative stress.

Citrate synthase activity measured in muscle total homogenates was markedly reduced in treated animals compared to control animals whereas measurements performed in mitochondrial fractions showed similar activity levels between control and A23187 groups (data not shown). These data suggest a decrease in mitochondria number. This decrease could be due to the elimination of damaged mitochondria.

Several observations indicate that oxidative stress was under control at the time of measurement: mitochondrial respiration was normal, mitochondrial protein carbonyl content was unchanged, and no sign of oxidative stress was apparent outside the mitochondrial compartment since catalase and Cu-Zn SOD activities remained unchanged. Accordingly, muscle mass was unchanged, even 48 h after treatment. However, the negative correlation between MCC and muscle mass that was specifically observed in old rats confirms the hypothesis that MCC could be a factor involved in the progression of age-related sarcopenia.

In conclusion, the capacity to maintain calcium homeostasis is altered during aging: calcium ionophore injection had no effect in adult rats, but it induced an increase in MCC in old rat gastrocnemius muscle. This mitochondrial Ca\(^{2+}\) overload was able to stimulate MHR, probably leading to autophagy of damaged mitochondria [31]) as suggested by the decrease in muscular citrate synthase activity. This Ca\(^{2+}\) induced mitochondrial oxidative stress could lead to a loss of muscle mass through apoptosis, necrosis or stimulation of proteolysis after protein oxidation. However, 48 h after injection, muscle mass remained unaffected, suggesting that muscle loss was prevented or delayed. The significant negative correlation observed between MCC and muscle weight indicates that an increase in MCC could be involved in muscle atrophy at least on a long term basis.
Acknowledgments: We thank Hélène Lafar ge for literature management and UENC staff for animal care.

Grants: This project was funded by a "Fonction mitochondriale et biologie du muscle" grant from INRA.

REFERENCES

18. McCormack JG, Denton RM. Role of Ca^{2+} ions in the regulation of intramitochondrial metabolism in rat heart. Evidence from studies with isolated mitochondria that adrenaline activates the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes by increasing the intramitochondrial concentration of Ca^{2+}. Biochem J 1984; 218: 235-47.


Received: February 2, 2005
Accepted: July 4, 2005

Author’s address: Luc Demaison, Laboratoire de Bioénergétique Fondamentale et Appliquée, INSERM E221, Université J. Fourier, BP 53, 38041 Grenoble Cédex 09, France. Tél 33 4.76.63.57.36, Fax 33 4.76.51.42.18.
E-mail: Luc.Demaison@ujf-grenoble.fr