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Impaired enhancement of insulin action in cultured skeletal muscle cells from insulin resistant type 2 diabetic patients in response to contraction using electrical pulse stimulation

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ABSTRACT

Aims: Skeletal muscle insulin resistance is a characteristic feature of type 2 diabetes. The aim of this study was to examine the effect of contraction on insulin action using electrical pulse stimulation (EPS) in cultured skeletal muscle cells from insulin resistant type 2 diabetic patients.
Methods: Skeletal muscle cell cultures were established from 6 insulin resistant type 2 diabetic subjects and age and BMI matched non-diabetic control subjects. Day 7 differentiated myotubes were treated with or without EPS for 16 h, after which glucose uptake and AS160 phosphorylation were measured in the presence or absence of insulin.
Results: In control myotubes, EPS resulted in increased phosphorylation of AMPKThr^{172} (vs no EPS; $p < 0.01$), and this was associated with increased glucose uptake ($p < 0.05$). Insulin in the absence of EPS increased glucose uptake and AS160Thr^{642} phosphorylation, and both effects were significantly enhanced by prior EPS. In the absence of EPS, AMPK activation was significantly increased ($p < 0.01$) in the diabetic vs control myotubes. Despite a comparable degree of AMPK activation following EPS, the action of insulin on glucose uptake ($p < 0.05$) and AS160Thr^{642} phosphorylation ($p < 0.001$) was decreased in the diabetic vs control myotubes.
Conclusion: EPS mediated AMPK activation enhances the effect of insulin on glucose uptake and AS160Thr^{642} phosphorylation in control myotubes replicating key metabolic benefits of exercise on insulin action in man. Conversely, insulin mediated glucose uptake and AS160Thr^{642} phosphorylation remain significantly decreased in diabetic vs control myotubes despite a comparable degree of AMPK activation following EPS.

1. Introduction

Insulin resistance is a characteristic feature of type 2 diabetes. Skeletal muscle is responsible for >80% of glucose disposal after meals in response to insulin in non-diabetic subjects,[1,2] and is the main peripheral tissue site of insulin resistance in type 2 diabetes. Key phenotypic characteristics of native skeletal muscle fibres are retained in multinucleated myotubes in culture.[3] We and others have shown that decreased insulin action in relation to glucose uptake and metabolism is retained in primary skeletal muscle cell cultures from type 2 diabetes patients and their non-diabetic 1st degree relatives,[4–7] in line with a genetic/epigenetic contribution to the peripheral insulin resistance in type 2 diabetes.

Skeletal muscle contraction through a single or repeated bouts of exercise has been shown to directly increase glucose uptake and enhance the action of insulin on glucose metabolism in non-diabetic subjects.[8,9] Activation of AMPK and AS160 at (Thr^{642} and Ser^{588}) has been shown to play a key role in exercise-mediated glucose uptake in skeletal muscle in non-diabetic subjects.[10,11] The beneficial effects of exercise on glucose metabolism and insulin action have been reported in T2D.[12,13] However, there is heterogeneity in the metabolic (insulin sensitivity and muscle mitochondrial function) and anthropometric (body fat or BMI) responses to exercise in patients with type 2 diabetes.[14]

For the last decade, a number of in vitro models that induce muscle contraction using electrical pulse stimulation (EPS) of cultured myotubes have been developed.[15–17] Early studies showed that EPS increased glucose uptake and oxidation,[16] similar to the adaptive changes observed during exercise in vivo.[18,19] We have developed a 16 h EPS protocol that induces contraction and AMPK activation in myotubes from non-diabetic subjects.[17] EPS also increased cytokine release replicating the known effects of exercise on cytokine release in vivo.[20] Furthermore, a new study by Park, Turner[21] suggested that, myotubes from insulin resistant severely obese women express a reduced exercise adaptation using similar EPS system. The aim of this study was to

Abbreviations: AMPK, 5' AMP-activated protein kinase.

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investigate the metabolic and signalling responses to EPS in cultured skeletal muscle cells from a selected group of markedly insulin resistant type 2 diabetes patients with a positive family history (at least one first-degree relative) of type 2 diabetes.

2. Material and methods

2.1. Chemicals and reagents

Cell culture media was obtained from Lonza. FBS and trypsin-EDTA were obtained from Life Technologies (Paisley, UK). Chick embryo extract was purchased from Sera Labs International (Sussex, UK). Phospho-AS160 Thr642 [EPR2733] and Phospho-AS160 S588 [ab65754] were purchased from Abcam. Phospho-AMPK Thr172 (40H9) rabbit antibodies, AMPKα (F6) mouse antibodies, and total AS160 rabbit (C96A7) were supplied by New England Biolabs (Herts, UK. 2-Deoxy-D-[2, 6-3H] glucose was purchased from Hartmann Analytic (Germany). IL-6 and IL-8 ELISA kits were obtained from Qiagen (Sussex, UK).

2.2. Study subjects

Muscle biopsies were taken from 6 type 2 diabetic patients with strong clinical evidence of insulin resistance, taking >100 U insulin/day with HbA1c 9.0 ± 0.5 and had at least one first-degree relative with type 2 diabetes. After diagnosis, patients had been treated with diet and oral hypoglycaemic drug for >3 years before starting insulin. All enrolled patients had a body mass index (BMI) < 32 kg/m^2 (30 ± 0.7) to exclude the effects of marked obesity on insulin sensitivity (glucose uptake) and/or exercise adaptation.

Skeletal muscle biopsies were taken from six, age and BMI, matched non-diabetic control subjects with no family history of type 2 diabetes. The study was approved by the Newcastle and North Tyneside Joint Ethics Committee and all the participants gave written informed consent. Metabolic and anthropometric features of recruited subjects were previously reported Table 1.

2.3. Cell culture

Muscle biopsies were obtained (from the vastus lateralis muscle of diabetic subjects by needle biopsy under local anesthesia while Control muscle was obtained from the vastus lateralis muscle at the time of hip surgery under general anesthesia) and satellite cells isolated as described previously.[22] Cultures were purified as described previously[4,23] by the use of a magnetic bead system (Miltenyi Biotec). In brief, the harvested cells were resuspended in PBS containing 2 mM EDTA and 5% FBS and to recognize a muscle specific cell surface antigen, cells were incubated with anti-CD56 antibody. Then, after washing the cell suspension was incubated with secondary antibody goat anti-mouse antibody conjugated to microbeads. After incubation, the cell suspension was applied to an MS column within a magnetic field and the cells attached to microbeads were retained, and other cells passed through the column. The cells retained in the column were eluted and re-cultured. Muscle cell origin was confirmed by using immunohistochemistry (muscle-specific protein desmin). Myoblasts were cultured in growth media (Ham's F-10 medium supplemented with 20% FBS, 2% chick embryo extract and 1% penicillin-streptomycin in an atmosphere of 5% CO_2 in air. When the cell monolayers reached approximately 90% confluence, differentiation to myotubes was induced by changing the culture media to minimal essential media supplemented with 2% FBS. All experiments were performed on passage 6 cells and day 7 of differentiation.

Table 1
Metabolic and anthropometric characteristics of recruited subjects.

	T2D	Control
Age (years)	59 ± 7	59 ± 11
Sex (males/females)	5/1	3/3*
Time to insulin treatment (years)	10 ± 5	
Units of insulin/day	131.2 ± 9.6	
Hb A1c, %	9.0 ± 0.5	5.2 ± 0.1**
BMI, kg/m^2	30 ± 0.7	28.5 ± 1.0
Waist/hip ratio	1.0 ± 0.03	0.9 ± 0.02**
Total cholesterol, mmol/l	4.8 ± 0.2	5.94.80.3**
Triglycerides (mmol/l)	3.3 ± 0.5	1.6 ± 0.4
Fasting serum insulin (mU/l)		7.1 ± 0.6
Fasting plasma glucose (mmol/l)		5.4 ± 0.2

Data are presented as means ± SE; T2D vs. control. T2D subjects had significantly higher HbA1c, waist/hip ratio, and triglycerides. Controls had significantly higher total cholesterol.
* $p < 0.05$.
** $p < 0.01$.

2.4. Electrical pulse stimulation

Electrical pulse stimulation (EPS) was performed using a C-Pace EP cell culture pacer (IonOptix, Dublin) using a two-step protocol (alternation between a period of high frequency and low frequency electrical pulses). Cells were plated in 35 mm dishes, and at day 7 of differentiation, media was changed to serum free media and subjected to in vitro exercise. EPS was applied via carbon electrodes at 5 V, 24 ms, 2 Hz for 1 h followed immediately by 5 V, 24 ms, 0.2 Hz for 1 h. This alternation in frequency was continued for 16 h (period of exercise).

2.5. Glucose uptake

Measurement of 2-deoxy-D-[2, 6-3H] glucose uptake took place in day 7 myotubes. Cells were incubated in serum-free media and divided into 2 groups for treatment; with or without 16 h EPS at 37 °C. After 16 h, cells were incubated in Krebs' buffer (136 mM NaCl, 4.7 mM KCl, 1.25 mM MgSO4, 1.2 mM CaCl2, 20 mM HEPES, pH 7.4) with or without 100 nM insulin or Cytochalasin B (10 μM) for 20 min. 0.1 mM 2-deoxy-

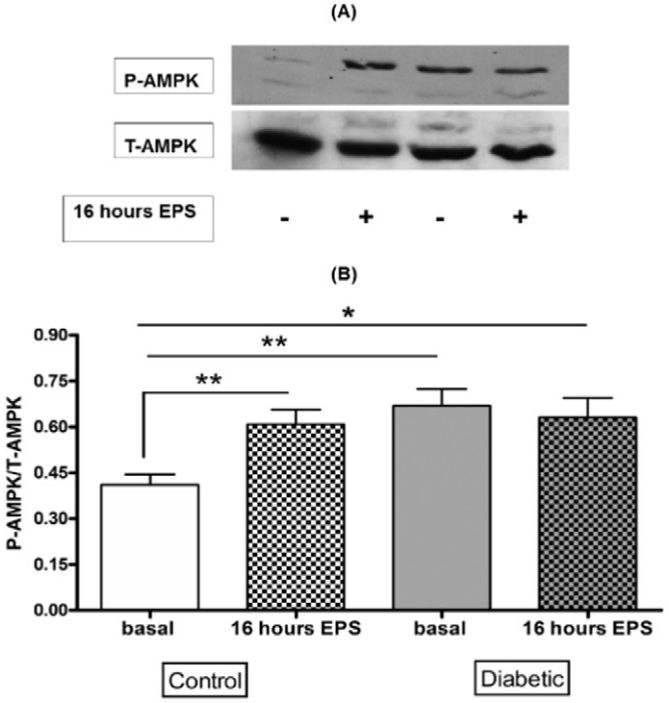

Fig. 1. AMPK activation in response to 16 h EPS in cultured myotubes. Panel (A) shows a representative blots of AMPK phosphorylation at Thr^{172} and total AMPK for basal (no EPS) and 16 h EPS for control and diabetic myotubes. Panel (B) shows the densitometric analysis of the ratio of phospho/total AMPK for both control and diabetic myotubes for basal (no EPS) and 16 h EPS. Values are expressed as the Mean ± SEM (n = 6), *p < 0.05, **p < 0.01.

glucose and 0.5 μCi (2,6-3H) 2-deoxyglucose were added to each well and incubated for a further 10 min. Reactions were stopped by washing with ice-cold phosphate buffered saline (PBS). Cells were lysed in 0.05% SDS before scintillation counting and protein determination.

2.6. Western blot

AMPK activation was measured by Western blot using phospho-specific antibodies against threonine residue (Thr172) which is essential for the full activation of kinase activity.[24] After 16 h incubation with or without EPS, cells were washed with ice-cold phosphate buffered saline (PBS) and then scraped into protein extraction buffer (100 mM Tris-HCl, pH 7.4, 100 mM KCl, 1 mM EDTA, 25 mM KF, 1 mM benzamidine, 0.5 mMNa3VO4, 0.1% (v/v) Triton X-100, 1× protease inhibitor cocktail (Pierce), sonicated briefly and centrifuged at 13,400 rpm for 5 min at 4 °C. Protein concentration was determined using the modified Bradford dye binding colorimetric method at 595 nm. 10 μg samples were loaded on 10% SDS-PAGE gels in loading buffer (0.125 M Tris-HCl, pH 6.8, 4% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol, and 0.004% (w/v) bromophenol blue). After separation, proteins were transferred onto a nitrocellulose membrane (0.45 μm pore size), using a mini-Hoeffer wet transfer system. Non-specific sites on the nitrocellulose membranes were blocked by incubation in Tris buffered saline tween (TBS-T) containing 5% (w/v) milk as a blocking buffer for 1 h for AMPK and 3% (w/v) BSA for AS160 at room temperature. Membranes were incubated with monoclonal primary antibody (1°Ab) diluted in 0.5% blocking buffer TBS-T/0.5% (w/v) milk or BSA, overnight at 4 °C. After washing, membranes were incubated with a horseradish peroxidase (HRP) conjugated secondary antibody IgG, diluted in 0.5% blocking buffer. Detection took place using enhanced chemiluminescence and densitometry measurements performed using the Bio-RAD Molecular Imager GS-800 calibrated densitometer and Quantity One software. Phospho and native AMPK antibodies were used at a 1:1000 and phospho and native AS160 were 1:500 dilutions.

2.7. IL-6 and IL-8 ELISA

Secretion of IL6 and IL-8 were determined by enzyme-linked immunosorbent assay (ELISA) using the Single-Analyte ELISArray (Qiagen). Media was changed to serum free media and day 7 differentiated cells (diabetes and control) were either subjected to EPS or kept in the

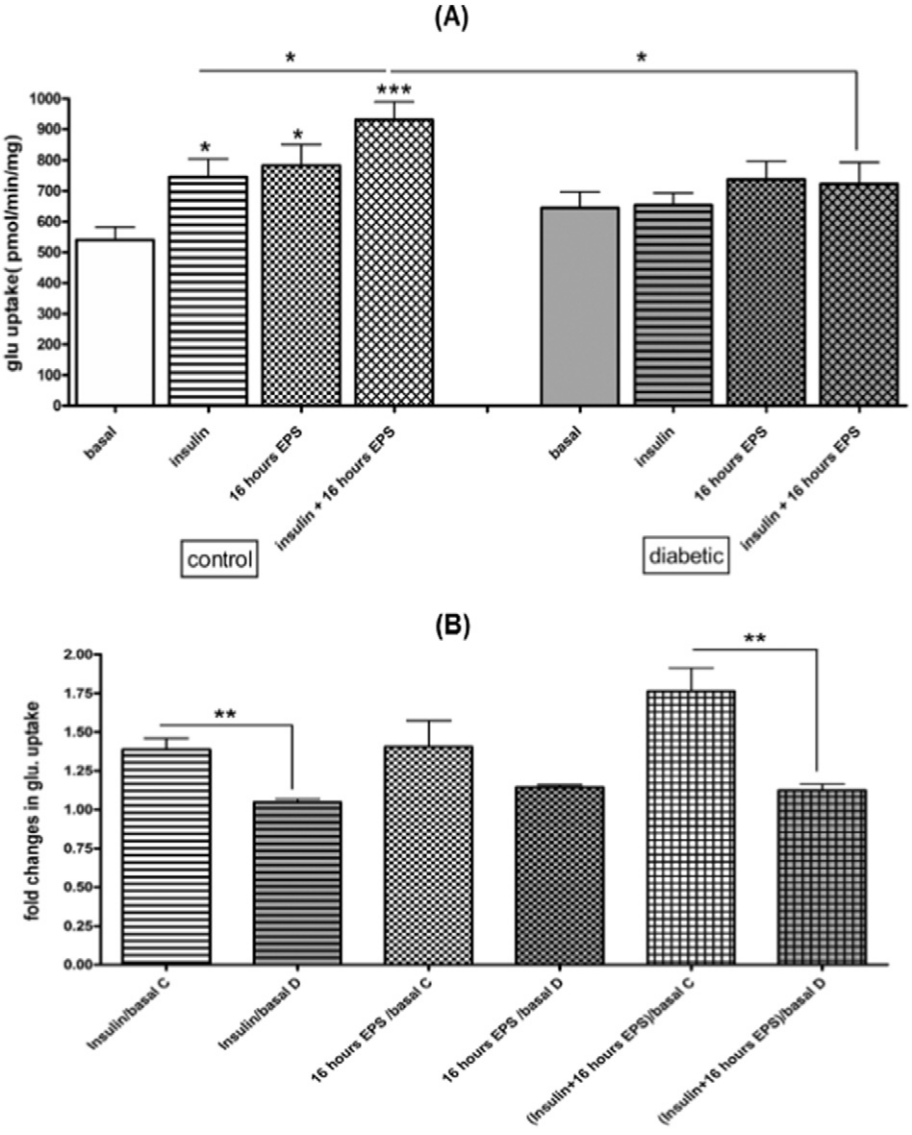

Fig. 2. Glucose uptake by control and diabetic myotubes under the following conditions: basal (no EPS), insulin (no EPS followed by insulin), 16 h EPS (EPS and no insulin), insulin & 16 h EPS (EPS followed by insulin). Panel (A) and (B) show absolute uptake and fold changes relative to basal, respectively. The letter C in panel (B) represents control and D is diabetic. Values are expressed as the mean ± SEM (n = 6), *p < 0.05, **p < 0.01, ***p < 0.001.

incubator for 16 h. After EPS, media was removed, centrifuged at 1000 *g* for 10 min and assayed for secretion of IL6 and IL-8 according to the manufacturer's protocol. A standard curve was generated using the provided antigen standard by serial dilution standard and absorbance read at 450 nm. Background absorbance was subtracted from the values and the protein concentrations of the samples calculated from the standard curve.

2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism (California) software. The results of the study are expressed as mean ± standard error of the mean (SEM), ($n = 6$) represents six independent experiments repeats from sex different cell lines (type 2 diabetes and control) of primary cell cultures. All data were tested for normal distribution using the Kolmogorov-Smirnov test. The variables presented were shown to be normally distributed, Data were analysed using one-way ANOVA and, where significant, followed up by unpaired *t*-test between groups. p Value <0.05 was considered statistically significant.

3. Results

3.1. AMPK activation

In control myotubes, (Fig. 1A and B), 16 h EPS significantly increased phosphorylation of AMPK^{Thr172} Mean ± SEM 0.608 ± 0.048, ($p < 0.01$) in comparison to no EPS (designated basal) 0.411 ± 0.034. In contrast, basal AMPK phosphorylation (no EPS) was significantly higher in the diabetic myotubes (0.736 ± 0.070) compared with the basal control myotubes ($p < 0.01$). However, there was no further activation of AMPK in the diabetic cells in response to EPS. AMPK phosphorylation was comparable in the control and diabetic myotubes following EPS.

3.2. Glucose uptake

Fig. 2A shows glucose uptake in control and diabetic myotubes. In control myotubes, EPS increased glucose uptake in comparison to no EPS (designated basal) ($p < 0.05$). Insulin alone (no prior EPS) increased glucose uptake to 744.3 ± 59.3 pmol/min/mg ($p < 0.05$) vs basal 539.8 ± 42.1. Furthermore, 16 h EPS caused an increase in glucose uptake to significant level 782.9 ± 68.1 pmol/min/mg over basal uptake ($p < 0.05$). The combination of EPS followed by insulin increased glucose uptake to 931 ± 58 pmol/min/mg, which was significantly higher than insulin alone ($p < 0.05$).

In diabetic myotubes, neither EPS nor insulin alone increased glucose uptake in comparison to basal uptake. Furthermore, EPS did not enhance the effect of insulin when compared to insulin alone.

As basal glucose uptake tended to be higher in the diabetic myotubes, glucose uptake was calculated as fold change over basal for each stimulus. Fig. 2B summarises the data for control and diabetic myotubes. Fold increase for insulin alone and insulin after EPS was significantly higher in controls vs diabetic myotubes ($p < 0.01$ for both comparisons).

3.3. AS160

AS160 activation was investigated by Western blot by measuring Thr^{642} and Ser^{588} phosphorylation. Fig. 3 summarises the AS160Thr^{642} phosphorylation. In control myotubes, insulin alone significantly increased AS160Thr^{642} phosphorylation over total AS160 (Mean ± SEM 0.725 ± 0.034) compared with basal (0.336 ± 0.023), ($p < 0.01$). Prior EPS enhanced the effect of insulin (0.926 ± 0.032) on AS160Thr^{642} phosphorylation compared with insulin alone ($p < 0.05$). In diabetic myotubes, insulin alone increased AS160Thr^{642} phosphorylation over total AS160 (0.659 ± 0.038) compared with basal ($p < 0.01$), but prior

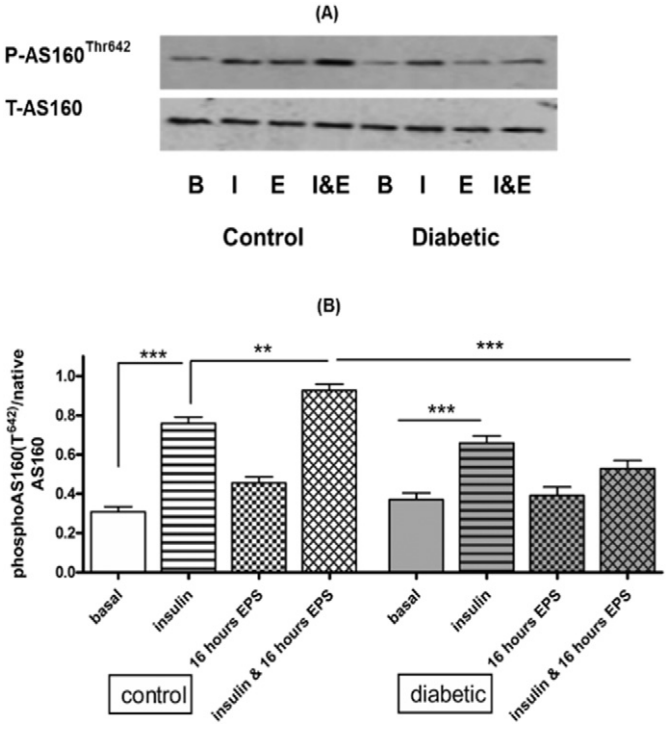

Fig. 3. AS160 Thr^{642} phosphorylation in cultured myotubes. Panel (A) shows representative blots of AS160 Thr^{642} phosphorylation and total AS160 for the following conditions; B basal (no EPS) and I insulin (no EPS followed by insulin), E 16 h EPS (EPS and no insulin), I&E insulin & 16 h EPS (EPS followed by insulin) for both control and diabetic myotubes. Panel (B) represents the densitometric analysis of the ratio of AS160 Thr^{642} phosphorylation/total AS160 for control and diabetic myotubes for the same conditions. Values are expressed as the mean ± SEM ($n = 6$), *$p < 0.05$, ***$p < 0.001$.

EPS did not enhance the effect of insulin. AS160Thr^{642} phosphorylation after EPS plus insulin was significantly higher in the control vs diabetic myotubes ($p < 0.001$).

Fig. 4 summarises the AS160Ser^{588} phosphorylation. In control myotubes, insulin alone and EPS both increased phosphorylation compared with basal (both $p < 0.05$). However, the effect of insulin was not enhanced by prior EPS. In the diabetic myotubes, none of the treatments increased AS160Ser^{588} phosphorylation compared with basal. Furthermore, AS160Ser^{588} phosphorylation after insulin alone was significantly higher in the control vs diabetic myotubes ($p < 0.05$).

3.4. Cytokines release

Skeletal muscle produces cytokines in response to contraction. The effect of EPS on IL-6 and IL-8 release is shown in Fig. 5A and B, respectively. IL-6 release was significantly increased from both control and diabetic myotubes after 16 h EPS compared with basal conditions (both $p < 0.05$). There was no difference in IL-6 release between control and diabetic myotubes. The same pattern was observed with IL-8 release, with EPS increasing release in both control and diabetic myotubes compared to basal (both $p < 0.05$).

4. Discussion

To our knowledge, this is the first study to investigate AMPK activation in cultured diabetic myotubes using EPS as an in vitro model of muscle contraction.

The EPS protocol activated AMPK and increased glucose uptake in the control myotubes from non-diabetic subjects. This is in keeping with our previous work and that of others that used EPS to induce muscle cell contraction leading to AMPK activation and increased glucose metabolism.[16,17] As shown in Fig. 2, insulin alone increased glucose

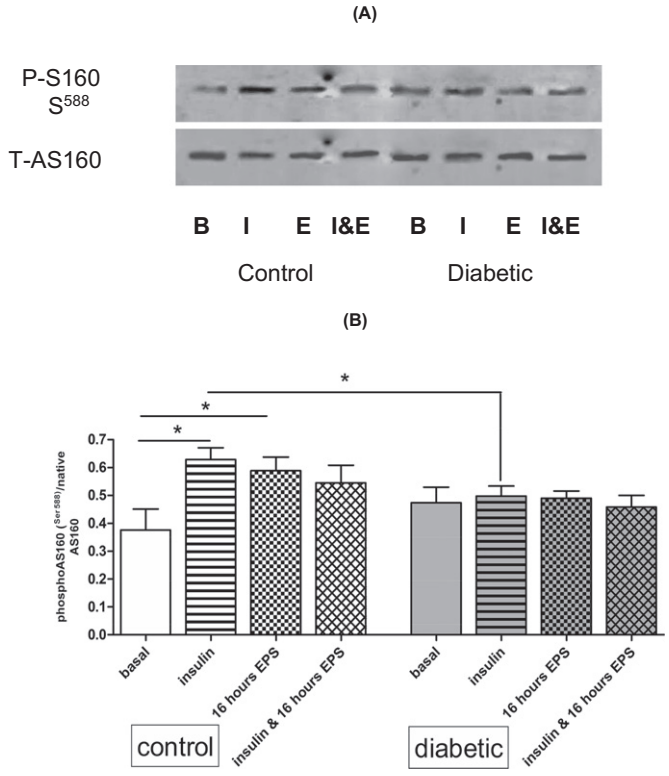

Fig. 4. AS160 Ser^{588} phosphorylation in cultured myotubes. Panel (A) shows representatives blots of AS160SerS^{588} phosphorylation and total AS160 for the following conditions; B basal (no EPS) and I insulin (no EPS followed by insulin), E 16 h EPS (EPS and no insulin), I&E insulin & 16 h EPS (EPS followed by insulin) for both control and diabetic myotubes. Panel (B) shows the densitometric analysis of the ratio of phospho Ser^{588}/total AS160 for control and diabetic myotubes for the same conditions. Values are expressed as the mean ± SEM, n = 6 for each group, *p < 0.05.

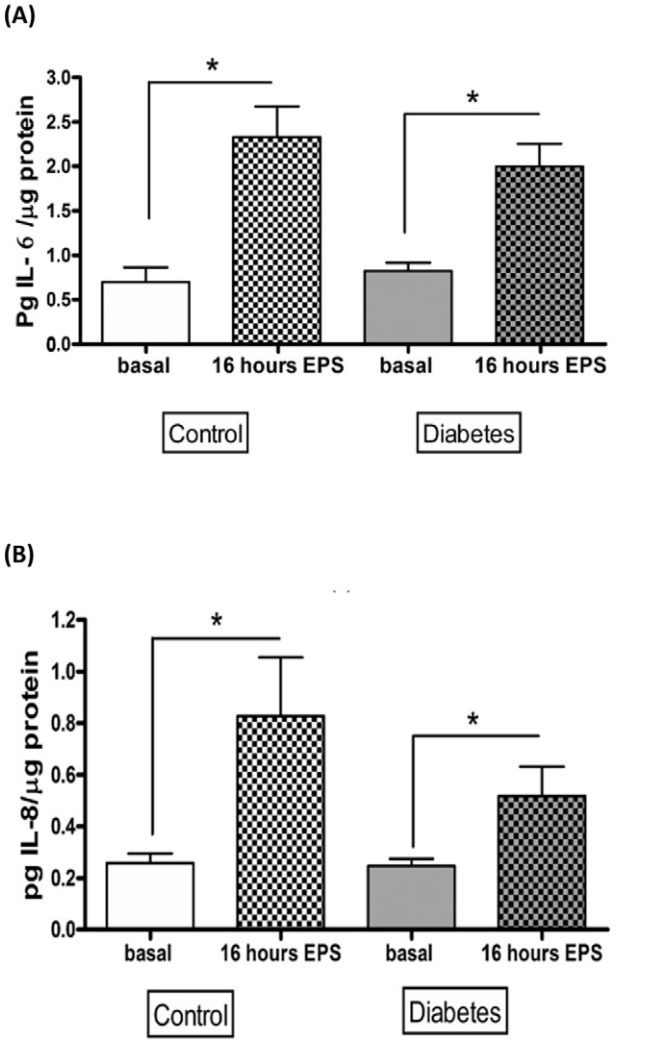

Fig. 5. IL-6 and IL-8 release in response to 16 h EPS in cultured myotubes. Myotubes were studied in the basal (no EPS) state and after 16 h EPS. Media was collected and IL-6 and IL-8 concentration assayed by ELISA. Panel (A) shows IL-6 release normalised to the protein content of the cells. Panel (B) shows IL-8 release normalised to the protein content of the cells. Results are expressed as mean ± SEM. * p < 0.05.

uptake in the control myotubes, and the effect of insulin was enhanced by prior EPS. This is consistent with the observation that muscle contraction enhances insulin action in vivo. A single bout of exercise increases glucose uptake into skeletal muscle,[25] and improves insulin sensitivity in the post-exercise period.[12,26] The cellular processes underlying the post-exercise insulin sensitisation involve an increase in GLUT4 translocation and availability at the cell membrane.[27]

A key finding was that in the absence of EPS AMPK phosphorylation was significantly greater in the diabetic compared with the control myotubes (Fig. 1). After EPS, AMPK phosphorylation was comparable in the control and diabetic myotubes. A number of diabetes studies have reported an increase in AMPK activity in response to exercise or AICAR,[28–33] but two studies[34,35] found no enhancement with exercise in line with our findings. The first study was conducted in a diabetic dog model characterised by marked hyperglycaemia and metabolic decompensation[34]; basal AMPK activity was increased and failed to increase further after exercise. In the second study,[35] basal AMPK activity was increased in muscle samples from obese type 2 diabetic versus lean control subjects to a degree comparable to that seen in our study, and similarly showed a failure of AMPK activity to increase in response to exercise in the diabetic patients. This contrasted with the group's earlier findings in lean type diabetic patients in which AMPK activity increased in response to exercise,[28] and led them to propose that the obese, insulin resistant state might affect AMPK activity in type 2 diabetes. It is worth noting that the diabetic cultures used in the present study were derived from type 2 diabetic patients who were characterised by marked insulin resistance based on an average insulin requirement of 130 units/day.

In the control myotubes, the action of insulin on glucose uptake was enhanced by prior EPS. While AMPK was activated to the same degree by EPS in the control and diabetic myotubes (Fig. 1), the action of insulin on glucose uptake was markedly diminished in the diabetic myotubes (Fig. 2). This illustrates that the impaired action of insulin in diabetic myotubes was not modulated by prior EPS. It is recognised that some patients with type 2 diabetes do not realize the full metabolic benefits of regular exercise, and have been referred to as "non-responders".[14] Böhm and colleagues studied non-diabetic 1st degree relatives of type 2 diabetic patients, and identified a group of "non-responders" who failed to show an improvement in insulin sensitivity after exercise.[36] A reduced exercise adaptation was observed in myotubes from insulin resistant severely obese women using EPS system.[21]

AS160 is a family of kinases activated by both insulin and exercise, and act as a convergence point for both insulin dependent and independent signalling pathways.[37] Insulin and exercise significantly increase AS160 phosphorylation in human skeletal muscle.[38] Phosphorylation of AS160 was shown to increase in response to exercise in both rat[39] and human muscle[40] and was associated with enhanced insulin stimulated glucose uptake several hours after exercise. Multiple phosphorylation sites of AS160 have been reported to be activated by distinct upstream kinases including Akt and AMPK.[38,41] Thr^{642} and Ser^{588} are key residues regulating GLUT4 translocation,[42] and mutations at these sites decrease insulin stimulated GLUT4 translocation.[42,43]

In control myotubes, AS160Thr^{642} phosphorylation was increased in response to insulin, and this effect was significantly enhanced by prior EPS (Fig. 3). These results are in agreement with earlier studies that showed a sustained post exercise activation in distal insulin signalling molecules in skeletal muscle.[44–46]

AS160Thr ^{642} phosphorylation increased in response to insulin in the diabetic myotubes (Fig. 3), but the effect of insulin was not enhanced by prior EPS. This is similar to the finding in a rodent model in which the effect of exercise to enhance insulin mediated AS160Thr^{642} phosphorylation was impaired in skeletal muscle from insulin resistant compared with insulin sensitive animals.[47]

Regarding AS160^{Ser588} phosphorylation, this increased in response to both insulin and EPS alone in the control myotubes, but the insulin effect was not enhanced by EPS. Two studies showed that insulin and exercise stimulate AS160^{Ser588} phosphorylation,[46,47] and both showed an additive effect for the co-activation of insulin and exercise as measured with insulin stimulation after 3 h post exercise. The differences from the results of the current study are not immediately apparent, but may relate to the differences in the muscle contraction protocol. AS160Ser^{588} phosphorylation following insulin alone was decreased in diabetic compared with control myotubes. This is in line with the findings of Vind and colleagues[48] who found that insulin stimulated AS160Ser^{588} phosphorylation was decreased in skeletal muscle from obese type 2 diabetic patients compared with BMI matched control subjects.

Finally, it is widely appreciated that muscle contraction results in the release of cytokines.[20] As shown in Fig. 5, EPS resulted in the release of IL-6 and IL-8 from both control and diabetic myotubes. This is in keeping with the observation that IL-6 secretion from skeletal muscle in response to contraction is mediated in part through an AMPK independent mechanism.[49] These results are consistent with data obtained from previous studies performed on control human muscle cells that showed increased IL-6 and IL-8 release with EPS.[17,50]

In summary, EPS activated AMPK in control myotubes and enhanced the action of insulin on glucose uptake and AS160Thr^{642} phosphorylation. In the diabetic myotubes, AMPK was activated to the same degree before and after EPS. Despite achieving a comparable degree of AMPK activation after EPS in the diabetic and control myotubes, the action of insulin on glucose uptake and AS160Thr^{642} phosphorylation was markedly decreased in the diabetic myotubes. It would seem therefore, that the beneficial effects of EPS and AMPK activation on insulin action are not apparent in myotubes from insulin resistant type 2 diabetic patients. This is in line with the concept that some patients with type 2 diabetes may not realize the full metabolic benefits of muscle contraction and exercise.

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Contribution statement

Each author was involved in the study design, data analysis and interpretation, and the drafting and critique of the manuscript. Each author has read and approved the final version of the submitted paper.

Declaration of competing interest

The authors confirm that there are no dualities of interests relating to this manuscript.

References

1. Thiebaud D, Jacot E, Defronzo RA, Maeder E, Jequier E, Felber J-P. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. Diabetes 1982;31:957-63.
2. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 1981;30:1000-7.
3. van der Ven PF, Schaart G, Jap PH, Sengers RC, Stadhouders AM, Ramaekers FC. Differentiation of human skeletal muscle cells in culture: maturation as indicated by titin and desmin striation. Cell Tissue Res 1992;270:189-98.
4. McIntyre E, Halse R, Yeaman S, Walker M. Cultured muscle cells from insulin-resistant type 2 diabetes patients have impaired insulin, but normal 5-amino-4-imidazolecarboxamide riboside-stimulated, glucose uptake. J Clin Endocrinol Metabol 2004;89:3440-8.
5. Jackson S, Bagstaff SM, Lynn S, Yeaman SJ, Turnbull DM, Walker M. Decreased insulin responsiveness of glucose uptake in cultured human skeletal muscle cells from insulin-resistant nondiabetic relatives of type 2 diabetic families. Diabetes 2000;49:1169-77.
6. Henry RR, Abrams L, Nikoulina S, Ciaraldi TP. Insulin action and glucose metabolism in nondiabetic control and NIDDM subjects: comparison using human skeletal muscle cell cultures. Diabetes 1995;44:936-46.
7. Gaster M, Petersen I, Højlund K, Poulsen P, Beck-Nielsen H. The diabetic phenotype is conserved in myotubes established from diabetic subjects: evidence for primary defects in glucose transport and glycogen synthase activity. Diabetes 2002;51:921-7.
8. Devlin JT, Hirshman M, Horton ED, Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. Diabetes 1987;36:434-9.
9. Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action. Acta Physiologica 2008;192:127-35.
10. Kjøbsted R, Munk-Hansen N, Birk JB, Foretz M, Viollet B, Björnholm M, et al. Enhanced muscle insulin sensitivity after contraction/exercise is mediated by AMPK. Diabetes 2017;66(3):598-612. db160530.
11. Treebak JT, Pehmøller C, Kristensen JM, Kjøbsted R, Birk JB, Schjerling P, et al. Acute exercise and physiological insulin induce distinct phosphorylation signatures on TBC1D1 and TBC1D4 proteins in human skeletal muscle. J Physiol 2014;592:351-75.
12. Henriksen EJ. Invited review: effects of acute exercise and exercise training on insulin resistance. J Appl Physiol 2002;93:788-96.
13. Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K, et al. Increased glucose transport–phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. New England Journal of Medicine 1996;335:1357-62.
14. Sparks LM. Exercise training response heterogeneity: physiological and molecular insights. Diabetologia 2017;60:2329-36.
15. Nikolić N, Görgens S, Thoresen G, Aas V, Eckel J, Eckardt K. Electrical pulse stimulation of cultured skeletal muscle cells as a model for in vitro exercise–possibilities and limitations. Acta Physiologica 2017;220(3):310-31.
16. Feng YZ, Nikolić N, Bakke SS, Kase ET, Guderud K, Hjelmesæth J, et al. Myotubes from lean and severely obese subjects with and without type 2 diabetes respond differently to an in vitro model of exercise. American Journal of Physiology-Cell Physiology 2015;308. (C548-C56).
17. Brown AE, Jones DE, Walker M, Newton JL. Abnormalities of AMPK activation and glucose uptake in cultured skeletal muscle cells from individuals with chronic fatigue syndrome. PloS one 2015;10, e0122982.
18. Santos JM, Ribeiro SB, Gaya AR, Appell HJ, Duarte JA. Skeletal muscle pathways of contraction-enhanced glucose uptake. Int J Sports Med 2008;29:785-94.
19. Hayashi T, Wojtaszewski JFP, Goodyear LJ. Exercise regulation of glucose transport in skeletal muscle. American Journal of Physiology-Endocrinology And Metabolism 1997;273.(E1039-E51).
20. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol 2012;8:457-65.
21. Park S, Turner KD, Zheng D, Brault JJ, Zou K, Chaves AB, et al. Electrical pulse stimulation induces differential responses in insulin action in myotubes from severely obese individuals. J Physiol 2019;597:449-66.
22. Blau HM, Webster C. Isolation and characterization of human muscle cells. Proc Natl Acad Sci 1981;78:5623-7.
23. Al-Bayati A, Lukka D, Brown AE, Walker M. Effects of thrombin on insulin signalling and glucose uptake in cultured human myotubes. J Diabetes Complications 2016;30:1209-16.
24. Scott JW, Norman DG, Hawley SA, Kontogiannis L, Hardie DG. Protein kinase substrate recognition studied using the recombinant catalytic domain of AMP-activated protein kinase and a model substrate. J Mol Biol 2002;317:309-23.
25. Richter EA, Mikines KJ, Galbo H, Kiens B. Effect of exercise on insulin action in human skeletal muscle. J Appl Physiol 1989;66:876-85.
26. Frøsig C, Richter EA. Improved insulin sensitivity after exercise: focus on insulin signaling. Obesity 2009;17:S15-20.
27. Wojtaszewski JFP, Hansen BF, Kiens B, Markuns JF, Goodyear LJ, Richter EA. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. Diabetes 2000;49:325-31.
28. Musi N, Fujii N, Hirshman MF, Ekberg I, Fröberg S, Ljungqvist O, et al. AMP-activated protein kinase (AMPK) is activated in muscle of subjects with type 2 diabetes during exercise. Diabetes 2001;50:921-7.
29. Kjobsted R, Pedersen AJ, Hingst JR, Sabaratnam R, Birk JB, Kristensen JM, et al. Intact regulation of the AMPK signaling network in response to exercise and insulin in skeletal muscle of male patients with type 2 diabetes: illumination of AMPK activation in recovery from exercise. Diabetes 2016;65:1219-30.
30. Wojtaszewski JFP, Birk JB, Frøsig C, Holten M, Pilegaard H, Dela F. 5′ AMP activated protein kinase expression in human skeletal muscle: effects of strength training and type 2 diabetes. J Physiol 2005;564:563-73.
31. Koistinen HA, Galuska D, Chibalin AV, Yang J, Zierath JR, Holman GD, et al. 5-amino-imidazole carboxamide riboside increases glucose transport and cell-surface GLUT4

content in skeletal muscle from subjects with type 2 diabetes. Diabetes 2003;52:1066-72.
32. Cao S, Li B, Yi X, Chang B, Zhu B, Lian Z, et al. Effects of exercise on AMPK signaling and downstream components to PI3K in rat with type 2 diabetes. PloS One 2012;7, e51709.
33. Barnes BR, Ryder JW, Steiler TL, Fryer LGD, Carling D, Zierath JR. Isoform-specific regulation of 5′ AMP-activated protein kinase in skeletal muscle from obese Zucker (fa/fa) rats in response to contraction. Diabetes 2002;51:2703-8.
34. Christopher MJ, Chen Z-P, Rantzau C, Kemp BE, Alford FP. Skeletal muscle basal AMP-activated protein kinase activity is chronically elevated in alloxan-diabetic dogs: impact of exercise. J Appl Physiol 2003;95:1523-30.
35. Sriwijitkamol A, Coletta DK, Wajcberg E, Balbontin GB, Reyna SM, Barrientes J, et al. Effect of acute exercise on AMPK signaling in skeletal muscle of subjects with type 2 diabetes a time-course and dose-response study. Diabetes 2007;56:836-48.
36. Böhm A, Hoffmann C, Irmler M, Schneeweiss P, Schnauder G, Sailer C, et al. TGF-β contributes to impaired exercise response by suppression of mitochondrial key regulators in skeletal muscle. Diabetes 2016;65:2849-61.
37. Cartee GD, Wojtaszewski JFP. Role of Akt substrate of 160 kDa in insulin-stimulated and contraction-stimulated glucose transport. Appl Physiol Nutr Metab 2007;32:557-66.
38. Howlett KF, Mathews A, Garnham A, Sakamoto K. The effect of exercise and insulin on AS160 phosphorylation and 14-3-3 binding capacity in human skeletal muscle. American Journal of Physiology-Endocrinology and Metabolism 2008;294. (E401-E7).
39. Funai K, Schweitzer GG, Sharma N, Kanzaki M, Cartee GD. Increased AS160 phosphorylation, but not TBC1D1 phosphorylation, with increased postexercise insulin sensitivity in rat skeletal muscle. American Journal of Physiology-Endocrinology and Metabolism 2009;297. (E242-E51).
40. Treebak JT, Frøsig C, Pehmøller C, Chen S, Maarbjerg SJ, Brandt N, et al. Potential role of TBC1D4 in enhanced post-exercise insulin action in human skeletal muscle. Diabetologia 2009;52:891-900.
41. Geraghty KM, Chen S, Harthill JE, Ibrahim AF, Toth R, Morrice NA, et al. Regulation of multisite phosphorylation and 14-3-3 binding of AS160 in response to IGF-1, EGF, PMA and AICAR. Biochem J 2007;407:231-41.
42. Sano H, Kane S, Sano E, Mïinea CP, Asara JM, Lane WS, et al. Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. J Biol Chem 2003;278:14599-602.
43. Mïinea CP, Sano H, Kane S, Sano E, Fukuda M, Peränen J, et al. AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. Biochem J 2005;391:87-93.
44. Kjøbsted R, Treebak JT, Fentz J, Lantier L, Viollet B, Birk JB, et al. Prior AICAR stimulation increases insulin sensitivity in mouse skeletal muscle in an AMPK-dependent manner. Diabetes 2015;64:2042-55.
45. Arias EB, Kim J, Funai K, Cartee GD. Prior exercise increases phosphorylation of Akt substrate of 160 kDa (AS160) in rat skeletal muscle. American Journal of Physiology-Endocrinology and Metabolism 2007;292. (E1191-E200).
46. Schweitzer GG, Arias EB, Cartee GD. Sustained postexercise increases in AS160 Thr642 and Ser588 phosphorylation in skeletal muscle without sustained increases in kinase phosphorylation. J Appl Physiol 2012;113:1852-61.
47. Castorena CM, Arias EB, Sharma N, Cartee GD. Postexercise improvement in insulin-stimulated glucose uptake occurs concomitant with greater AS160 phosphorylation in muscle from normal and insulin-resistant rats. Diabetes 2014;63:2297-308.
48. Vind BF, Pehmøller C, Treebak JT, Birk JB, Hey-Mogensen M, Beck-Nielsen H, et al. Impaired insulin-induced site-specific phosphorylation of TBC1 domain family, member 4 (TBC1D4) in skeletal muscle of type 2 diabetes patients is restored by endurance exercise-training. Diabetologia 2011;54:157-67.
49. Lauritzen HPMM, Brandauer J, Schjerling P, Koh H-J, Treebak JT, Hirshman MF, et al. Contraction and AICAR stimulate IL-6 vesicle depletion from skeletal muscle fibers in vivo. Diabetes 2013;62:3081-92.
50. Scheler M, Irmler M, Lehr S, Hartwig S, Staiger H, Al-Hasani H, et al. Cytokine response of primary human myotubes in an in vitro exercise model. American Journal of Physiology-Cell Physiology 2013;305. (C877-C86).