Mechanisms of insulin resistance in Type I and Type II muscle fibres from Type 2 diabetics

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Skeletal muscle is critical for maintaining whole body glucose homeostasis. Impaired glucose regulation can result in type 2 diabetes (T2D). Skeletal muscle is a heterogeneous tissue composed of different fibre types classified according to their myosin heavy chain isoform (MHC). Type I and Type II fibres each have distinctive contractile and metabolic characteristics. Studies in rodents show that insulin-stimulated glucose uptake in the oxidative Type I dominant muscle is higher than in muscle with a higher degree of glycolytic Type II fibres (James, Jenkins & Kraegen, 1985; Marette *et al.*, 1992). In human muscle strips, insulin-stimulated glucose transport was associated with the relative Type I fibre content (Zierath *et al.*, 1996). These studies suggest that fibre type is a direct contributor to glucose regulation. We hypothesized that these differences are due to fibre type-specific expression / regulation of glucose signalling proteins and changes to the abundance or diffusibility of these proteins could be a confounding factor in T2D.

Segments of single fibres were obtained from biopsies of the *vastus lateralis* muscles, following 1% lignocaine injection into skin and fascia, from T2D subjects (n=11, mean ± SEM: 57.7 ±2.7 yr) and age-matched control subjects (n=9, mean ± SEM: 64.6 ±11.2 yr) before and after a euglycaemic hyperinsulinemic clamp. To determine fibre type dependency of proteins (Glut4, AMPK β2, glycogen branching enzyme (GBE), glycogen debranching enzyme (GDE), and glycogen phosphorylase (GP)), individual muscle fibre segments were dissected and analysed using quantitative Western blotting. Fibre typing was determined using antibodies for specific MHC isoforms. When Type I and Type II fibres from T2D subjects were compared for protein abundance, Type I fibres showed increased Glut 4 (~30%), decreased GP and GDE (~20% each) and similar AMPK β2 and GBE. These levels were not altered following the insulin clamp.

Protein diffusibility was determined using mechanically-skinned fibres whereby the surface membrane is removed, allowing any freely diffusible proteins (*i.e.* cytoplasmic proteins) to move out of the fibre and into the wash solution. The skinned-fibre segment and the matched 10 min wash solution were run on Western blots and the diffusibility of Glut4, AMPK β2, GDE, GBE and GP could be determined.

*Percentage of a given protein that appeared in the diffusible fraction relative to the total amount (mean ± S.E.M). * P<0.05 different from Con same fibre type, † different from pre for given fibre type, ‡ fibre type difference for a given group.*

Protein	Control (pre)		Type 2 Diabetes			
	Type I	Type II	Type I		Type II	
			Pre	Post	Pre	Post
Glut4	7 ± 2%	8 ± 3%	17 ± 2% *	11 ± 1% †	10 ± 3% ‡	10 ± 2%
AMPKβ2	76 ± 4%	68 ± 6%	76 ± 4%	68 ± 7%	75 ± 3%	63 ± 6%
GBE	52 ± 10%	48 ± 11%	72 ± 7% *	78 ± 5%	85 ± 3% *	77 ± 5%
GP	40 ± 6%	24 ±4% ‡	49 ± 8% *	37 ± 5% †	18 ± 5% *‡	42 ±6% †
GDE	22 ± 8%	12 ±4%	54 ± 8% *	37 ± 5% †	25 ± 5% ‡	26 ± 6%

Overall, these findings demonstrate substantial fibre type differences in T2D subjects. They suggest an enhanced (Type I) and diminished (Type II) glucose handling in specific fibres from diabetic subjects. The findings also show differences in the diffusibility of various glucose signalling proteins, which may impair a cell's ability to regulate glucose and thus be a determining factor in the prognosis of T2D.

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