Altered mitochondrial apparent affinity for ADP and impaired function of mitochondrial creatine kinase in gluteus medius of patients with hip osteoarthritis

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Muscles gluteus medius; myosin heavy chains; mitochondrial respiration; energy transfer

HIP OSTEOARTHRITIS (OA) characterized by pain and stiffness of the hip joint is one of the most common chronic diseases in the elderly. The disease is linked to neuromuscular deficit, because the patients exhibit reduced abduction, adduction, and flexion muscle strength (3). These changes are largely related to dysfunction of *musculus gluteus medius* (MGM) (9, 12, 17), for this muscle plays an important role in hip abduction, gait, and stabilization of the joint and pelvis (9, 16). On the other hand, functionally normal MGM is vital to prevent the implant from loosening and hasten the recovery after total hip arthroplasty in OA patients (6, 11, 21).

Notwithstanding the potentially significant role of dysfunctional MGM in the pathogenesis of OA, only a few studies have explored the underlying cellular mechanisms. The results show selective type II (fast-twitch) muscle fiber atrophy and increase in relative content of type I (slow-twitch) fibers (23, 31, 36). Because the fast-to-slow transition of muscle cells is generally associated with changes in muscle oxidative capacity and regulation of oxidative phosphorylation (22, 35, 41), one may expect altered mitochondrial function in diseased MGM, as well. However, this issue has not yet been addressed.

The objective of this study was to assess the parameters of oxidative phosphorylation and energy transfer systems mediated by creatine and adenylate kinases (CK and AK) in MGM in relation to the grade of OA. The respiratory parameters were studied in saponin-permeabilized (skinned) muscle fibers, which allows the avoidance of artifacts due to isolation of mitochondria, enables analysis of mitochondria in the small specimens of human muscle biopsy, and preserves the natural interactions of mitochondria with other cellular structures in situ (25, 29).

MATERIALS AND METHODS

Subjects. Sixty sedentary subjects (31 males and 29 females, 65 ± 2.4 and 66 ± 2.2 years old, respectively) participated in this study. All subjects voluntarily gave informed, written consent, and the study was undertaken in accordance with the Declaration of Helsinki (39) and approved by the Tartu University Ethics Committee. The patients were divided into three groups. The control group (*n* = 15, 10/5 male/female ratio, age 68 ± 5.2) comprised the patients undergoing surgical correction of traumatic hip fracture. The two other groups included the patients with unilateral or bilateral hip replacement for OA of radiographic grade 3 (*n* = 11, 83, age 66 ± 4.1) and grade 4 (*n* = 34, 13/21, age 65 ± 1.2), estimated according to Kellgren and Lawrence (15). The muscle specimens (50–100 mg) were taken during surgery from the middle portion of the MGM. A part of each specimen was rapidly frozen in liquid nitrogen and stored at −70°C for enzyme and myosin heavy chain (MHC) analyses, whereas the rest was permeabilized (skinned) by saponin (5, 25, 29, 30) and used for oxygraphical studies. Because of the limited sample availability, the number of patients participating in different experiments varied (see figures).

Analysis of the system of oxidative phosphorylation and its coupling to mi-CK. The skinned fibers were incubated in solution B containing (in mM): 2.77 Ca<sub>2+</sub>EGTA, 7.23 K<sub>2</sub>EGTA, 1.38 MgCl<sub>2</sub>, 0.5 DTT, 100 K-Mes, 20 imidazole, 20 taurine, 3 K<sub>2</sub>HPO<sub>4</sub>·10

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weight. Coupling between oxidative phosphorylation and mi-CK was
removed from the chamber and dried overnight at 105°C. The rates of
oxidation were normalized per milligram of dry muscle
contents of MHC were not statistically different between the
grades 3
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M MGM groups studied. However, a grade 4 group (n = 29)
contained a subgroup of nine patients characterized by the lack of
either one (MHCIIIX, see third panel, inset) or both fast
isoforms (MHCIIIX and MHCIIA, see fourth panel, inset), the
latter change observed in seven patients out of nine. Such a
fiber-type dropout was not observed in the other two patient
groups. Thus progression of the disease from grade 3 to 4 was
associated with a complete loss of type II fibers in a subgroup
of patients with the severest grade of disease, this change being
in line with predominantly type II fiber atrophy observed
earlier (23, 31, 36).

To assess whether OA is associated with altered mitochon-
drial functions, the parameters of oxidative phosphorylation and
regulation of respiration by ADP and creatine in skinned
MGM fibers were studied. Figure 2 shows that in control
MGM fibers ADP (1 mM) produced an increase in the respi-
ration rate (V_{Pyr}) by 7.8 times (RCI_{Pyr}) compared with
the levels of v0 in the presence of pyruvate and malate, which
points to highly coupled state between oxidation and phosphor-
ylation in situ. The respiratory parameters with succinate
(V_{succ} and RCI_{succ}) were not statistically different (ANOVA)
from their counterparts with NADH-related substrates. Atrac-
tyloside, an inhibitor of ANT, effectively suppressed the suc-
cinate oxidation, and the atractyloside-insensitive respiration
(V_{ATR}) was mostly caused by a proton leak; these data indicate
preserved intactness of the inner mitochondrial membrane in
skinned muscle fibers. A comparison of the respiratory data
revealed no significant difference between the controls (grade
0) and diseased patients (grades 3 and 4). Thus OA did not
affect the systems of oxidative phosphorylation in MGM cells.

Figure 3A shows that in control patients, the kinetics of
activation of mitochondrial respiration by exogenous ADP
followed the Michaelis-Menten equation, with the apparent K_m
for ADP of 331 μM in the absence of creatine. A mean value
of that parameter for the control group (282 ± 56 μM, Fig. 3B)
corresponds well to that in oxidative muscles such as heart and
m. soleus but largely exceeds that in glycolytic muscles and
isolated mitochondria (10–20 μM; Refs. 18 and 38). To test
whether the CK-phosphotransfer system functions in the MGM
cells, the effect of creatine on Vo2 vs. [ADP] relationship was
determined. Creatine significantly shifted this relationship to-

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**RESULTS**

Figure 1 demonstrates that the mean values of the relative
contents of MHC were not statistically different between the

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**Fig. 1.** Relative contents of myosin heavy chain (MHC) isoforms in *musculus
gluteus medius* (MGM) of traumatic patients as controls (grade 0, n = 10) and
patients with grades 3 (n = 10) and 4 (n = 29) of osteoarthritis (OA). The
means ± SE values (as elsewhere) per group are given. *Inset:* examples of
SDS-PAGE analysis of MHC isoform distribution in MGM.

**Fig. 2.** Characterization of the parameters of oxidative phosphorylation in
skinned fibers of MGM. v0, basal respiration rate; V_{Pyr}, ADP-dependent
respiration rate in the presence of pyruvate; RCI_{Pyr}, ADP-dependent respiration
rate; RCI_{succ}, respiratory control index in the presence of pyruvate; V_{succ},
ADP-dependent respiration rate in the presence of succinate and rotenone;
V_{ATR}, respiration rate in the presence of atractyloside; RCI_{succ}, respiratory control
index in the presence of succinate and rotenone. Vo2, oxygen consumption; d.w., dry weight. n = 5–18 per

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**Fig. 3.** A shows that in control patients, the kinetics of
activation of mitochondrial respiration by exogenous ADP
followed the Michaelis-Menten equation, with the apparent K_m
for ADP of 331 μM in the absence of creatine. A mean value
of that parameter for the control group (282 ± 56 μM, Fig. 3B)
corresponds well to that in oxidative muscles such as heart and
m. soleus but largely exceeds that in glycolytic muscles and
isolated mitochondria (10–20 μM; Refs. 18 and 38). To test
whether the CK-phosphotransfer system functions in the MGM
cells, the effect of creatine on Vo2 vs. [ADP] relationship was
determined. Creatine significantly shifted this relationship to-
ward lower [ADP] (Fig. 3A), thus producing a fourfold decrease in the mean apparent $K_m$ for ADP (Fig. 3B) but exerting no effect on $V_{max}$ (Fig. 3A) or $V_0$ of respiration (not shown). This effect of creatine on apparent $K_m$ strongly indicates the coupling of mi-CK to ANT, a key process in the CK-phosphotransfer system (26).

OA (grade 4) was associated with decreased total CK activity due to reduced activities of all CK isoforms (Table 1). The mi-CK activity decreased in earlier phase of the OA (stage 3) and to a greater extent than did other isoforms. Analysis of regulation of respiration with ADP and creatine revealed a different kinetics compared with normal muscles (Fig. 3B): the apparent $K_m$ for ADP was decreased in the absence of creatine, and creatine exerted less effect on that parameter, thus giving rise to a decreased creatine index. These changes suggest increased access of exogenous ADP to mitochondria and impaired coupling of mi-CK to ANT during the progression of OA (see DISCUSSION). Table 1 shows that OA was also associated with decreased total AK activity in MGM.

DISCUSSION

To our knowledge, this study is the first to characterize the cellular energy metabolism in human MGM and its alterations associated with OA. The important finding was that mitochondria in skinned fibers of MGM of control patients exhibited an apparent $K_m$ for ADP ($282 \pm 56 \mu M)$, which is much higher value than registered for isolated mitochondria ($10–20 \mu M$) in regulation of respiration. The principal question here is whether the phenomenon of low apparent affinity of mitochondria to exogenous ADP in situ represents an artifact due to experimental conditions and limited ADP diffusion owing to long diffusion distances within the muscle fiber, or it reflects intrinsic mechanisms of intracellular regulation of mitochondrial function. In these experiments, 5 mg/ml BSA was added to the incubation medium to protect mitochondria from uncoupling caused by free fatty acids (FFA) (19, 24) produced by the phospholipases (4). Because BSA binds not only FFA, but also adenine nucleotides ($K_d = 120 \mu M$ at pH 7.4; Ref. 34), partial binding of ADP may shift the $V_0$ vs. [ADP] to the right, which gives rise to high apparent $K_m$ for ADP. Several observations contradict this interpretation, however. 1) Considering that ATP binds BSA stronger than ADP (34), the dependency of $V_0$ vs. [ATP] should have resulted in higher values of $K_m$ than that found from $V_0$ vs. [ADP], but we have registered similar $K_m$ values for ATP and ADP in skinned heart fibers (2). 2) In our experiments the incubation medium contained 3 mM inorganic phosphate, a competitive inhibitor of adenine nucleotide binding ($K_i = 1 \text{mM}$; Ref. 34), thus hindering ADP from binding to BSA. 3) We have found that in the presence of similar concentrations of BSA, isolated mitochondria always exhibit much lower $K_m$ for ADP than mitochondria in situ, in skinned slow-twitch muscle fibers (18). 4) Liobikas et al. (20) have demonstrated that the addition of 10% BSA (i.e., 20 times more than in our experiments) exerted no effect on $K_m$ for ADP in saponin-skinned heart fibers compared with control conditions with 0.2% BSA. Altogether, these data make it highly unlikely that high apparent $K_m$ for ADP in regulation of respiration in skinned MGM results from partial binding of ADP to BSA.

A number of arguments also exclude the possibility that high $K_m$ for ADP in skinned MGM is related to long diffusion distances for ADP ($R_{diff}$) from medium into cell core. In correctly performed experiments, combined mechanical and chemical (saponin) treatment results in separation of skinned muscle fibers (cells) from each other, thus making the mean

Table 1. Activities of AK and CK and distribution of the CK isoenzymes in MGM

<table>
<thead>
<tr>
<th>Disease Grade</th>
<th>Mi-CK</th>
<th>MM-CK</th>
<th>MB-CK</th>
<th>Total CK</th>
<th>Total AK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (n = 5–7)</td>
<td>44±20.4</td>
<td>823.4±30</td>
<td>64.2±23.2</td>
<td>798.6±89.24</td>
<td>86.1±6.5</td>
</tr>
<tr>
<td>3 (n = 7–9)</td>
<td>5.7±1.5*</td>
<td>651.0±62.2</td>
<td>96.2±23.8</td>
<td>724.7±79.5</td>
<td>57.2±4.9*</td>
</tr>
<tr>
<td>4 (n = 25–28)</td>
<td>17.9±3.8*</td>
<td>471.4±35.7†‡</td>
<td>47.4±7.1†</td>
<td>547.4±39.7§</td>
<td>50.7±6.1*</td>
</tr>
</tbody>
</table>

Values are presented as means± SE. Adenylate kinase (AK) and creatine kinase (CK) values are given in μmol min⁻¹ g⁻¹ wet wt. MGM, *P < 0.05, †P < 0.01 compared with disease grade 0, ‡P < 0.05 compared with OA grade 3, §P < 0.05 compared with osteoarthritis grades 0 and 3; n, number of muscles assessed.

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R_{\text{dif}} comparable to half of the fiber’s diameter (29). In permeabilized cardiac cells, type I MGM fibers, and type I m. soleus fibers, the maximum R_{\text{dif}} is 10 \mu m (29), 24 \mu m (31), and 50 \mu m (40), respectively. In spite of fivefold differences in R_{\text{dif}}, these muscle specimens exhibit similarly high \( K_m \) values for ADP in regulation of mitochondrial respiration (200–400 \mu m; Ref. 29 and Fig. 3). Furthermore, skinned fast twitch glycolytic muscle fibers having longer R_{\text{dif}} (40–50 \mu m) than MGM fibers display a very high affinity to ADP (\( K_m = 8–22 \mu m \)) comparable to that in isolated mitochondria (29). From these data, it is clear that high \( K_m \) for ADP in regulation of mitochondrial respiration in situ in skinned MGM fibers results not from specific composition of polarographic medium or morphological properties of the muscle but rather from specific organization of the intracellular energy metabolism, which may be similar to other oxidative muscles (18, 30, 38). It has been suggested that in oxidative muscle cell mitochondria, ATPases and part of the cellular adenine nucleotides are compartmentalized into functional complexes (also called intracellular energetic units, ICEUs), most probably by cytoskeletal proteins (25, 30). By creating localized restrictions on diffusion of ADP, these proteins limit the access of exogenous ADP to mitochondria, which underlies low apparent affinity of mitochondria to this nucleotide (25, 30) (Fig. 3). Within the ICEUs, the mitochondria communicate with ATPases via CK- and AK-phosphotransfer systems and/or direct transfer of adenosine nucleotides. In normal heart cells the CK-phosphotransfer system plays a predominant role in energy transduction (8, 26). The functional coupling between ANT and mi-CK converting ATP that is generated by the mitochondria to PCr and provides local/endogenous ADP to stimulate respiration via ANT represents a key step in this system. Owing to this mechanism, the mitochondrial respiration becomes less dependent on the limited ADP flux from the cytoplasm, this effect is seen as a decrease in \( K_m \) for exogenous ADP after addition of creatine (Fig. 3). Our study revealed that MGM of control patients possesses a significant amount of mi-CK activity (4.3 \pm 1.8\% of total CK activity, Table 1), which is close to that in m. soleus (6.8 \pm 2.8\%; Ref. 1). Likewise, creatine caused a fourfold decrease in apparent \( K_m \) for ADP (Fig. 3), thus showing that mi-CK is functionally coupled to mitochondrial oxidative phosphorylation (18, 26). Thus, by its type of respiratory regulation and key enzyme activities, the MGM represents a novel muscle belonging to the class of oxidative muscles. Most likely, it is the specific unitary organization of energy metabolism ensuring precise regulation of mitochondrial ATP synthesis in response to its use (25, 26) that enables chronically strong twitches of MGM for stabilization of the hip joint and pelvis during gait.

We found that \( K_m \) for ADP decreased in MGM more than twice and that creatine exerted a diminished effect on that parameter after development of OA (Fig. 3). Given the independence of \( K_m \) for ADP of muscle cell geometry (see above), these changes cannot be attributed to the modest decrease in MGM fiber diameter (4–32\%, depending on patient’s age and fiber type) accompanying the muscle atrophy in patients with OA (31). It seems more likely that OA is associated with loosening of the cytoskeletal restrictions for ADP diffusion on the borders of or inside the ICEU, because this compound added exogenously reaches mitochondria more easily than in normal muscle, thus resulting in decreased apparent \( K_m \) for ADP in the absence of creatine (Fig. 3B). An excess ADP flux from the medium/cytoplasm to the intermembrane space of mitochondria may inhibit PCR synthesis in mi-CK reaction, whereas diminished mi-CK activity may result in the same effect via impaired coupling to ANT. Clearly, both mechanisms should reduce the effectiveness of CK energy transfer between mitochondria and ATPases in MGM. Because similar defects have been revealed under different diseases [e.g., ischemia/reperfusion impairment and heart failure (7, 14)] and in oxidative muscles genetically devoid of dystrophin or desmin (5, 13), they probably represent a universal type of alteration resulting from disintegration of the ICEUs. It has been suggested that in case of a failing CK system, activation of AK-phosphotransfer can play a compensatory role (8). In diseased MGM, however, this mechanism may also become limited, as suggested by decreased AK activity (Table 1).

In conclusion, the present study demonstrates that energy metabolism in MGM cells is organized similarly to that in oxidative muscles, probably in the form of complexes of mitochondria and ATPases. Pathogenesis of OA involves disintegration of these complexes, which results in dysfunction of CK-phosphotransfer system and increased access of exogenous ADP to mitochondria. In these conditions, the mitochondrial ATP synthesis becomes dependent on fluctuations of the cytoplasmic [ADP], which reduces both the mitochondrial PCr synthesis and the effectiveness of ATP usage at the ATPase sites (25) and therefore may contribute to decreased hip muscle strength (3) in OA patients. In clinical terms, this study infers that arthroplasty undertaken before development of the grade 3 OA may improve the postsurgical rehabilitation by anticipating the deterioration of the intracellular energy transfer processes in MGM cells.

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