

Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients

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Summary

Background Maintenance of normoglycaemia by use of insulin reduces morbidity and mortality of patients in surgical intensive care. Studies on mitochondrial function in critical illness or diabetes suggest that effects of intensive insulin therapy on mitochondrial integrity contribute to the clinical benefits.

Methods Enzyme activities of the respiratory-chain complexes and oxidative-stress-sensitive glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured by spectrophotometry in 36 snap-frozen samples of liver and skeletal muscle obtained after death from patients who had been randomly assigned intensive (normoglycaemia) or conventional (hyperglycaemia) insulin therapy and who were similar in terms of admission diagnoses and causes of death. Mitochondrial ultrastructure was examined by electron microscopy in a random subgroup (n=20).

Findings In the liver, hypertrophic mitochondria with an increased number of abnormal and irregular cristae and reduced matrix electron density were observed in seven of nine conventionally treated patients. Only one of 11 patients given intensive insulin treatment had these morphological abnormalities ($p=0.005$). The effect on ultrastructure was associated with higher activities of respiratory-chain complex I (median 1.53 [IQR $1.14-3.01$] *vs* 0.81 [$0.54-1.43$] U/g liver; $p=0.008$) and complex IV (1.69 [$1.40-1.97$] *vs* 1.16 [$0.97-1.40$] U/g; $p=0.008$) in the intensive group than in the conventional group. There was no detectable difference in GAPDH activity. In skeletal muscle, mitochondrial ultrastructure and function were not affected by intensive insulin therapy.

Interpretation Strict glycaemic control with intensive insulin therapy prevented or reversed ultrastructural and functional abnormalities of hepatocyte mitochondria. The lack of effect on skeletal-muscle mitochondria suggests a direct effect of glucose toxicity and glucose control, rather than of insulin, as the likely explanation.

Relevance to practice Maintenance or restoration of mitochondrial function and cellular energetics is another therapeutic target, in addition to optimisation of cardiac output, systemic oxygen delivery, and regional blood flow, that might improve outcome for critically ill patients. Our findings could help to explain the mechanism underlying the reduction in mortality found when normoglycaemia was maintained with insulin, and further support use of intensive insulin therapy in this setting.

Introduction

Hyperglycaemia is common in critically ill patients, as a result of stress-induced insulin resistance and accelerated glucose production.^{1,2} Maintenance of normoglycaemia with insulin during intensive care was recently shown to reduce mortality while in the intensive-care unit and in hospital of patients in surgical intensive care. Intensive insulin therapy also improved morbidity,^{2,3} reducing the risks of sepsis, excessive inflammation,⁴ and multiple organ failure, transfusion requirements, and dependence on mechanical ventilation and intensive care. The mechanisms underlying these beneficial clinical effects remain incompletely understood.

Cellular function requires energy supplied by ATP. Under aerobic conditions, most of the ATP necessary to supply organs and tissues with energy is generated by the mitochondrial oxidative phosphorylation system. Electrons derived from oxidation of glucose or fatty acids are transferred through the respiratory-chain complexes I–IV. At complexes I, III, and IV, protons are

pumped out of the mitochondrial matrix into the intermembrane space. This action results in the generation of an electrochemical proton gradient, which is used by a fifth enzyme complex (ATP synthase) to drive ATP synthesis. A dysfunctional mitochondrial respiratory chain can affect all organs and tissues and cause a wide variety of disorders.⁵ Several lines of evidence support the hypothesis that cellular energy metabolism is disturbed in sepsis and critical illness. This disturbance was originally ascribed to inadequate tissue perfusion leading to cellular hypoxia. Recent studies, however, point to a disturbance in oxygen utilisation rather than delivery, which has been labelled “cytopathic hypoxia”.^{6–9} Such an abnormality in cellular energy metabolism in critically ill patients is likely to cause organ system dysfunction, the most common cause of death in intensive-care units. In diabetes mellitus, hyperglycaemia-induced overproduction of superoxide by the mitochondrial respiratory chain, inhibiting glyceraldehyde-3-phosphate dehydrogenase

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	Conventional insulin therapy (n=18)	Intensive insulin therapy (n=18)	p
Demography and anthropometry			
M/F	11/7	13/5	0.7
Mean (SD) age, years	68 (13)	69 (15)	0.7
Median (IQR) body-mass index, kg/m ²	25.5 (24.4–27.5)	24.0 (20.9–27.7)	0.3
History			
Diabetes	2 (11%)	0	0.5
Malignant disease	4 (22%)	6 (33%)	0.7
Reason for admission to intensive-care unit			
Cardiac or complicated aortic surgery	9	6	0.5
Complicated abdominal surgery or peritonitis	2	5	
Cerebral or multiple trauma	3	5	
Complications after oesophageal or pulmonary surgery	3	2	
Other	1	0	
Blood glucose			
Mean (SD) blood glucose on admission, mmol/L	10.9 (5.3)	9.1 (3.9)	0.3
Number with hyperglycaemia (≥ 11 mmol/L) on admission	5 (28%)	5 (28%)	>0.9
Mean (SD) morning blood glucose, mmol/L	9.9 (0.9)	5.6 (0.4)	<0.0001
Mean (SD) blood glucose on last day, mmol/L	10.3 (2.2)	5.7 (1.1)	<0.0001
Insulin			
Median (IQR) daily dose, IU	14.4 (2.1–34.0)	44.2 (23.1–87.0)	0.005
Median (IQR) dose on last day, IU	0 (0–30.0)	29.0 (21.0–66.0)	0.002
Scores on clinical assessments			
APACHE II >15 during first 24 h	4 (22%)	11 (61%)	0.04
Median (IQR) APACHE II during first 24 h	11 (9–13)	17 (12–18)	0.08
Median (IQR) TISS-28 during first 24 h	41 (35–49)	38 (32–43)	0.1
Median (IQR) APACHE II on last day	21 (14–28)	24 (16–28)	0.6
Median (IQR) TISS-28 on last study day	40 (34–44)	36 (33–41)	0.6
Comorbidity and treatment			
Critical illness polyneuropathy	10 (56%)	5 (28%)	0.3
Renal replacement therapy	11 (61%)	9 (50%)	0.7
Inotropic/vasoactive support	17 (94%)	17 (94%)	>0.9
Median (IQR) days treated with inotropes	8 (4–13)	9 (4–12)	0.8
Median (IQR) days treated with norepinephrine	11 (2–20)	10 (6–14)	0.8
Glucocorticoid treatment	14 (78%)	10 (56%)	0.3
Thyroid hormone treatment	5 (28%)	9 (50%)	0.3
Days in ICU before death			
Median (IQR) days in ICU	34 (12–42)	12 (7–24)	0.03
Cause of death			
Cardiovascular collapse	2	2	>0.9
Multiple organ failure with sepsis and/or SIRS	15	15	
Severe brain damage	1	1	

Unless otherwise indicated, data are number of patients. APACHE II=acute physiology and chronic health evaluation; TISS-28=therapeutic intervention scoring system; SIRS=systemic inflammatory response syndrome.

Table 1: Characteristics of patients

(GAPDH), has been linked to vascular damage to organs and tissues.¹⁰

Oxidative stress
Disequilibrium between pro-oxidants and antioxidants in biological systems.

Since increased **oxidative stress** and bioenergetic failure contribute to multiple organ failure in critically ill patients, we hypothesised that a protective effect of intensive insulin therapy on mitochondrial integrity has a role in its potential to improve outcome. We therefore studied mitochondrial ultrastructure, respiratory-chain function, and GAPDH activity in samples of liver and skeletal muscle from patients who had been randomly assigned conventional or intensive insulin therapy.²

Methods

Patients

For this study, we selected a subgroup of patients who had been included in a large randomised controlled trial

(n=1548) studying the effects of intensive insulin therapy in adult, mechanically ventilated patients admitted to the surgical intensive-care unit.² In that trial, patients were randomly assigned either intensive insulin therapy to maintain blood glucose concentrations between 4.4 mmol/L and 6.1 mmol/L (normoglycaemia) or conventional insulin therapy, in which insulin was administered only if glucose concentrations exceeded 11.9 mmol/L, with the aim of keeping concentrations at 10.0–11.1 mmol/L. Written informed consent was obtained from the closest family member. Post-mortem biopsy samples of liver (lower right quadrant) and skeletal muscle (right musculus rectus abdominis) were taken from 74 of the 98 patients who died in the intensive-care unit. The samples were snap-frozen in liquid nitrogen within 30 min (SD 20) of death and stored at –80°C until analysis. The study protocol was approved by the Institutional Review Board of the Catholic University of Leuven.

For analysis of mitochondrial function, we selected 36 patients, 18 from each group, who were matched for demographic characteristics, reason for admission to the intensive-care unit, invasive therapeutic strategies, and cause of death, and from whom biopsy samples were available (table 1). All deaths occurred after a multidisciplinary decision to restrict therapy when further treatment was judged to be futile.

Mitochondrial ultrastructure was studied by electron microscopy in a random subselection of 20 biopsy samples. The investigators who independently examined morphology and mitochondrial function in the samples were unaware of patients' treatment assignment.

Procedures

Small fragments of frozen liver and skeletal-muscle tissue were thawed in fixative solution consisting of 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.2, and stored overnight at 4°C. After 1 h of post-fixation in 1% osmium tetroxide in 0.1 mol/L phosphate buffer, the samples were dehydrated in increasing concentrations of ethanol, and embedded in epoxy resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 10 electron microscope.

Liver and muscle homogenates (1 in 15 weight/volume) were prepared from 50–100 mg tissue, centrifuged, subjected to five freeze-thaw cycles, and sonicated if necessary.¹¹ The activity of citrate synthase (a mitochondrial marker enzyme) was measured before centrifugation and in the postnuclear fraction. The latter fraction was used to assess the individual activities of complexes I–V.

The enzymatic reactions were carried out at 30°C and were followed by the change in absorbance detected with a Lambda 25 UV/VIS spectrometer (Perkin Elmer, Norwalk, CT, USA).¹² For the assay of complex V, we used a 40 mmol/L Tris-bicarbonate buffer with

10 mmol/L ethylene glycol bis (2-aminoethylether) tetraacetic acid, pH 8.0, to which we added 200 μ mol/L NADH, 2.5 mmol/L phosphoenolpyruvate, 5 μ mol/L antimycin A, 5 mmol/L magnesium chloride, 27.5 U/mL lactate dehydrogenase, and 10 U/mL pyruvate kinase. After preincubation for 2 min with 2.5 mmol/L ATP, the reaction was initiated by addition of the sample. For liver, 2 μ mol/L oligomycin was added to the blank; for skeletal muscle, a sample blank without ATP and an ATP blank without sample were prepared. The recovery of citrate synthase activity in the supernatant relative to its activity in the crude homogenate was used to correct the complex activities expressed per g of tissue for mitochondrial recovery. GAPDH activity was measured in the crude homogenate at 30°C as previously described.¹³

Statistical analysis

Differences between study groups were analysed by Fisher's exact test for comparison of proportions, Student's *t* test for comparison of normally distributed data, and the Mann-Whitney *U* test or Wilcoxon's signed rank test for data that were not normally distributed. Correlations between variables were studied by regression analysis. Statistical analyses were done with StatView 5.0.1 for Macintosh.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

As a consequence of the better survival with intensive insulin therapy, the patients in this treatment group who died had been more severely ill on admission to the intensive-care unit than those in the conventional group. The greater severity is shown by a higher score on the acute physiology and chronic health evaluation (APACHE II)¹⁴ during the first 24 h after admission to the intensive-care unit (table 1). They also died sooner. The APACHE II score in the patients assigned intensive insulin treatment remained stable during the first 7 days (median 17 [IQR 12–18] to 16 [12–20], $p=0.7$), whereas it deteriorated in those assigned conventional treatment (11 [9–13] to 17 [13–21], $p=0.04$). At the time of treatment withdrawal, however, APACHE II scores were similar in both groups. The scores on the simplified therapeutic intervention scoring system (TISS-28)¹⁵ were similar for the two groups on admission and at the time of treatment withdrawal (table 1). According to the study protocol, the mean morning blood glucose concentration during intensive care was higher in the conventional group than in the intensive insulin group (table 1), a difference achieved with higher median daily

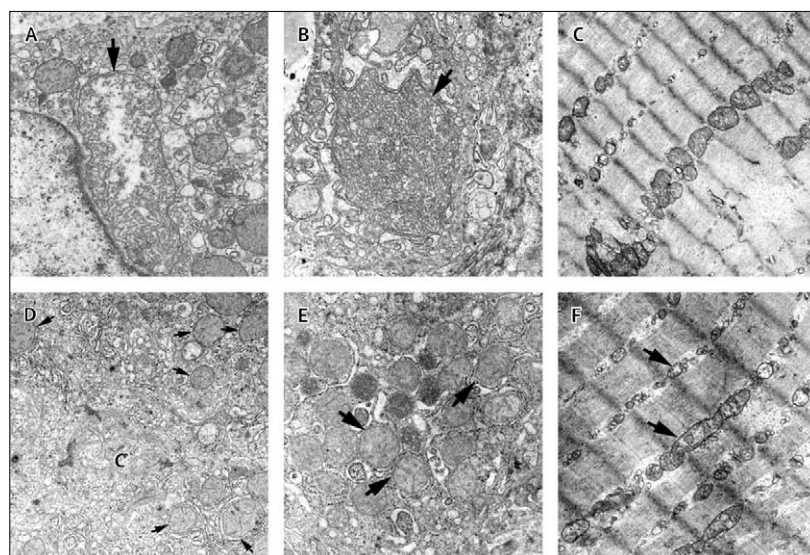
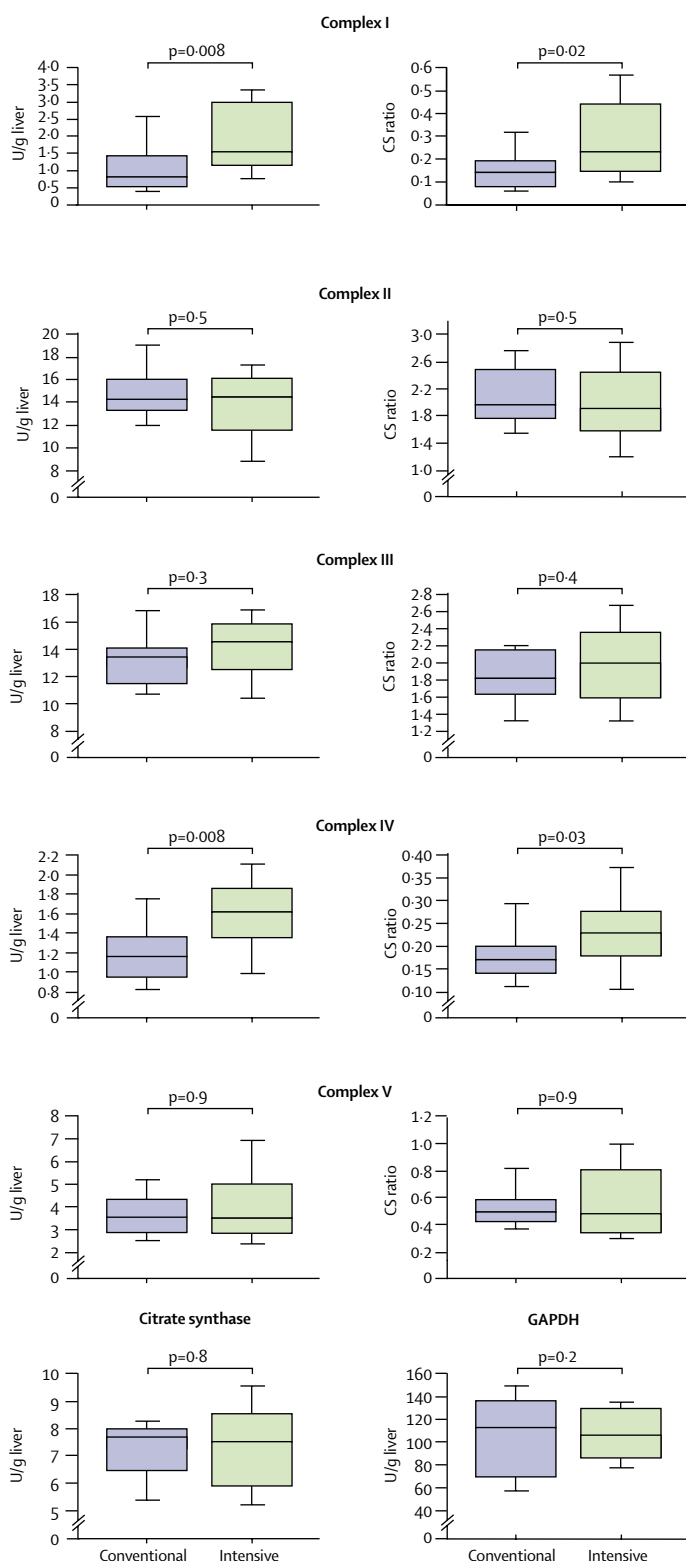


Figure 1: Mitochondrial ultrastructure in liver and skeletal muscle of critically ill patients

Electron micrographs show greatly enlarged mitochondria with an increased number of disarrayed cristae and reduced electron density of the matrix in hepatocytes adjacent to normal mitochondria (A, B) contrasting with normal mitochondrial morphology in skeletal muscle (C) of conventionally treated patients. In most of the intensively treated patients hepatocyte mitochondrial ultrastructure was normal (D [c=canaliculus], E), as in all muscle biopsy samples from these patients (F). Original magnification $\times 23\,000$.

doses of insulin in the intensive group. Glucose control was maintained until the last study day (table 1).

The overall ultrastructure of the liver samples was well maintained with only minor autolytic artefacts, which are unavoidable with post-mortem material. Thus, the biopsy samples were of acceptable quality. We did not observe overall architectural differences between the treatment groups. The mitochondrial compartment, however, showed striking differences. In seven of the nine (78%) liver samples from conventionally treated patients, there were mitochondria in which cristae had lost contact with the outer mitochondrial membrane and were oriented in varying oblong and oblique directions in the matrix. The latter had a patchy, electron-lucent appearance. These ultrastructural abnormalities were identified in 20–30% of the hepatocytes, each with 10–35% morphologically abnormal mitochondria. The size of the aberrant mitochondria varied from one to ten times the normal size. Megamitochondria (more than three times the normal size), with an increased number of cristae arranged in differing directions (figure 1 A, B), were observed in 5–10% of the hepatocytes. Occasional enlarged dense granules and small lipid droplets were present in the matrix. By contrast, similar abnormalities were found in only one of the 11 (9%) biopsy samples from patients who were assigned intensive insulin therapy ($p=0.005$); all other samples were negative (figure 1 D, E) after extensive investigation. In both treatment groups, discrete characteristics of post-mortem changes were seen, including some hydropic swelling and some flocculent densities in the matrix.



The skeletal-muscle samples obtained from patients in both groups had normal ultrastructure. The striated-muscle cells contained many sarcomers with normal Z bands and fibrils. The mitochondria were disposed in longitudinal rows between the myofibrils and were slightly more numerous near the peripherally located nuclei. They were elongated or round and had numerous transverse cristae with a normal pattern in both treatment groups (figure 1 C, F).

The median activity of respiratory-chain complex I in liver was 89% higher for patients assigned intensive insulin therapy than for those assigned conventional therapy (median 1.53 [IQR 1.14–3.01] vs 0.81 [0.54–1.43] U/g liver; $p=0.008$); activity of complex IV was 40% higher (1.69 [1.40–1.97] vs 1.16 [0.97–1.40] U/g; $p=0.008$; figure 2). No significant differences were found for complexes II, III, and V or for the mitochondrial marker citrate synthase. The results were similar after normalisation of the results for citrate synthase (figure 2) or complex II (data not shown), which corrects for the number of mitochondria, or for total protein (data not shown). There was no correlation between the length of stay in the intensive-care unit and the activities of complex I and complex IV, in either treatment group (data not shown). The activity of GAPDH was similar in the two groups (figure 2). Total protein concentrations were also similar for the conventional and intensive insulin therapy groups (median 126 [IQR 119–132] vs 128 [120–134] mg/g liver; $p=0.7$). In univariate analysis, samples from patients with a proven septic focus on post-mortem examination had 26% lower median activity of complex IV ($p=0.008$) and 26% lower median activity of complex I ($p=0.07$), expressed as citrate synthase ratio.

In contrast to the findings in liver, none of the enzyme activities in skeletal muscle were significantly affected by intensive insulin therapy (table 2). Skeletal-muscle total protein concentrations, however, were higher in patients who were assigned intensive insulin therapy than in those assigned conventional treatment (median 118 [IQR 111–128] vs 110 [99–116] mg/g skeletal muscle; $p=0.06$). When septic and non-septic patients were compared for complex I activity in muscle, the median activity was slightly lower for the septic patients when expressed as citrate synthase ratio (16% lower; $p=0.1$) or as complex II ratio (9% lower; $p=0.05$), whereas the activity of complex IV was the same whether or not a septic focus was present (data not shown).

Figure 2: Activities of mitochondrial enzymes and GAPDH in liver of critically ill patients who received conventional (n=18) or intensive (n=17) insulin therapy

Activities are presented as box plots; central line indicates median, the box the IQR, and the whiskers the 10th and 90th percentiles. The activities of respiratory-chain complexes are expressed in U/g liver, corrected for mitochondrial recovery with the recovery of citrate synthase, and as the corresponding CS ratios—ie, the ratios of the respiratory-chain enzyme to citrate synthase activities measured in the postnuclear fraction. No liver biopsy was available for analysis from one of the selected patients in the intensive treatment group.

Discussion

Strict maintenance of normoglycaemia with insulin infusion during intensive care beneficially affected the hepatocyte mitochondrial compartment of critically ill patients in a surgical intensive-care unit. The prevention or reversal of mitochondrial ultrastructural abnormalities in hepatocytes with intensive insulin therapy was associated with functional correlates thereof, such as higher activity of respiratory-chain complexes I and IV. By contrast, electron microscopy showed no major abnormalities in the mitochondria of skeletal muscle, and morphology and respiratory-chain activity were not detectably affected by insulin therapy.

Altered activity of one or more of the respiratory-chain complexes has been reported in critical illness, particularly in sepsis.⁶ Depending on the species, the model, and the severity and duration of the insult, data have been conflicting.⁶ In models of longer duration and greater severity of insult, however, mitochondrial function is uniformly depressed. Mitochondrial dysfunction and the associated bioenergetic failure have been regarded as factors contributing to multiple organ dysfunction, the most common cause of death in sepsis and long-lasting critical illness. Brealey and colleagues⁸ showed mitochondrial respiratory-chain dysfunction (30% reduction in complex I activity) and decreased ATP concentrations in skeletal-muscle biopsy samples from patients with sepsis in intensive care as compared with patients after elective hip replacement. More severe abnormalities were associated with a higher risk of organ failure and adverse outcome of septic shock. Furthermore, recovery from organ failure was associated with improvement of mitochondrial function in septic patients.¹⁶ In a resuscitated long-term rat model of sepsis, bioenergetic abnormalities were also observed in the liver and related to lethal outcome.¹⁷

These studies suggested a novel therapeutic target—besides optimisation of cardiac output, systemic oxygen delivery, and regional blood flow—to prevent lethal organ failure in intensive-care units. Indeed, maintenance or restoration of mitochondrial function and cellular energetics (ie, prevention or reversal of cytopathic hypoxia) might improve outcome for critically ill patients. Our findings in the liver suggest that intensive insulin therapy could be such an adjuvant therapy. A major drawback of our study, however, is the limitation of the analyses to non-survivors. For obvious ethical reasons, liver samples were not taken from survivors. However, the non-survivors in the intensive insulin group we studied were more, not less, severely ill on admission to the intensive-care unit than non-survivors in the conventional group, as shown by the higher APACHE II score. Furthermore, neither the reason for admission nor the cause of death accounted for the differences in mitochondrial integrity, since these features were similar for both treatment groups. Hence, the improved mitochondrial function and

	Median (IQR) activity		p
	Conventional insulin therapy (n=17)	Intensive insulin therapy (n=18)	
Activity, U/g skeletal muscle*			
Complex I	1.47 (1.14–1.76)	1.41 (1.20–1.88)	0.8
Complex II	2.68 (1.83–2.87)	2.09 (2.00–2.73)	0.7
Complex III	6.29 (4.38–7.21)	5.85 (4.97–6.84)	0.9
Complex IV	1.34 (1.11–2.02)	1.49 (1.34–1.78)	0.4
Complex V	12.8 (10.1–13.8)	12.3 (10.6–14.3)	0.5
Citrate synthase	8.60 (7.12–9.89)	7.87 (6.77–8.85)	0.7
GAPDH	238 (138–329)	232 (185–330)	0.9
Activity, CS ratio†			
Complex I	0.17 (0.15–0.20)	0.19 (0.15–0.24)	0.3
Complex II	0.29 (0.26–0.35)	0.27 (0.26–0.37)	0.8
Complex III	0.74 (0.65–0.88)	0.78 (0.68–0.85)	0.7
Complex IV	0.17 (0.14–0.22)	0.20 (0.18–0.23)	0.2
Complex V	1.40 (1.26–1.94)	1.47 (1.28–1.71)	0.8

*After correction for mitochondrial recovery by use of the recovery of citrate synthase.

†Ratio of the respiratory-chain enzyme activities to citrate synthase activity measured in the postnuclear fraction. No skeletal-muscle biopsy was available for analysis from one of the patients selected in the conventional treatment group.

Table 2: Activities of mitochondrial enzymes and GAPDH in skeletal muscle of critically ill patients

*After correction for mitochondrial recovery by use of the recovery of citrate synthase.

†Ratio of the respiratory-chain enzyme activities to citrate synthase activity measured in the postnuclear fraction. No skeletal-muscle biopsy was available for analysis from one of the patients selected in the conventional treatment group.

Table 2: Activities of mitochondrial enzymes and GAPDH in skeletal muscle of critically ill patients

ultrastructure are highly unlikely to be explained by factors other than the intensive insulin therapy. Nevertheless, because of the inherent weakness of studying tissue obtained after death, we cannot provide evidence for a causal link between the observed effect on mitochondrial integrity and the better outcome with intensive insulin therapy. Similarly, we cannot say whether respiratory-chain enzyme activities were restored to normal by intensive insulin therapy, because no samples from patients who were not critically ill were available. Comparison with previous publications is also difficult, owing to variability in the reported results and the limitations of post-mortem biopsies.

Studies in models of diabetes mellitus also point to hyperglycaemia, hypoinsulinaemia, or both as a trigger for mitochondrial dysfunction. Diabetes mellitus induced in rats by streptozotocin or alloxan treatment or in cats by pancreatectomy has been reported to impair mitochondrial respiration and disturb energy production in liver, skeletal muscle, heart, and diaphragm.^{18–22} Mitochondrial protein synthesis was greatly decreased in skeletal muscle of streptozotocin-treated rats.²¹ When insulin was administered, mitochondrial protein synthesis and function were restored to normal.^{18–22}

Besides the functional changes, we also observed ultrastructural mitochondrial changes in hepatocytes. The morphological abnormalities were largely prevented or restored by intensive insulin therapy during intensive care. In animal models of diabetes, insulin also improves or completely reverses mitochondrial ultrastructural changes.^{19,22} In diabetic rat heart, swelling of mitochondria and a reduced density of the matrix were observed, whereas mitochondria were only slightly swollen in insulin-treated animals.²² Much variation in

size and density of mitochondria has been described in liver of diabetic rats; many of the mitochondria were visible as large, pale spheres, and a normal appearance was regained after only 3 days of insulin therapy.¹⁹

Whether the morphological changes are cause or consequence of the mitochondrial functional impairment is not clear. The inner mitochondrial membrane could proliferate to compensate for reduced efficiency of the mitochondrial respiratory unit, which could result in overabundant, dissolving cristae, enlargement of the organelle, and mitochondrial membrane disintegration.²³ By contrast, Wakabayashi²⁴ showed that free radicals can contribute to the formation of "megamitochondria" or "mitochondrial giants". When intracellular concentrations of **reactive oxygen species** are sufficiently suppressed, normal structure and function of megamitochondria returns. Several factors can lead to the induction of mitochondrial swelling, including opening of the mitochondrial permeability transition pore (a non-specific pore in the inner mitochondrial membrane) or direct mitochondrial membrane damage.^{25,26}

In contrast to our observations in the liver, the mitochondrial compartment in skeletal muscle appeared morphologically intact, and ultrastructure and respiratory-chain activity were not detectably affected by intensive insulin therapy. This difference can be explained by the different mechanisms of glucose uptake in liver and skeletal muscle. Whereas the non-insulin-dependent **GLUT-2**-mediated glucose uptake in liver is directly proportional to blood glucose concentrations, insulin action is required to increase **GLUT-4**-mediated glucose uptake in skeletal muscle.²⁷ Hence, in the hyperglycaemic, insulin-resistant patients of the conventional insulin group, glucose could have readily entered hepatocytes but not skeletal myocytes. Intracellular glucose overload might thus have induced direct toxic effects in the liver and not the skeletal muscle. Intensive insulin therapy lowered circulating glucose concentrations, most likely by increasing GLUT4-mediated uptake in the muscle,²⁸ which might have prevented the glucose toxicity in the liver. The higher total protein concentrations in skeletal muscle of patients assigned intensive insulin treatment suggests an anabolic response to insulin. Together the data support the hypothesis that the prevention of hyperglycaemia, rather than a direct effect of insulin on tissues, explains the beneficial effect on mitochondrial ultrastructure and function in liver. Furthermore, they suggest that other mechanisms should be considered to explain the mitochondrial damage observed in skeletal muscle of patients with severe sepsis.⁸ Many factors have been shown to affect mitochondrial function adversely, including cytokines, nitric oxide, and increased generation of reactive oxygen species, all of which are involved in sepsis.⁶

Several factors promote the development of increased

oxidative stress in critical illness, which is reflected by reduced antioxidant defence systems and increased oxidative stress-induced damage.^{29,30} Hyperglycaemia could be an important factor to consider.^{10,31,32} High intracellular glucose concentrations might increase transition-metal-catalysed glucose oxidation, mitochondrial superoxide production, and NADPH oxidase activity, among other mechanisms, leading to production of reactive oxygen species.^{10,33,34} Overproduction of superoxide by the mitochondrial respiratory chain might inhibit the glycolytic enzyme GAPDH, in turn possibly explaining increased polyol pathway flux, increased formation of advanced glycation end products, activation of protein kinase C, and increased hexosamine pathway flux; these four biochemical pathways are implicated in the pathogenesis of diabetic hyperglycaemic damage.¹⁰ We measured GAPDH activity in liver and skeletal muscle of critically ill patients, but we found no significant difference in the activity of this enzyme between the intensive insulin and conventional insulin groups. Whether intensive insulin therapy reduces oxidative stress in critically ill patients remains to be investigated.

Further analyses are needed to link the beneficial effect of intensive insulin therapy on mitochondrial integrity to an effect on oxidative stress state, to prevention of organ failure, and to overall survival in critical illness. A recently developed animal model of long-lasting critical illness will be suitable to test this hypothesis.³⁵

Contributors

All the authors contributed to the interpretation of the results and the writing of the report. G Van den Berghe and P J Wouters did the biopsies and collected clinical data. I Vanhorebeek did the biochemical analyses, and R De Vos and C De Wolf-Peeters were responsible for the ultrastructural investigation.

Conflict of interest statement

G Van den Berghe holds an unrestricted Catholic University of Leuven Novo Nordisk Chair of Research.

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Reactive oxygen species

Reactive intermediates derived from oxygen in aerobic metabolism, either radicals (eg, superoxide anion radical, hydroxyl radical) or non-radical compounds (eg, hydrogen peroxide) able to damage biological macromolecules.

GLUT-2

Facilitative glucose transporter present in hepatocytes, renal tubular cells, pancreatic β cells, and gastrointestinal mucosa; it has a high K_m and V_{max} and allows glucose to enter cells directly in proportion to the extracellular glucose concentration, independently of insulin.

GLUT-4

Insulin-dependent facilitative glucose transporter present in tissues such as skeletal muscle, myocardium, and adipose tissue.

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