Glycogen depletion and lactate accumulation in human intercostal muscles after administration of succinylcholine

M. Mizuno and N. H. Secher

Summary

I.m. glycogen content, lactate concentration and staining intensity for glycogen in slow- (ST) and fast-twitch (FTa and FTb) fibres were determined in the external and internal intercostal muscles after thoracotomy. Thirteen patients received a precurarizing dose of a non-depolarizing neuromuscular blocking agent followed by the depolarizing agent, succinylcholine, to facilitate intubation of the trachea (succinylcholine group). Nine patients received pancuronium or gallamine (control group). There were no significant differences in ventilatory functional capacity between the two groups. In the external intercostal muscles, lower i.m. glycogen content was observed in the succinylcholine compared with the control group \((P < 0.05)\). I.m. glycogen was depleted from the FT fibres and in particular from FTb fibres, while little depletion was noted in ST fibres. In both the external and internal intercostal muscles, a higher lactate concentration was observed in the succinylcholine than in the control group \((P < 0.05)\). The results indicate that the use of succinylcholine for tracheal intubation stimulated breakdown of i.m. glycogen, particularly in fast-twitch muscle fibres, and caused accumulation of lactate in intercostal muscles. (Br. J. Anaesth. 1998; 80: 302–307)

Keywords: metabolism, glycogen; metabolism, lactate; muscle skeletal, metabolism; neuromuscular block, pancuronium; neuromuscular block, succinylcholine

Depolarizing or non-depolarizing neuromuscular blocking agents can be used to facilitate intubation of the trachea. After administration of a depolarizing agent, plasma potassium concentration increases.\(^1\)\(^3\) The efflux of intracellular potassium induced by the depolarizing agent may be caused by muscle fasciculations at the onset of neuromuscular block.\(^4\) Even after pretreatment with a non-depolarizing agent such as tubocurarine, pancuronium or gallamine, followed by succinylcholine, plasma potassium concentration is higher than after the use of a non-depolarizing agent alone.\(^1\)\(^3\) Fasciculation of ventilatory and other skeletal muscles is observed after combined administration of a non-depolarizing and depolarizing agent.\(^5\)\(^7\)

In a preliminary report, we noted a high lactate concentration and a low glycogen content in the intercostal muscles of surgical patients in whom the depolarizing agent, succinylcholine, was used to facilitate intubation of the trachea.\(^8\) This occurred even though a precurarizing dose of a non-depolarizing agent was used. No control group was available, but the difference was marked compared with reported values from spontaneously breathing patients.\(^9\) These results indicate that muscle fasciculations induced by succinylcholine cause i.m. metabolic changes similar to those observed in limb skeletal muscles during exercise. A breakdown of i.m. glycogen and accumulation of lactate occurs in proportion to the intensity and duration of exercise\(^10\)\(^11\); it also depends on the type of muscle fibres recruited.\(^12\)

Human skeletal muscles,\(^13\) including ventilatory muscles,\(^14\) consist of two major fibre types: slow- (ST or type I) and fast-twitch (FT or type II), with FT fibre subgroups (FTa and FTb). ST fibres contract more slowly and fatigue less during repetitive contractions compared with FTb fibres.\(^15\) ST fibres have higher mitochondrial and lower glycolytic enzyme activities than FTb fibres. Fatigue characteristics and metabolic properties of FTa fibres resemble those of ST fibres, but they contract at a speed comparable with FTb fibres.

Histochemical evaluation of muscle fibre types and estimation of i.m. glycogen in individual muscle fibres allows investigation of glycogen depletion in specific types of muscle fibres.\(^16\)\(^18\) We have evaluated the effect of a precurarizing dose of a non-depolarizing agent followed by succinylcholine on glycogen content and lactate concentration in external and internal intercostal muscles of patients undergoing thoracotomy. Histochemical evaluation of staining intensity for i.m. glycogen in ST and FT fibres was performed and data were compared with those obtained from similar biopsies of patients who received only a non-depolarizing agent.

Patients and methods

We studied 22 patients with no history of neuromuscular disease who fasted overnight and underwent thoracotomy for benign \((n = 6)\) or malignant \((n = 16)\) pulmonary disease (table 1). In order to obtain data...
Table 1  Physical and ventilatory characteristics, and glycogen content and lactate concentration in external and internal intercostal muscles for two groups of patients undergoing thoracotomy with and without the use of succinylcholine (mean (SD or range) or number). FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity; MBC = maximal breathing capacity. *P<0.05 between groups

<table>
<thead>
<tr>
<th></th>
<th>Succinylcholine group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>2/11</td>
<td>2/7</td>
</tr>
<tr>
<td>Tumour (benign/malignant)</td>
<td>2/11</td>
<td>4/5</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>56 (35–71)</td>
<td>54 (28–75)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175 (9)</td>
<td>171 (6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 (13)</td>
<td>70 (18)</td>
</tr>
<tr>
<td>FEV1 (litre s(^{-1}))</td>
<td>7.5 (0.7)</td>
<td>3.4 (0.6)</td>
</tr>
<tr>
<td>FVC (litre)</td>
<td>3.7 (0.7)</td>
<td>3.4 (0.6)</td>
</tr>
<tr>
<td>MBC (litre min(^{-1}))</td>
<td>94 (24)</td>
<td>98 (19)</td>
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Lactate (µmol g\(^{-1}\) dry wt)

<table>
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<tr>
<th></th>
<th>External</th>
<th>Internal</th>
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<tbody>
<tr>
<td></td>
<td>233.0 (60.7)*</td>
<td>305.8 (60.0)</td>
</tr>
<tr>
<td></td>
<td>298.7 (148.3)</td>
<td>315.8 (80.2)</td>
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Lactate (µmol g\(^{-1}\) dry wt)

<table>
<thead>
<tr>
<th></th>
<th>External</th>
<th>Internal</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>23.6 (10.7)*</td>
<td>6.5 (3.1)</td>
</tr>
<tr>
<td></td>
<td>24.8 (11.2)*</td>
<td>11.4 (3.5)</td>
</tr>
</tbody>
</table>

using two non-depolarizing neuromuscular blocking agents, in four patients intubation of the trachea was facilitated by a precurarizing dose of pancuronium 1 mg, and in nine patients, gallamine 20 mg. A mean dose of succinylcholine 130 mg (range 100–150 mg) was then administered (succinylcholine group). In the other nine patients, pancuronium 8 mg (n = 1) or gallamine 160 mg (120–240 mg) (n = 8) was used for tracheal intubation (control group). Patients were allocated randomly to receive succinylcholine. The study was approved by the local Ethics Committee. In all patients, induction of anaesthesia comprised fentanyl 3 µg kg\(^{-1}\), midazolam 0.07 mg kg\(^{-1}\) and thiopental 3 mg kg\(^{-1}\), and was maintained with 70% nitrous oxide in oxygen. We did not use halothane or isoflurane as these agents may influence glycogen depletion and lactate accumulation in skeletal muscle after the use of succinylcholine because of “desensitization” of nicotinic cholinergic receptors.19

**BIOPSY SAMPLING**

Surgical biopsies of the external and internal intercostal muscles were obtained from the fourth to the seventh intercostal space in the mid-axillary line. The muscle sample was dissected immediately into two parts. One piece for biochemical analysis was frozen directly in liquid nitrogen. The remaining portion for histochemical analysis was mounted in an embedding medium (OCT Compound, Miel’s Tissue-Tek), frozen in isopentane and cooled to freezing point by liquid nitrogen. These samples were stored at −80 °C until analyses were performed. Biochemical and histochemical analyses of muscle biopsies were performed without knowledge of the drug(s) used for tracheal intubation.

**BIOCHEMISTRY**

The muscle samples for quantitative determination of glycogen content and lactate concentration were freeze-dried in a thermoelectric freeze-dryer (Heto-sic CD 52) for 48 h at −50 °C at a pressure of 0.01 Torr. Thereafter, each muscle sample was dissected under a microscope to remove blood, fat and connective tissue in a room where the temperature (20 °C) and humidity (<30%) were controlled. The dissected fibre fragments of 1.5 (0.5–3.0) mg dry weight for glycogen and 1.3 (0.5–2.7) mg dry weight for lactate were hydrolysed with HCl 1 mol litre\(^{-1}\). Samples for glycogen were hydrolysed further at 100 °C for 2 h; glycogen was analysed as glucose residues. Glucose and lactate concentrations were measured fluorometrically.20 The coefficients of variance of double determinations of glycogen content and lactate concentrations were 1.8% and 5.6%, respectively.

**HISTOCHEMISTRY**

The frozen muscle sample was replaced in a Cryo-cut Cryostat Microtome (American Optical). In order to evaluate the relative glycogen content of individual muscle fibres, one transverse section (16 µm thick) was cut at −20 °C and stained for glycogen by the periodic acid–Schiff (PAS) reaction.21 Under light microscopy the staining intensity of the fibres was rated as “dark”, “moderate”, “light” or “negative” and ranked 3 to 0, respectively.17 The proportion of the four-rated intensities and mean staining intensity as an average of ranked values were determined for each muscle biopsy. The rating was performed on 392 (287–539) fibres in each muscle biopsy.
Five serial transverse sections (10 μm thick) were also cut and incubated for myofibrillar adenosine triphosphatase (ATPase) reaction at pH 9.4,22 after preincubation at pH 4.3, 4.6 or 10.3.23 The remaining two sections were incubated with reduced nicotinamide adenine dinucleotide tetrazolium (NADH-TR)24 or alpha-glycerophosphate dehydrogenase (α-GPD)25 as markers for oxidative and glycolytic potentials, respectively. These sections were used to evaluate coupling between contractile and metabolic characteristics of individual fibres. Based on myofibrillar ATPase staining, individual fibres were classified under light microscopy as ST, FTa, FTb or unidentified.26 The relative occurrence of each fibre type was determined from 544 (187–937) fibres in each biopsy. Unidentified fibres (range 0.3–1.5 %) were not included in the analysis for histochemical evaluation of i.m. glycogen.

STATISTICAL ANALYSIS

Values are expressed as mean (sd). Glycogen content, lactate concentration and glycogen staining were compared between the two groups using the Mann–Whitney U test.27 Spearman's coefficient of rank correlation was used to evaluate interdependencies. A level of 5% was considered significant for two-tailed tests.

Results

There was no significant difference in patient characteristics or ventilatory functional capacity between the two groups (table 1). Mean time from the start of anaesthesia to biopsy sampling was 35 min (range 20–55 min); there was no difference between the two groups.

GLYCOGEN AND LACTATE CONCENTRATION

In the external intercostal muscle, the succinylcholine group showed a 20% lower glycogen content and a four-fold higher lactate concentration than the control group (table 1). Similarly, lactate concentration in the internal intercostal muscle was two-fold higher in the succinylcholine than in the control group, while no significant difference in i.m. glycogen content was observed. A significant correlation could not be demonstrated between lactate concentration and glycogen content or between lactate concentration determined in external or internal intercostal muscles and time of sampling of the biopsy. For both groups, comparison between external and internal intercostal muscles revealed no significant difference in glycogen content or lactate concentration. There was no difference in muscle biochemistry between patients with benign or malignant disease.

GLYCOGEN DISTRIBUTION IN MUSCLE FIBRE TYPES

The relative occurrence of ST, FTa and FTb fibres was 62 (9), 17 (9) and 21 (10) % for external intercostal muscles and 59 (18), 39 (15) and 2 (3) % for internal intercostal muscles (ns between groups).
For external intercostal muscles, staining intensity for glycogen of ST fibres was similar in the succinylcholine and control groups (dark 65 (21) vs 78 (20) %; moderate 32 (14) vs 21 (19) %), while FTa fibres stained weaker in the succinylcholine than in the control group (dark 23 (25) vs 64 (28) %; moderate 38 (20) vs 34 (24) %; light 33 (22) vs 2 (7) %; negative 6 (1) vs 0 (0) %) (figs 1–3). In particular, FTb fibres were stained the weakest (dark 0 (0) vs 45 (35) %; moderate 31 (26) vs 38 (23) %; light 60 (23) vs 17 (25) %; negative 9 (13) vs 0 (0) %). Mean staining intensity for glycogen in FT fibres was lowest in the succinylcholine group (FTa 1.8 (0.6) vs 2.6 (0.3) %; FTb 1.2 (0.3) vs 2.3 (0.6) % (P<0.01)), while a similar value was obtained for ST fibres (2.6 (0.3) vs 2.7 (0.3) %).

In the internal intercostal muscles, for which ST and FTa fibres were mainly identified, both fibre types were stained more weakly in the succinylcholine than in the control group (ST: dark 50 (33) vs 96 (8) %; moderate 39 (22) vs 4 (8) %; light 11 (14) vs 0 (0) %; FTa: dark 34 (33) vs 89 (23) %; moderate 52 (26) vs 11 (21) %; light 14 (19) vs 11 (21) %). Thus mean staining intensity for glycogen in both fibre types was also low in the succinylcholine group (ST 2.3 (0.5) vs 3.0 (0.1) %; FTa 1.9 (0.8) vs 2.9 (0.3) %).

ST fibres were stained darker for NADH-TR activity than FT fibres in both muscles, whereas the opposite was seen for α-GPD (figs 1 and 2). There was no difference in staining intensity for these enzyme activities between the two groups.

Discussion

We have demonstrated that the use of succinylcholine for intubation of the trachea in patients undergoing thoracotomy caused substantial breakdown of i.m. glycogen in external intercostal muscles and an increase in lactate concentration in both external and internal intercostal muscles. This was the case although a precurarizing dose of a non-depolarizing agent (either pancuronium or gallamine) was used before succinylcholine. Biochemical changes are more likely to be produced if succinylcholine alone has been administered, as the level of muscle fasciculation and increase in plasma potassium are larger without pretreatment with non-depolarizing agents. The degree of glycogen depletion in individual muscle fibres was evaluated in histochemical transverse sections stained by the PAS technique. The results indicated that i.m. glycogen in FT fibres, particularly in FTb fibres, was more depleted than in ST fibres for both external and internal intercostal muscles. The predominant effect of succinylcholine on FT fibres in this study agrees with results from human limb skeletal muscles.28 29

Glycogen and lactate concentration

Evaluation of glycogen depletion was based on the assumption that, before operation, the level of glycogen was similar in the two groups of patients. Similar i.m. glycogen content for external and internal intercostal muscles (250 and 309 μmol g⁻¹ dry weight, respectively) was reported in biopsies obtained from patients with chronic obstructive pulmonary disease during thoracotomy after administration of both depolarizing and non-depolarizing neuromuscular blocking agents.30 In contrast, approximately half of that value (170 μmol g⁻¹ dry weight) was observed in biopsies obtained from the external intercostal muscle of healthy controls under local anaesthesia during spontaneous breathing.9 Thus glycogen content of intercostal muscles may be affected by the ventilatory capacity of the patient. It is unlikely, however, that the lower glycogen content and higher lactate concentration observed in the succinylcholine group were caused by a difference in ventilatory capacity, as FEV₁, FVC and MBC were similar in the two groups. Further, in this study, approximately 70% of subjects had malignant disease. This is in accordance with a previous observation30 that malignant disease does not appear to affect i.m. glycogen content and lactate concentration of external and internal intercostal muscles.

In the control group, lactate concentrations in external (7 μmol g⁻¹ dry weight) and internal (11 μmol g⁻¹ dry weight) intercostal muscles were similar to reported values (7–12 μmol g⁻¹ dry weight), while those observed in the succinylcholine group (25 μmol g⁻¹ dry weight) were higher.3 A similar value to that determined in the control group (10 μmol g⁻¹ dry weight) was also reported in biopsies obtained from both external and internal intercostal muscles after the use of succinylcholine.30 Why lactate concentration was lower in their study compared with the succinylcholine group reported here is not obvious. One explanation could be a shorter time between the use of succinylcholine and biopsy in this study (35 min) compared with a previous study, although the time was not given.30 Alternatively, a smaller precurarizing dose of a non-depolarizing agent could have been administered in this study. Lactate concentration determined in thigh muscles 30 min after intense exercise11-12 corresponds to that obtained in our succinylcholine group (25 μmol g⁻¹ dry weight). During recovery from extremely heavy bicycling, muscle lactate concentration reaches approximately 80 μmol g⁻¹ dry weight.32

Fibre type specificity of neuromuscular blocking agents

A preferential effect of succinylcholine on FT fibres was indicated in this study. This finding is supported by results from animal experiments, demonstrating that the neuromuscular blocking agent decamethonium13-14 and succinylcholine15 have a preference for muscles dominated by FT fibres. In contrast, the non-depolarizing neuromuscular blocking agent, tubocurarine, has a preference for muscles dominated by ST fibres.33-34 Similar findings are available from human extremity skeletal muscles with respect to the isometric mechanogram and force during maximal voluntary contraction16-17, endurance time18 and electromyographic activity during a sustained contraction19; glycogen depletion pattern during cycling20; and 31P-NMR detected energy metabolism during rhythmic forearm exercise.29

Coupling between the pattern of i.m. glycogen depletion and involvement of a specific type of muscle fibre is demonstrated during low intensity exercise with selective glycogen depletion from ST
fibres, while high intensity exercise results in depletion of glycogen from FT fibres, and in particular from FTb fibres. However, there may not be a direct link between the pattern of i.m. glycogen depletion and involvement of specific muscle fibres during muscle fasciculations after the use of succinylcholine. Quantitative analyses of single human muscle fibres reveal that FT fibres have larger glycolytic potentials than ST fibres. In addition, a subgroup of FT fibres (FTb fibres) show the largest potentials. Furthermore, FT fibres use more ATP and thereby deplete a larger amount of glycogen than ST fibres during repeated electrical stimulation-induced contractions. Thus there is a metabolic bias to support the preferential effect of succinylcholine on FT and, in particular, on FTb fibres. Therefore, we conclude that after administration of succinylcholine, depletion of glycogen and accumulation of lactate take place predominantly in the fast-twitch fibres of human skeletal muscles.

What would be the clinical implications of selective glycogen depletion and accumulation of lactate in intercostal muscles after the use of succinylcholine? When the glycogen concentration in exercising muscles during bicycling becomes significantly low (≤80 μmol g⁻¹ dry weight), it is associated with reduced performance. However, the reduction in i.m. glycogen in external intercostal muscle is less, and is not therefore expected to affect ventilation. Equally, the increase in muscle lactate, approximately 30 min after the use of succinylcholine, may not influence patients by the time the operation is completed, as the level of muscle lactate would by then have returned to resting levels. However, with the combined use of depolarizing and non-depolarizing neuromuscular blocking agents during surgery, postoperative ventilatory function may be impaired by the combined effects of persistent neuromuscular block of, in particular, ST fibres by the non-depolarizing agent and weakness caused by the development of fatigue in glycogen-depleted FT fibres by the depolarizing agent.

Acknowledgements
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References


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