Post-operative effects on insulin resistance and specific tension of single human skeletal muscle fibres

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ABSTRACT

Surgery and accidental trauma are associated with a transient period of insulin resistance, substrate catabolism and muscle weakness. In the present study, we evaluated the changes in the force-generating capacity of chemically skinned single muscle fibres following abdominal surgery. Biopsies of the m. vastus lateralis were obtained in three patients 1 day before and 3 or 6 days after surgery. Part of the biopsy was frozen for histochemical analysis of the fibre cross-sectional area (FCSA) and myofibrillar protein content, and another part was used for single-fibre contractile measurements. All patients developed insulin resistance following surgery. The maximum velocity of unloaded shortening of single muscle fibres did not change following surgery. The FCSA did not decrease after surgery, as determined either from histochemical sections or from single fibres measured at a fixed sarcomere length of 2.76 ± 0.09 μm (mean ± S.D.). Further, the force-generating capacity of the single fibres, measured as maximal Ca²⁺-activated force ($P_0$) or as $P_0$ normalized to FCSA (specific tension), remained unchanged, as did the myofibrillar protein content of the muscle. In conclusion, the muscle weakness associated with post-operative insulin resistance is not related to a decreased specific tension or a loss of myofibrillar proteins. Other potential cellular mechanisms underlying post-operative weakness are discussed.

INTRODUCTION

Two characteristics of metabolism following injury and surgery are protein catabolism [1–4] and hyperglycaemia [5,6]. From a theoretical point of view, both of these events can be linked to a reduced effectiveness of insulin in exerting its normal actions. The elevation in blood glucose following stress is caused by a decrease in insulin sensitivity [5–7]. This insulin resistance occurs mainly in peripheral tissues such as skeletal muscle, and may be induced by increased levels of stress hormones such as adrenaline, cortisol and glucagon [3–5,8,9]. However, increased insulin resistance has been reported to develop after abdominal surgery, despite the presence of normal levels of stress hormones, indicating that other factors also initiate this part of the stress response [7]. The increased catabolism of proteins in the post-operative phase can, at least theoretically, be explained by the increase in insulin resistance, since insulin inhibits protein degradation [4,10,11]. After surgery, insulin resistance is present, as is reduced muscle function, which may be related to proteolysis [2–4,12–15].

Key words: post-operative insulin resistance, single fibres, skeletal muscle, specific tension.

Abbreviations: FCSA, fibre cross-sectional area; GIR, glucose infusion rate; $P_0$, maximal Ca²⁺-activated force; $V_0$, maximum velocity of unloaded shortening.

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It is well established that muscle weakness occurs after surgery [13–15]. The mechanism underlying this impairment is not fully understood. It is not known whether impaired excitation–contraction coupling or preferential degradation of myofibrillar proteins causes the decrease in muscle function. The latter would result in a reduction in specific tension, i.e. a decrease in the maximal force per cross-sectional area, if fibre size did not change proportionally. Measurements of skeletal muscle function \textit{in vivo} are limited by factors that tend to obscure the behaviour of individual muscle fibres, such as: (1) intramuscular differences in fibre orientation, (2) differences in the mechanical leverage provided by the bony anatomy of the joint, (3) the elasticity of the muscle and its tendons and, during voluntary contractions, (4) differences in patterns of motor unit recruitment and (5) activation of antagonistic muscles [16]. These confounding factors are circumvented in skinned fibre preparations, which allow investigation of the function of the myofilament proteins in a cell with an intact filament lattice under near-physiological conditions.

The aim of the present study was to improve our understanding of impaired muscle function in the post-operative period when insulin resistance is present. It is hypothesized that enhanced myofibrillar protein degradation and decreased specific tension, or impaired excitation–contraction coupling, or both, primarily cause the impaired muscle function. This study is specifically designed to investigate the effects of surgery on the force-generating capacity of single muscle fibres.

**MATERIALS AND METHODS**

**Subjects and protocol**

Three female patients undergoing major abdominal surgery were studied. Clinical characteristics are given in Table 1. Insulin sensitivity was measured using the hyperinsulinaemic euglycaemic clamp method on 1 day before surgery and 3 or 6 days after surgery (see below). About 1 h after clamps, muscle biopsies were taken for histochemical analysis and determination of contractile properties of single muscle fibres. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and has been approved by the local Ethical Committee, Karolinska Hospital, Stockholm, Sweden. All patients gave their informed consent.

**Glucose clamp**

The hyperinsulinaemic glucose clamp method was used to measure insulin sensitivity [17]. Briefly, after an overnight fast, short-acting insulin (Actrapid; Novo Nordisk, Malmö, Sweden) was infused at a fixed rate (0.8 m-unit·min\(^{-1}·kg\(^{-1}\)), resulting in a serum insulin concentration of approx. 60 \(\mu\)-units·ml\(^{-1}\). A 20 % (w/v) glucose solution (Glucos Pharmacia 200 mg·ml\(^{-1}\); Pharmacia & Upjohn, Stockholm, Sweden) was infused at a variable rate to maintain (clamp) the circulating glucose concentration at 4.5 mmol·l\(^{-1}\). Arterialized venous samples (forearm heating sleeve; Kanthal Medical Heating AB, Stockholm, Sweden) [18] were collected every 3–5 min from an antecubital intravenous catheter. The concentration of glucose was analysed immediately (glucose oxidase method; Yellow Springs Instruments, Yellow Springs, OH, U.S.A.) [19] and the rate of glucose infusion was adjusted. At 50–70 min after the start of the insulin infusion, steady-state levels of glucose and the glucose infusion rate (GIR) were achieved; the clamp was continued for a further 1 h. Insulin sensitivity is expressed as the average GIR during this 1 h, in mg·min\(^{-1}·kg\(^{-1}\) [7]. In all patients insulin sensitivity was determined about 1 week before the operation, as well as 3–6 days after the operation.

**Biopsies**

Biopsies of the m. vastus lateralis were taken under local anaesthesia by the percutaneous conchotome method 1 day before and 3 or 6 days after surgery. One muscle biopsy specimen was frozen in freon cooled in liquid nitrogen for subsequent histochemical analysis. Another biopsy specimen was placed in relaxing solution (see below) for subsequent dissection of fibre bundles for single-fibre contractile measurements.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg·m(^{-2}))</th>
<th>Surgery</th>
<th>GIR (mg·min(^{-1}·kg(^{-1}))</th>
<th>Relative change in GIR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>161</td>
<td>53</td>
<td>20</td>
<td>Ileal pouch procedure</td>
<td>8.20</td>
<td>3.02 (3 days)</td>
</tr>
<tr>
<td>36</td>
<td>173</td>
<td>75.5</td>
<td>25</td>
<td>Perineal rectal resection</td>
<td>4.18</td>
<td>2.69 (3 days)</td>
</tr>
<tr>
<td>70</td>
<td>162</td>
<td>68</td>
<td>26</td>
<td>Anterior rectal resection</td>
<td>2.29</td>
<td>1.03 (6 days)</td>
</tr>
</tbody>
</table>

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**Histochemistry**
Cross-sections of 10 µm were cut with a cryostat (2800 Frigocut E; Reichert-Jung G.m.b.H., Heidelberg, Germany). The sections were stained for myofibrillar ATPase after pre-incubation at pH 4.35. Dark fibres were classified as type I and light fibres as type II (for details, see [20]), while intermediately stained fibres were classified as type I/II fibres. In each individual the fibre cross-sectional area (FCSA) before and after surgery was determined on 100 fibres of each type by using a digitizing tablet (Bioquant IV; R & M Biometrics Inc., Nashville, TN, U.S.A.). The relative number of fibres of each type (%) was calculated by counting the absolute number of fibres in 0.94 mm² of tissue.

**Myofibrillar protein content**
The same biopsy specimen that was used for histochemistry was analysed for myofibrillar protein content. The mounting medium, and also the part of the biopsy that was in the mounting medium, were cut away to prevent bias that may occur because of protein degradation as a consequence of thawing during mounting for histochemistry. After the wet weight of the frozen biopsy had been determined, the specimen was speed-freeze-dried for 1 h in a Speedvac concentrator SVC100H (Savant Instruments Inc., Farmingdale, NY, U.S.A.), and the dry weight determined. Subsequently, the dry/wet weight ratio was determined as an indicator of potential oedema formation following surgery. Then the muscles were homogenized in a solution containing 250 mM sucrose, 100 mM KCl, 5 mM EDTA and 20 mM imidazole at pH 6.8. The total protein content was determined by using bicinchoninic acid (BCA) reagents (Pierce, Rockford, IL, U.S.A.) and expressed as mg of protein per g wet weight. The samples were subsequently used for the preparation of washed myofibrils, using the method of Tsika et al. [21]. The myofibrillar protein content was determined by using the BCA reagents, and expressed as mg of myofibrillar protein per g wet weight.

**Contractile measurements**
The force-generating capacity and maximum velocity of unloaded shortening (\(V_o\)) of single fibres were determined as described previously [22–24]. Briefly, the biopsies were transferred to a relaxing solution at 4 °C (see below), and bundles of about 50 fibres were dissected free and tied to glass capillary tubes at a slightly stretched length. The bundles were chemically skinned for 24 h in relaxing solution containing 50% (v/v) glycerol (skinning solution) at 4 °C, and subsequently stored at −20 °C for up to 4 weeks.

The experimental procedure and the apparatus have been described in detail previously [23,25]. Briefly, fibres were placed for 30 min in relaxing solution (see below) containing 0.5% Brij-58 (polyoxyethylene 20 cetyl ether; Sigma), before they were mounted in the experimental apparatus, leaving an average fibre segment length of 2.47 mm (S.D. 0.71) exposed to the solution between connectors to a force transducer (Model 403; Cambridge Technology Inc., Cambridge, MA, U.S.A.) and a DC torque motor (Model 300H; Cambridge Technology Inc.). The apparatus was mounted on the stage of an inverted microscope (Olympus IX70; Olympus America Inc., Melville, NY, U.S.A.). While the fibre was in relaxing solution, the sarcomere length was set at 2.76 µm (S.D. 0.09), which is the optimal length, by adjustment of the overall segment length. Sarcomere length was measured from videoprints taken with a video copy processor (P67E; Mitsubishi Electric Corp., Tokyo, Japan). Fibre diameter was measured from a videoprint, and the depth was measured by recording the vertical displacement of the microscope nosepiece while focusing on the top and bottom surfaces of the fibre. FCSA was determined on 100 fibres of each type by using a digitizing tablet (Bioquant IV; R & M Biometrics Inc., Nashville, TN, U.S.A.). The relative number of fibres of each type (%) was calculated by counting the absolute number of fibres in 0.94 mm² of tissue.

The relaxing solution contained 10 mM imidazole, 1 mM MgCl₂, 2 mM EGTA and 4.5 mM Na₇₂₄coliATP. The activating solution contained 5.3 mM Na₇₂₄coliATP, 1 mM free Mg²⁺, 20 mM imidazole, 7 mM EGTA and 19.6 mM phosphocreatine. The free Ca²⁺ concentration in the activating solution was 10⁻⁴.⁵ M, and is expressed as pCa (−log([Ca²⁺])). Immediately before each activation, the fibre was submerged for 15 s in a solution similar to the activating solution, except that the pCa was 9.0 and the EGTA concentration was 0.5 mM. This results in more rapid attainment of a steady force during subsequent activation and better preservation of the regularity of the cross-striation. All solutions contained sufficient KCl to adjust the ionic strength to 180 mM. The pH was adjusted to 7.0.

In some fibres, \(V_o\) was measured with the slack-test [26]. All experiments were performed at 12 °C. When a steady tension was reached in the activating solution, slacks of different amplitude were rapidly applied, and the time taken to take up the slack was measured. After each slack, the fibre was re-extended to its resting length, while relaxed. The slope of the linear regression between the slack length and the time taken to take up the slack, based on four or more data points, is the \(V_o\). Fibres were excluded from analyses if the correlation coefficient was less than 0.97, if there was sarcomere non-uniformity, if sarcomere length during isometric tension development changed by > 0.1 µm compared with sarcomere length while relaxed, or if \(P_o\) decreased by more than 10%. The myosin heavy chain compositions were determined in all fibres accepted for the contractile measurements.
Myosin heavy chain isoform composition
After contractile measurements, each fibre was placed in an SDS sample buffer and stored at −20 °C for up to 1 week, and subsequently at −80 °C until analysed. The myosin heavy chain compositions of the fibres were determined by SDS/PAGE. Sample loads were kept small, equivalent to ~0.1 mm of a fibre segment. The total acrylamide and bisacrylamide concentrations were 4% (w/v) in the stacking gel and 7% (w/v) in the running gel, and the gel matrix included 30% (v/v) glycerol. Electrophoresis was performed at 120 V for 25 h with a tris(hydroxymethyl)aminomethane-glycine electrode buffer (pH 8.3) (SE 600 vertical slab gel unit; Hoefer Scientific Instruments) at 15 °C [22–24].

Statistics
All data are means ± S.D., unless otherwise stated. We applied a t-test to test for differences between values obtained before and after surgery for the single-fibre measurements, and a paired t-test for the histochemically determined FCSA. Differences were considered significant at P < 0.05.

RESULTS
The data obtained from the 70-year-old woman were not significantly different from those from the younger ones. Therefore the data from this woman were pooled with the data from the younger women for further analysis.

Insulin sensitivity
All patients in the present study developed insulin resistance following the operation, as reflected by a 36–63% post-operative decrease in GIR (Table 1).

FCSA values
Figure 1 shows representative myofibrillar ATPase-stained muscle biopsy sections before and 3 days after surgery. The 3–6 day post-operative period had no significant impact on the FCSAs of either type I or type II muscle fibres (Figures 1 and 2). However, the interpretative value of FCSA measurements from biopsy cross-sections is limited, because it is not known whether the sections are perpendicular to the longitudinal axis of the muscle fibres, and the degree of contraction (sarcomere length) may vary considerably between muscle biopsies (see [20,27]). In the single fibres, on the other hand, FCSA measurements are made perpendicular to the longitudinal axis of the fibre, and at a fixed sarcomere length. These measurements were performed both while the fibre was suspended in air and when it was in solution. The FCSA was systematically greater when measured in air than in solution (Table 2). However, no significant changes in FCSA were observed after surgery with either method (Table 2).
Table 2  Contractile properties and FCSAs of human single muscle fibres before and 3 or 6 days after surgery

Pre, pre-operative; Post, post-operative. FCSA1 and FCSA2 were determined by method 1 (fibre in relaxing solution) and method 2 (fibre suspended in air) respectively; ST1 and ST2 are specific tension based on FCSA1 and FCSA2 respectively. Ranges are indicated below each mean ± S.D. Difference between values obtained with method 1 and method 2: *P < 0.0001.

<table>
<thead>
<tr>
<th>Subject</th>
<th>P0 (µN)</th>
<th>FCSA1 (µm²)</th>
<th>ST1 (N · cm⁻²)</th>
<th>FCSA2 (µm²)</th>
<th>ST2 (N · cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 years;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre (n = 13)</td>
<td>561 ± 192</td>
<td>2393 ± 429</td>
<td>33.84 ± 10.04</td>
<td>4387 ± 808</td>
<td>19.16 ± 6.83</td>
</tr>
<tr>
<td>20 years;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 years;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre (n = 12)</td>
<td>463 ± 185</td>
<td>2440 ± 427</td>
<td>38.64 ± 11.70</td>
<td>4110 ± 937 (13)</td>
<td>22.74 ± 5.68 (13)</td>
</tr>
<tr>
<td>36 years;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post (n = 7)</td>
<td>304–998</td>
<td>1767–3901</td>
<td>19.30–62.29</td>
<td>2552–6362</td>
<td>14.60–32.53</td>
</tr>
<tr>
<td>70 years;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre (n = 9)</td>
<td>733 ± 195</td>
<td>3628 ± 978</td>
<td>29.39 ± 4.04</td>
<td>5192 ± 1648 (10)</td>
<td>21.76 ± 5.93 (10)</td>
</tr>
<tr>
<td>70 years;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post (n = 11)</td>
<td>600 ± 204</td>
<td>2954 ± 526</td>
<td>20.56 ± 5.07</td>
<td>3881 ± 1455 (6)</td>
<td>23.74 ± 6.48 (6)</td>
</tr>
<tr>
<td>All; Pre (n = 34)</td>
<td>337–1119</td>
<td>1572–3216</td>
<td>20.64–46.01</td>
<td>2463–4657</td>
<td>18.56–29.41</td>
</tr>
<tr>
<td>All; Post (n = 32)</td>
<td>293–998</td>
<td>1767–3913</td>
<td>19.30–62.29</td>
<td>2280–8012</td>
<td>14.60–32.53</td>
</tr>
</tbody>
</table>

Table 3  Dry/wet weight ratio, total protein content and myofibrillar protein content, and myofibrillar protein/total protein ratio before and 3 or 6 days after surgery

Data are presented as mean ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Dry/wet weight ratio</th>
<th>Total protein (mg · g⁻¹ wet weight)</th>
<th>Myofibrillar protein (mg · g⁻¹ wet weight)</th>
<th>Myofibrillar protein/total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>0.31 ± 0.08</td>
<td>127 ± 14</td>
<td>32 ± 11</td>
</tr>
<tr>
<td>Post-operative</td>
<td>0.30 ± 0.05</td>
<td>129 ± 7</td>
<td>42 ± 8</td>
</tr>
</tbody>
</table>

respectively; type I/II, 2.1 ± 3.2% and 0.4 ± 0.6% respectively).

Myofibrillar protein content

The unchanged dry/wet weight ratio indicates that there was no oedema formation in the muscle following surgery (Table 3). The total protein content and myofibrillar protein content were similar pre- and post-operatively, indicating that there was no post-operative protein loss or selective loss of myofibrillar proteins (Table 3).

Single-fibre contractility

Type I fibres were slower than type II fibres, but there was no significant change in the Vₜ₀ of either type I or type II fibres as a consequence of the surgery (before and after surgery: type I, 0.35 ± 0.07 ML · s⁻¹ (n = 6) and 0.41 ± 0.13 ML · s⁻¹ (n = 10) respectively; type II, 1.35 ± 0.61 ML · s⁻¹ (n = 4) and 0.96 ± 0.27 ML · s⁻¹ (n = 3) respectively, where ML is muscle length). Furthermore, neither P₀ nor the force-generating capacity (specific tension), i.e. force normalized to FCSA, were affected by surgery and the increase in insulin resistance (Table 2, Figure 3).

Figure 3  Specific tension in human single muscle fibres before and 3 or 6 days after major surgery

Specific tension was calculated from the width and depth of the fibre while in relaxing solution. Numbers in parentheses indicate numbers of fibres.
DISCUSSION

It has been reported that increased insulin resistance and muscle protein catabolism are part of the response to general surgery [2–4], and it is anticipated that this would result in muscle fibre atrophy and impaired muscle function. However, in spite of a post-operative increase in insulin resistance, similar to that reported previously after major abdominal surgery [7,28], we did not find any significant changes in fibre size, myofibrillar protein content or force-generating capacity at the single-fibre level in the early post-operative period.

It is unlikely that the absence of detectable muscle fibre atrophy in the early post-operative period is a result of oedema, since the dry/wet weight ratio and specific tension were both unaltered in the post-operative phase. It may be, however, that the insensitive morphometrical technique did not detect small changes in muscle fibre size in muscle biopsy cross-sections. However, fibre atrophy was not observed in single-fibre preparations either. Additional evidence for an unchanged FCSA following surgery comes from the unchanged maximal force-generating capacity of the single muscle fibres. It is accordingly concluded that the increased insulin resistance observed in the early post-operative phase is not accompanied by muscle fibre atrophy.

Several studies have indicated a loss of muscle protein after surgery [2–4]. However, the methods employed to determine protein loss do not discriminate between different origins, such as the contractile apparatus, membrane proteins, etc. The present results show that the early loss of muscle force following major surgery (see [12,15]) is not caused primarily by enhanced myofibrillar protein degradation or decreased specific tension. In the skinned-fibre preparation, myofibrillar proteins are well maintained and ATP is readily available, but membranes such as the sarcolemma and the sarcoplasmic reticulum are rendered inactive by treatment with glycerol and detergents. Insulin has been shown to have a stimulatory effect on the activity of the muscle membrane Na\(^+\),K\(^+\)-pump, and addition of insulin to K\(^+\)-depressed muscle has been demonstrated to result in force recovery [29]. Thus it is more likely that the muscle debilitation occurring during insulin resistance is related primarily to changes such as may occur in sarcolemma Na\(^+\),K\(^+\)-pump activity, and not to degradation of myofibrillar proteins.

The reduced post-surgical availability of glycogen as an energy source for the muscle most probably does not affect the force-generating capacity. The initial energy delivery for muscle contraction comes from phosphorylation and ATP stores in the muscle, and only after a few seconds does the delivery begin of energy via oxidative and glycolytic pathways, which consume glycogen. Therefore energy storage in the form of glycogen is not important for maximal force production. However, depletion of glycogen, independent of its role in energy production, has been suggested as a factor triggering functional changes in the sarcoplasmic reticulum [30]. Since the sarcoplasmic reticulum is important for Ca\(^{2+}\)-handling, it has been suggested as a factor underlying the decrease in force-generating capacity during prolonged exercise [30]. Similarly, the decreased insulin-induced glycogenesis in the muscle in post-operative insulin-resistant patients [31] may provide an additional explanation for the reduced force-generating capacity of muscle reported in the post-operative phase (see [12,13,15]).

Finally, glucocorticoids have a catabolic effect on nitrogen metabolism, and may decrease any potential stimulatory effect of insulin on protein synthesis and blunt the anti-proteolytic action of insulin [10,11,32]. It is therefore possible that the absence of any indications of changes in muscle function or muscle wasting may be related to relatively low levels of glucocorticoids in our patients [7,31].

In conclusion, the size, myofibrillar protein content, force-generating capacity and \(V_c\) of individual muscle fibres are unchanged for up to 6 days after surgery, despite the presence of marked insulin resistance. The loss of muscle function during the first few days following surgery is thus most likely attributable to factors other than loss of myofibrillar proteins.

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455Post-operative specific tension of muscle fibres


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