INTRODUCTION: In diabetes type II (DM2) alterations of microvasculature are the main cause of complications as retinopathy, nephropathy and peripheral neuropathy [1,2,3]. Large scale therapeutic trials have demonstrated that a tight control of glycaemia might reduce or delay the incidence of all complications related to diabetic microangiopathy [4,5]. Nevertheless, the determinants of early markers of microangiopathy in those patients at risk would be useful in improving their management. Moreover, peripheral angiopathy has also been suggested to contribute to impaired oxidative phosphorylations in DM2 [7]. We proposed to use interleaved multi-parametric functional NMR (mpf-NMR) [8,9] to investigate the metabolic and vascular response to stress in calf muscle of DM2 patients, separated according to degree of microangiopathic complications. Our objectives were to observe possible alterations of perfusion and oxidative metabolism in skeletal muscle and to establish whether these factors could relate to incidence of microangiopathy.

METHODS: Ninety-six voluntary DM2 patients were distributed into 3 groups according to glycaemia, HbA1c dosage, retinopathy, nephropathy and peripheral nerve screenings, and compared to 36 controls (C) matched for gender and BMI (Table 1). For patients D2 and D3, therapeutic treatments were interrupted at least 48h prior to NMR examination. Patients D1 were all untreated.

RESULTS: During exercise, the level of PCR depletion was comparable between groups but the ratios work/muscle section area (W/S) show that patients D1 tended to work less than controls and other patients (p>0.05). Their PCR resynthesis time constants (tPCR) measured after exercise and corrected to intramuscular pH [11,12] were higher compared to controls (p=0.01, Table 2). Post-exercise refuelling was lowered in diabetic groups (Figure 1A). Peak perfusion (fmax) was reduced in D2 and D3 patients compared to controls (p<0.01) and the area under the perfusion curve (integral f(t)) was more important in controls compared to patients, significantly versus D3 (Table2). BOLD profiles on the other hand did not differ between patients and controls (Figure 1B), whereas the resaturation time of Mb (tdMb) was significantly shorter in D2 versus D3 (Table 2; p<0.05). Importantly, we found no correlation between any of perfusion, PCR and tdMb, either altogether or within each group.

DISCUSSION: Evidence of altered skeletal muscle perfusion was found in DM2 patients whether they presented microangiographic complications or not, even if this did not reach significance for the smaller D1 group. BOLD, on the other hand, reflecting capillary oxygenation, was unaffected by the diabetic state, suggesting oxygen supply was sufficient to match O2 demand in muscle of patients. In D3 patients, in whom perfusion was also the most altered and dMb resaturation rate was slower, an alternative explanation may be that a normal BOLD contrast could reflect impaired O2 diffusion in case of more severe microvascular structural changes. These results obtained simultaneously to phosphate energetics also indicate that altered perfusion cannot be responsible for the impaired mitochondrial activity observed in patients D1. This is further substantiated by absence of correlation between parameters of O2 supply by perfusion and those reflecting O2 uptake (tdMb) and consumption (tPCR), although somewhat moderate correlation between tPCR and NIRS reoxygenation rates have been reported [13]. The fact that reduced oxidative capacity is observed only in patients D1 with uncontrolled glycaemia concords with observations of correlation between tPCR and plasma glucose concentrations in [7], and may help explain that patients with better controlled diabetes [14] showed no reduction in mitochondrial activity. There is some debate over mitochondrial dysfunction in DM2 [14,15], but impaired oxidative phosphorylations in D1 could yet result from a reduction in quantity of mitochondria and could yet possibly be explained by typological changes in calf muscles in favour of the more glycolytic type 2 fibers [16, 17].

CONCLUSION: The integrated study of altered oxidative and mitochondrial impaired muscle and reduced oxidative capacity in type 2 diabetes

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Table 1 : Characterization parameters of different groups of diabetic patients (D1, D2 and D3) and controls (C).

Table 2 : Principal functional parameters, during and after exercise. PCR end-depletion is r/comsumption relative to rest; W/S is the ratio of work output to section area of calf muscles; tPCR is the exponential time constant of PCR resynthesis corrected for pH; fmax is peak exercise post-exercise hyperemia; integ f(t) is the perfusion time integral; tdMb is the resaturation time of myoglobin; * for p<0.05 vs C; # for p<0.05 vs D2.

NMR acquisitions

Subjects underwent mpf-NMR in a 4T Bruker Biospec magnet following a short bout of single-leg ischemic plantar flexion arterial spin labeled (ASL) images providing perfusion and BOLD measurements of calf muscles, and were interleaved with 31P spectroscopy of the high energy phosphate metabolites and 1H spectroscopy of deoxy-myoglobin, as previously described [8-10]. Complete interleaved data sets were acquired every 1.5 s over 15 min of recovery from exercise.

Figure 1 : Mean perfusion (A) and BOLD signal (B) in all groups post-exercise

Figure 2 : Table 2 : Principal functional parameters, during and after exercise. PCR end-depletion is r/comsumption relative to rest; W/S is the ratio of work output to section area of calf muscles; tPCR is the exponential time constant of PCR resynthesis corrected for pH; fmax is peak exercise post-exercise hyperemia; integ f(t) is the perfusion time integral; tdMb is the resaturation time of myoglobin; * for p<0.05 vs C; # for p<0.05 vs D2.

Table 1 : Characterization parameters of different groups of diabetic patients (D1, D2 and D3) and controls (C).

<table>
<thead>
<tr>
<th>Group</th>
<th>Group size</th>
<th>% HbA1c</th>
<th>Diabetes duration (years)</th>
<th>BMI (kg/m2)</th>
<th>Age (years)</th>
<th>Micro-vascular complications : retino-, nephro-, peripheral neuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>n = 36</td>
<td>5.1 ± 0.4</td>
<td>27.4 ± 0.6</td>
<td>51 ± 2</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>D1</td>
<td>n = 16</td>
<td>12.5 ± 0.5</td>
<td>&gt;9%</td>
<td>9.5 ± 1</td>
<td>28 ± 1.0</td>
<td>No or only 1 moderate complication</td>
</tr>
<tr>
<td>D2</td>
<td>n = 58</td>
<td>7.0 ± 0.3</td>
<td>&lt;7%</td>
<td>8.5 ± 0.9</td>
<td>23 ± 0.5</td>
<td>No or only 1 moderate complication</td>
</tr>
<tr>
<td>D3</td>
<td>n = 24</td>
<td>9.0 ± 0.4</td>
<td>7% &lt; HbA1c &lt; 9%</td>
<td>13.0 ± 1.4</td>
<td>23 ± 0.8</td>
<td>2 or 3 complications</td>
</tr>
</tbody>
</table>

Table 2 : Principal functional parameters, during and after exercise. PCR end-depletion is r/comsumption relative to rest; W/S is the ratio of work output to section area of calf muscles; tPCR is the exponential time constant of PCR resynthesis corrected for pH; fmax is peak exercise post-exercise hyperemia; integ f(t) is the perfusion time integral; tdMb is the resaturation time of myoglobin; * for p<0.05 vs C; # for p<0.05 vs D2.