No relation between altered oxidative mitochondrial function and impaired muscle perfusion in type 2 diabetes

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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 [4-6]. Nevertheless, the determination 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 neuropathy screenings, and compared to 36 controls (C) matched for gender and BMI (Table 1). For patients D2 and D3, therapeutic treatments were interupted at least 48h prior to NMR examination. Patients D1 were all untreated.

	Micro-vascular complications : retino-, nephro-, peripheral neuropathy	Fasting glycaemia (mmol/l)	% HbA1c	Diabetes duration (years)	BMI (kg/m^2)	Age (years)	Group size
Group C	-	5.1 ± 0.4	-	-	27.4 ± 0.6	51 ± 2	n = 36
Group D1	No or only 1 moderate complication	12.5 ± 0.5	> 9%	9.5 ± 1.7	28.0 ± 1.0	53 ± 2	n = 16
Group D2	No or only 1 moderate complication	7.0 ± 0.3	< 7%	8.5 ± 0.9	27.3 ± 0.5	58 ± 1	n = 56
Group D3	2 or 3 complications	9.0 ± 0.4	7% < HbA1c < 9%	13.0 ± 1.4	27.3 ± 0.8	56 ± 2	n = 24

Table 1 : Characterization parameters of different groups of diabetic patients (D1, D2 and D3) and controls (C) ; Statistical significance was set at $^*p<0.05$, using a one-way ANOVA. Values in Tables are means ± SD.

NMR acquisitions

Subjects underwent mpf-NMR in a 4T Bruker Biospec magnet following a short bout of single-leg ischemic plantar flexionArterial spin labeled SATIR images providing perfusionand BOLD measurements of calf muscles, and were interleaved with ^{31}P spectroscopy of the high energy phosphate metabolites and ^{1}H 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.

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 rephosphorylation 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 reperfusion 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, tPCr and tdMb, either altogether or within each group.

Groups	% PCr depletion	W/S	tPCr (s)	fmax (ml.min^{-1}.100g^{-1})	integ f.t (ml.100g^{-1})	tdMb (s)
C	64 ± 3	12.7 ± 1.0	31 ± 2	49.2 ± 2.5	272 ± 19	11.8 ± 0.8
D1	60 ± 5	10.5 ± 1.4	42 ± 3*	40.4 ± 3.7	221 ± 28	12.5 ± 1.1
D2	61 ± 2	12.1 ± 0.8	36 ± 2	41.4 ± 2.0*	231 ± 15	10.3 ± 0.6
D3	60 ± 4	12.7 ± 1.2	38 ± 2	36.8 ± 3.0*	191 ± 23*	13.4 ± 0.9$^{\$}$

Table 2 : Principal functional parameters, during and after exercise. PCr end-exercise depletion is %consumption 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 rephosphorylation corrected for pH; fmax is peak perfusion post-exercise hyperemia ; integ f.t is the perfusion x time integral; tdMb is the resaturation time of myoglobin; * for $p< 0.05$ vs C; $^{\$}$ for $p<0.05$ vs D2

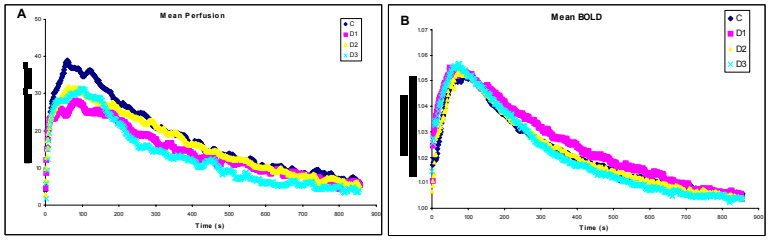

Figure 1 : Mean perfusion (A) and BOLD signal (B) in all groups post-exercise

DISCUSSION: Evidence of altered skeletal muscle perfusion was found in DM2 patients whether they presented microangiopathic 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 O_2 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 O_2 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 O_2 supply by perfusion and those reflecting O_2 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 muscle function in DM2 showed that reduced oxidative capacity was unrelated to perfusional alterations, but rather occurred with poor glycaemic control. Reduced perfusion was observed in all DM2 patients, yet did not seem to impair metabolism in well controlled patients, It was most reduced in D3 patients, with micro-angiographic complications, but also with longest standing diabetes.

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