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Contribution of Circulating Lipids to the Improved Outcome of Critical Illness by Glycemic Control with Intensive Insulin Therapy

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Compared with the conventional approach, which recommended only insulin therapy when blood glucose levels exceeded 12 mmol/liter, strict maintenance of blood glucose levels less than 6.1 mmol/liter with intensive insulin therapy has shown to reduce intensive care mortality, acute renal failure, critical illness polyneuropathy, and bloodstream infections in critically ill patients by about 40%. This study of 363 patients, requiring intensive care for more than 7 d and randomly assigned to either conventional or intensive insulin therapy, examines the effects of intensive insulin therapy on glucose and lipid homeostasis and their respective impact on the improved outcome. Intensive insulin therapy effectively normalized blood glucose levels within 24 h, both in survivors and nonsurvivors. Intensive insulin therapy also increased serum levels of low-density lipoprotein ($P = 0.007$) and high-density lipoprotein ($P = 0.005$), whereas it suppressed the elevated serum triglyceride concentrations ($P < 0.0001$). Multivariate logistic regression analysis, corrected for baseline univariate risk factors and the effect on inflammation, indicated that lipid rather than glucose control independently determined the beneficial effects of intensive insulin therapy on morbidity and mortality. In postmortem biopsies obtained from 74 patients who died in the intensive care unit, intensive insulin therapy increased mRNA levels of skeletal muscle glucose transporter 4 ($P = 0.02$) and hexokinase ($P = 0.03$), unlike those of hepatic glucokinase. In conclusion, our data suggest that intensive insulin therapy normalizes blood glucose levels through stimulation of peripheral glucose uptake and concomitantly partially restores the abnormalities in the serum lipid profile, which may have contributed significantly to the improved outcome of protracted critical illness. (*J Clin Endocrinol Metab* 89: 219–226, 2004)

CRITICAL ILLNESS HAS been associated with insulin resistance and hyperglycemia since the description of stress diabetes by Claude Bernard at the end of the 19th century. Initially, this response was regarded as adaptive, and, as such, beneficial for survival. However, a large prospective, randomized, controlled trial in 1548 critically ill patients showed a 40% decrease in mortality and morbidity through strict glycemic control less than 6.1 mmol/liter (110 mg/dl) with intensive insulin therapy compared with the conventional approach, which recommended insulin therapy only when blood glucose levels exceeded 12 mmol/liter (220 mg/dl) (1). Contrary to expectation, the tight control of blood glucose levels by intensive insulin therapy did not seem to be mediated through a suppression of hepatic gluconeogenesis because mRNA levels of phosphoenolpyruvate carboxykinase, the rate-limiting enzyme of the hepatic gluconeogenesis, were unaffected by the status of insulinization (2). The apparent hepatic insulin resistance was corroborated by the lack of suppression of serum IGF-binding protein-1 by intensive insulin therapy. Whether intensive insulin therapy normalizes blood glucose levels in the critically ill through stimulation of peripheral glucose uptake was unknown until now. On molecular level, insulin-stimulated glucose uptake in skeletal muscle is principally mediated through glucose transporter 4 (GLUT-4) and hexokinase II (HXK-II) (3). In the liver, the rate-limiting enzyme for insulin-mediated glucose uptake and glycogen synthesis is glucokinase (GK) (4).

As in diabetes mellitus, deranged metabolism during critical illness is reflected by not only hyperglycemia but also an abnormal serum lipid profile (5–7). Elevated triglyceride levels, because of an increase in very low-density lipoprotein (VLDL), and low circulating high-density lipoprotein (HDL) cholesterol are the most characteristic during critical illness (8). Low-density lipoprotein (LDL) cholesterol levels are also decreased (8). The latter is offset by an increase in circulating small dense LDL particles (9), which are supposedly more proatherogenic than the medium and large LDL particles (10). Although hepatic triglyceride production is always increased, VLDL clearance through lipoprotein lipase (LPL)-mediated lipolysis is inhibited only by high levels of endotoxin (11, 12). Within hours of critical illness, cholesterol content in LDL and HDL decreases through increased sequestration in the subendothelial space and accelerated catabolism, respectively (7). Increased cholesterol clearance is even more important in light of the elevated cholesterol production (13). Increased morbidity and mortality accompanying dysregulated lipid homeostasis in diabetes has been

Abbreviations: APACHE II, Acute physiology and chronic health evaluation II; CI, confidence interval; CRP, C-reactive protein; CV, coefficient of variation; GK, glucokinase; GLUT-4, glucose transporter 4; HDL, high-density lipoprotein; HXK-II, hexokinase II; LDL, low-density lipoprotein; LPL, lipoprotein lipase; OR, odds ratio; SREBP-1c, sterol regulatory element binding protein 1c; VLDL, very LDL.

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established for quite some time (14–17), and thus diet and drug interventions targeted at improving this deranged lipid profile have long been implemented. In contrast, in critically ill patients, so far only one noninterventional retrospective study described a U-shaped relationship between cholesterolemia and mortality (18). Hence, the prognostic value remains unclear (19, 20), and no effective strategies to improve the dyslipidemia of the critically ill patient are available. The effect of intensive insulin therapy on the lipid abnormalities of intensive care patients has not been investigated.

The goal of this study was 3-fold: 1) to examine whether intensive insulin therapy exerted its blood glucose-lowering effect through stimulation of peripheral glucose uptake; 2) to assess whether intensive insulin therapy ameliorates the dyslipidemia of the critically ill; and 3) to examine which of these metabolic effects, improvement of glucose or lipid homeostasis, is most important in explaining the amelioration of morbidity and mortality of critical illness.

Subjects and Methods

Subjects

Subjects of this study were part of a large randomized, controlled trial on intensive insulin therapy in intensive care patients (n = 1548), of which the treatment protocol and major clinical outcomes were published in detail previously (1, 21). In that trial, all mechanically ventilated, adult patients admitted to a mainly surgical intensive care unit were eligible for inclusion, after informed consent from the closest family member. The Institutional Review Board of the Catholic University of Leuven approved the protocol.

For the current analysis of the effect of intensive insulin therapy on serum lipid concentrations, all patients with an intensive care stay of more than 7 d (n = 363) were selected. The baseline characteristics of the two treatment groups are described in Table 1. Postmortem biopsies from 74 patients who died during the study were available and were analyzed after quality assessment of the RNA, as reported previously (2).

Clinical outcome measures

The primary outcome measure was death from any cause during the stay in the intensive care unit. Acute renal failure, critical illness polyneuropathy, prolonged mechanical ventilation, and the incidence of bacteremia were used as outcome measures for morbidity. Acute renal failure was present if the patient required renal replacement therapy either by continuous venovenous hemofiltration or dialysis.

The presence of critical illness polyneuropathy was assessed electromyographically on a weekly basis by one electrophysiologist who was unaware of the treatment assignments. Bacteremia was defined as the presence of a positive blood culture at any time during intensive care stay.

Serum analyses

Serum samples were taken on admission and daily at 0600 h. Serum C-reactive protein (CRP) as well as serum triglyceride, total cholesterol, and HDL-cholesterol concentrations were determined by routine clinical assays using commercial kits on an automated analyzer (Roche/Hitachi Modular, Mannheim, Germany). Normal levels are: CRP, <5 mg/liter; triglycerides, <200 mg/dl; total cholesterol, 110–220 mg/dl; HDL, 35–60 mg/dl. Serum LDL-cholesterol concentrations were measured using the Roche LDL-C plus second-generation kit because the Friedewald calculation of LDL is reliable only in serum from fasted subjects. Normal LDL levels range from 80 to 150 mg/dl. For this study, analyses were performed on serum samples obtained on admission, d 1, and d 8 of intensive care stay.

RNA isolation from liver biopsies and real-time PCR

For the assessment of the major site of glucose uptake, gene expression levels of GLUT-4 and HXK-II in skeletal muscle as well as hepatic GK, the rate-limiting enzyme for hepatic glucose uptake and glycogen synthesis, were measured.

Hepatic sterol regulatory element-binding protein 1c (SREBP-1c) and skeletal muscle LPL were used to define the major site of action of intensive insulin therapy on lipid metabolism.

Although SREBP-1c is central in the regulation of the lipogenic enzymes (22), LPL exerts the rate-limiting step in peripheral triglyceride uptake (23).

Liver biopsies were taken from the left lower quadrant, and skeletal muscle samples were obtained from the right musculus rectus abdominis. Time between death and freezing of the samples was 30.5 ± 20.1 min. RNA isolation and the generation of cDNA and external standards were performed as described previously (2). To avoid amplification of genomic DNA, primer pairs were designed to span an intron (Table 2). SREBP-1c detection was performed by using primers with published sequences (24). A 1:100 dilution of the resultant cDNA was prepared, and 5 μl of this template were used for sample cDNA quantification with the ABI PRISM 7700 sequence detector (PE Applied Biosystems, Foster City, CA). The reaction mixtures for GLUT-4, HXK-II, GK, and LPL contained 1 × Platinum Quantitative PCR-Supermix-UDG (Invitrogen, Carlsbad, CA), 200 nM forward primer, 200 nM reverse primer, 200 nM TaqMan probe, 2 mM $MgCl_2$, made up to a total volume of 25 μl with sterile water. In the SREBP-1c reaction 0.4 × SYBR green I (Sigma, St. Louis, MO) instead of 200 nM TaqMan probe was used. The real-time PCR protocol was 10 min at 95 C, 40 cycles of 15 sec at 95 C and 1 min at 60 C. To assess PCR specificity, reverse transcriptase minus samples were included. Gene expression was corrected for well-to-well loading variation by expressing data as a ratio of 18S ribosomal RNA, measured using the TaqMan rRNA kit (PE Applied Biosystems). All samples were analyzed in duplicate and percentage coefficient of variation (CV) was calculated. Individual samples with a copy number CV greater than 20% were reanalyzed. The within-assay CV on the copy number quantification, determined by ANOVA for the means of duplicates, was 9.2% for GLUT-4, 10.6% for HXK-II, 13.5% for GK, 12.0% for SREBP-1c, and 21.8% for LPL. All patient samples were analyzed in two runs.

TABLE 1. Clinical characteristics of all patients with an intensive care stay of more than 1 wk

Insulin treatment	Conventional	Intensive
No.	206	157
Male gender, n (%)	136 (66%)	110 (70%)
Age, yr (mean ± SEM)	61 ± 16	61 ± 15
BMI (kg/m^2)	26 ± 5	26 ± 5
On-admission APACHE-II [median (IQR)]	12 (9–15)	12 (7–16)
Pre-admission diabetes, n (%)	18 (9%)	17 (11%)
On-admission hyperglycemia >11 mM, n (%)	29 (14%)	20 (13%)
On-admission blood glucose level (mM)	8.2 ± 3.2	8.1 ± 3.0
On-admission total cholesterol (mg/dl)	86.6 ± 2.8	90.5 ± 3.4
On-admission HDL cholesterol (mg/dl)	20.9 ± 0.9	20.9 ± 0.8
On-admission LDL cholesterol (mg/dl)	42.0 ± 2.0	44.5 ± 2.3
On-admission triglycerides (mg/dl)	118.5 ± 6.0	126.7 ± 10.7

IQR, Interquartile range.

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TABLE 2. Primer sets used to quantitate *GLUT-4*, *HXK-II*, *GK*, and *LPL* gene expression by real-time PCR

GLUT-4	F	5′-ATGGCTGTGGCTGGTTTCTC-3′
	R	5′-ACCGCAAATAGAAGGAAGACGTA-3′
	P	5′-CCTCCGCAACATACTGGAAACCCATG-3′
HXK-II	F	5′-CGACACAGTCGGAACTATGATGA-3′
	R	5′-ATCTCCTCCATGTAGCAGGCATT-3′
	P	5′-TGCCAACAATGAGGCCAACTTCACAG-3′
GK	F	5′-CATCACTGTGGGCGTGGAT-3′
	R	5′-TCGATGAAGGTGATCTCGCA-3′
	P	5′-CCCAGCTTCAAGGAGCGGTTCCAT-3′
LPL	F	5′-TTGTGAAATGCCATGACAAGTCT-3′
	R	5′-AATTCACATGCCGTTCTTTGTTC-3′
	P	5′-AGATTCGCCCAGTTTCAGCCTGACTTCTTAT-3′

The primer sets for *GLUT-4* (NM001042), *HXK-II* (Z46376), *GK* (M69051), and *LPL* (NM000237) were designed to be intron spanning to avoid measuring genomic DNA contamination. F, Forward primer; R, reverse primer; P, Taqman probe.

A separate run was performed for repeat samples. Because only a limited number of runs were performed, interassay precision was not assessed formally.

Statistical analysis

Statistical analyses were performed using StatView 5.0.1 for Macintosh (SAS Institute, Cary, NC). Data are represented as mean ± SEM, and statistical significance was assumed for $P < 0.05$. Although differences between the treatment groups were analyzed by Mann-Whitney *U* test, changes in serum lipid concentrations over time were evaluated by factorial and repeated-measures ANOVA with Fisher's least significant difference test. Bonferroni correction for multiple testing was used where necessary. Multivariate logistic regression analysis was performed to assess which metabolic effect of intensive insulin therapy explains the improvement of critical illness mortality and morbidity.

Results

Lowering of blood glucose levels with intensive insulin therapy during critical illness coincided with an increase of peripheral glucose uptake

Intensive insulin therapy effectively normalized blood glucose levels within 24 h, both in survivors and nonsurvivors (Fig. 1A). Also on the last day of intensive care, and according to the study protocol, blood glucose levels in the nonsurvivors receiving intensive therapy (5.9 ± 0.3 mmol/liter) were significantly lower than in the conventionally treated patients (9.0 ± 0.5 mmol/liter) ($P < 0.0001$) (Fig. 2A). This blood glucose-lowering effect of intensive insulin therapy coincided with an increase of skeletal muscle GLUT-4 and HXK-II gene expression by 70 and 36%, respectively (Fig. 2, B and C). In contrast, gene expression levels of hepatic GK were not altered by insulin treatment (Fig. 2D).

Intensive insulin therapy modified the serum lipid profile of prolonged critically ill patients

On admission to the intensive care unit, serum total cholesterol levels were equal in both study groups (86.6 ± 2.8 mg/dl in the conventional treatment group and 90.5 ± 3.4 mg/dl in the intensive treatment group) (Fig. 3A). Compared with admission total cholesterolemia (88.3 ± 2.2 mg/dl), serum total cholesterol decreased on d 1 (73.6 ± 1.8 mg/dl, $P < 0.0001$), whereas levels were significantly higher on d 8 (108.9 ± 1.8 mg/dl, $P < 0.0001$). Insulin treatment did not affect total cholesterolemia.

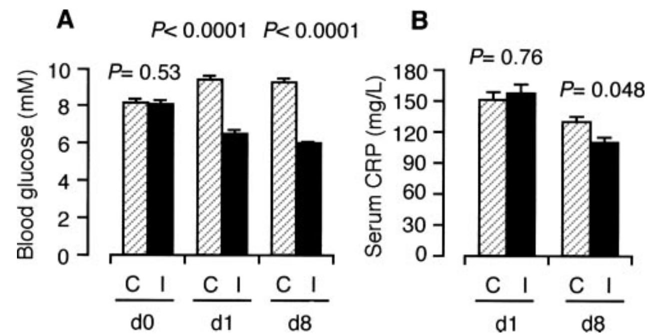

FIG. 1. Intensive insulin therapy lowers blood glucose levels and serum CRP levels in all prolonged critically ill patients. A, Blood glucose levels (mean ± SEM). B, Serum CRP levels (mean ± SEM). C, Conventional insulin therapy (n = 188); I, intensive insulin therapy (n = 150). Bonferroni correction for multiple testing.

On intensive care admission, serum triglyceride levels were equal in both treatment groups (119.1 ± 6.2 mg/dl *vs.* 126.7 ± 10.7 mg/dl, $P = 0.93$, Fig. 3B). On d 1 and d 8, serum triglyceride levels were lower in intensively insulin-treated as compared with the conventionally treated patients (Fig. 3B). Relative to the admission levels, only patients in the conventional therapy group showed a strong increase in serum triglycerides on d 8 (179.7 ± 6.1 mg/dl; $P < 0.0001$). In contrast, intensive insulin therapy resulted in a decrease of triglyceridemia on d 1 (102.2 ± 8.0 mg/dl; $P = 0.02$).

Although HDL-cholesterol levels decreased over time in both treatment groups ($P < 0.0001$; Fig. 3C), the decrease was less severe in the intensively insulin treated patients, resulting in significantly higher ($P = 0.005$) HDL levels on d 8 (17.4 ± 0.6 mg/dl) compared with the patients in the conventional treatment schedule (15.1 ± 0.6 mg/dl) (Fig. 3C).

LDL-cholesterol levels strongly reflected the total cholesterol levels (R = 0.73, $P < 0.0001$) with a decrease on d 1 and an elevation on d 8, in comparison with admission levels (Fig. 3D). However, on d 8, serum LDL-cholesterol levels were higher ($P = 0.007$) in intensively insulin treated patients (43.4 ± 2.3 mg/dl) as compared with the patients in the conventional treatment group (36.5 ± 2.2 mg/dl).

The above-mentioned changes in serum lipids occurred in light of a progressively increasing amount of parenteral/enteral feeding from admission to full steady-state nutritional support on d 8. The nonprotein caloric intake of 8.4 ± 0.4 kcal/kg·d on d 1 was increased to 23.8 ± 0.5 kcal/kg·d on d 8 ($P < 0.0001$). Glucose intake was gradually increased from 447 ± 14 glucose kcal/d on admission and 786 ± 18 glucose kcal/d on d 1 to 954 ± 21 glucose kcal/d on d 8.

On intensive care admission, lipid intake was negligible. It was more steeply built up from 159 ± 16 lipid kcal/d on d 1 to 760 ± 21 lipid kcal/d on d 8. Intravenous lipids were given as Intralipid 20% (Pharmacia-Upjohn, Stockholm, Sweden); 500 ml contain 100 g purified soybean oil, 12 g purified egg phospholipids, and 22 g anhydrous glycerol (energy content: 1100 kcal/500 ml). At any time, total, glucose, and lipid caloric intakes were equal between the two treatment groups. No differences in serum lipids could be detected between patients who were exclusively parenterally fed and those receiving enteral feeding (data not shown).

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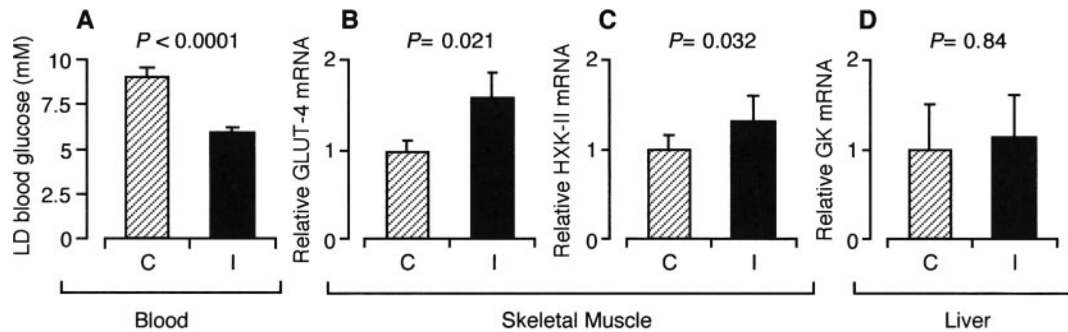

FIG. 2. GLUT-4 and HXK-II gene expression in skeletal muscle but not hepatic GK gene expression were increased by intensive insulin therapy in the nonsurvivors. A, Last day (LD) blood glucose levels (mean ± SEM) in the nonsurvivors, conventional (C) (n = 51), or intensive (I) insulin therapy (n = 31). B, Relative GLUT-4 gene expression for the two insulin treatment regimens, conventional (C) insulin therapy (n = 38) or intensive (I) insulin therapy (n = 24). C, Relative HXK-II gene expression in conventional (C) (n = 41) or intensive (I) (n = 24) insulin therapy. D, Relative GK gene expression in conventional (C) (n = 30) or intensive (I) (n = 17) insulin therapy.

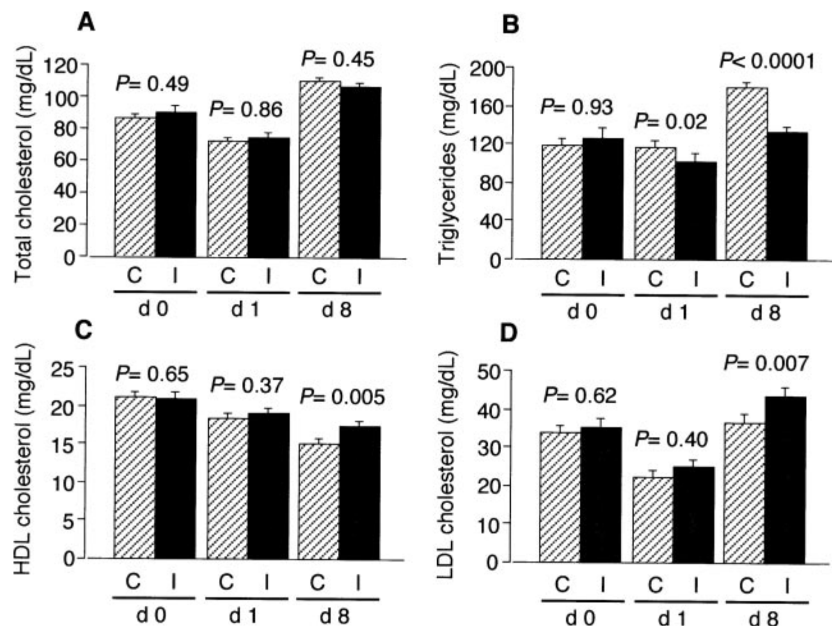

FIG. 3. Intensive insulin therapy modifies the serum lipid profile. A, Serum total cholesterol (mean ± SEM). B, Serum triglycerides (mean ± SEM). C, Serum HDL cholesterol (mean ± SEM). D, Serum LDL cholesterol (mean ± SEM). On d 0, 1, and 8: C, conventional insulin therapy (n = 188); I, intensive insulin therapy (n = 150). The relative increase in total cholesterol from baseline to d 8 was 52 ± 6% for C and 44 ± 7% for I. The relative increase in triglycerides from baseline to d 8 was 111 ± 12% for C and 49 ± 11% for I. Although HDL cholesterol relatively decreased 2.4 ± 6% from baseline in C, levels increased by 3.4 ± 6% in I. The relative increase in LDL cholesterol from baseline to d 8 was 143 ± 28% for C and 167 ± 29% for I. Bonferroni correction for multiple testing.

Quantification of hepatic SREBP-1c and skeletal muscle LPL gene expression

To examine whether insulin exerted its effects on circulating lipids predominantly in the liver or in skeletal muscle, gene expression levels of two important regulators of triglyceride homeostasis, hepatic SREBP-1c and skeletal muscle LPL, were determined in available tissue biopsies obtained from the nonsurvivors. Hepatic SREBP-1c (Fig. 4B) and skeletal muscle LPL (Fig. 4C) mRNA levels were not significantly altered by insulin treatment. In contrast to the suppressive effect on circulating triglycerides in the survivors, intensive insulin therapy also did not affect triglycerides on d 8 in the nonsurvivors ($P = 0.32$) (Fig. 4A).

The effect on serum lipids exerted by intensive insulin therapy explained its effect on mortality

As reported previously in the entire study population (1), also in this subset of protracted critically ill patients requiring more than 7 d of intensive care, intensive insulin therapy reduced intensive care mortality from 21.3% to 12.1% ($P = 0.02$).

Compared with the conventional approach, intensive insulin therapy decreased blood glucose ($P < 0.0001$) (Fig. 1A) and also CRP ($P = 0.048$) levels (Fig. 1B).

The relationship among serum triglycerides (Fig. 5A), LDL (Fig. 5B), and HDL (Fig. 5C) concentrations and risk of intensive care mortality was defined. Per 100 mg/dl increase in serum triglycerides, an almost linear correlation with intensive care mortality was observed. In contrast, for serum LDL and HDL concentrations, there appeared to be a cut-off level below which mortality strongly increased. This cut-off level was 20 mg/dl for LDL and 15 mg/dl for HDL. Intensive insulin therapy significantly reduced the number of patients with serum LDL levels less than 20 mg/dl from 37% in the conventional group to 23% ($P = 0.005$) and the number of patients with serum HDL levels less than 15 mg/dl from 54 to 34% ($P < 0.0001$).

To investigate which of the observed metabolic responses

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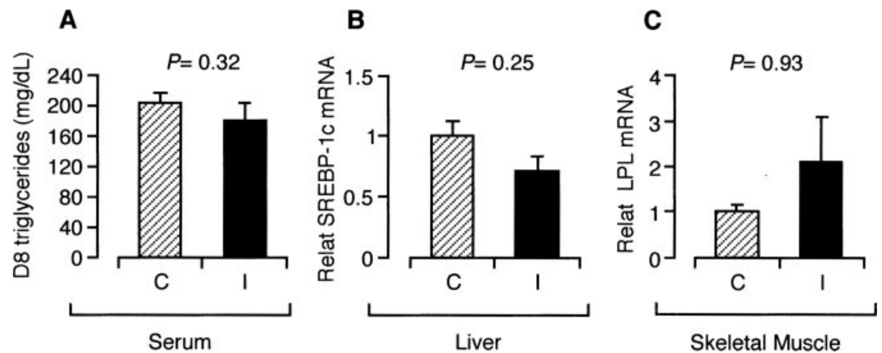

FIG. 4. Hepatic SREBP-1c and LPL in skeletal muscle were not increased by intensive insulin therapy in the nonsurvivors. A, Day 8 serum triglyceride levels (mean ± SEM) in the nonsurvivors (n = 62). B, Relative SREBP-1c gene expression for the two insulin treatment regimens, conventional (C) (n = 35) or intensive (I) insulin therapy (n = 20). C, Relative LPL gene expression in conventional (C) (n = 37) or intensive (I) (n = 24) insulin therapy.

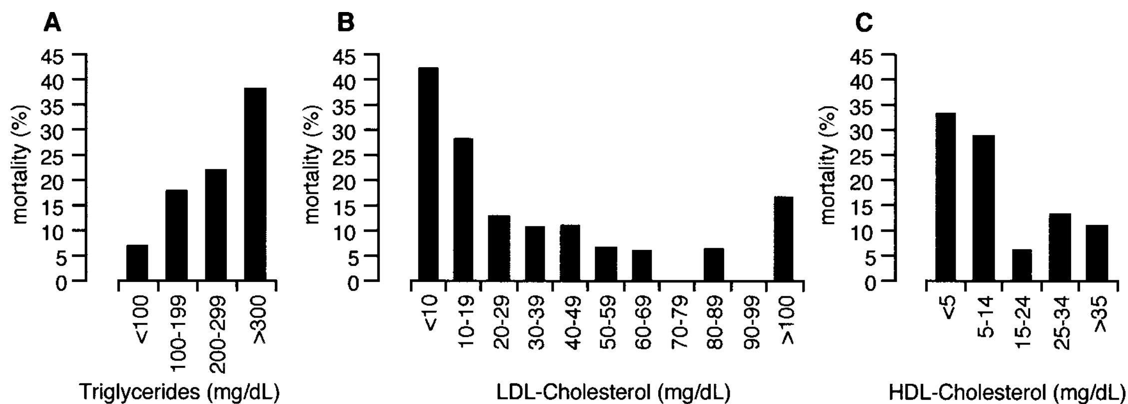

FIG. 5. Prevalence of mortality in patients stratified according to different serum lipid levels. A, Percentage mortality in stratified serum triglyceride concentrations: n = 88 (<100 mg/dl), 181 (100–199), 60 (200–299), 29 (>300). B, Percentage mortality in stratified serum LDL concentrations: n = 64 (<10 mg/dl), 46 (10–19), 39 (20–29), 47 (30–39), 45 (40–49), 31 (50–59), 34 (60–69), 17 (70–79), 16 (80–89), 7 (90–99), 12 (>100). C, Percentage mortality in stratified serum HDL concentrations: n = 6 (<5 mg/dl), 156 (5–14), 149 (15–24), 38 (25–34), 9 (>35).

(glucose or lipid control) best explained the mortality-reducing effect of intensive insulin therapy in protracted critically ill patients, they were entered into a multivariate logistic regression model (Table 3), together with all baseline univariate risk factors of adverse outcome [age; the first 24 h Acute Physiology and Chronic Health Evaluation (APACHE II) score with higher values reflecting a more severe illness (25); on admission hyperglycemia; history of diabetes; history of malignancy; noncardiac surgery], the intervention-related factors (randomized insulin therapy group and the insulin dose), and the previously reported effect on inflammation (CRP). From the baseline risk factors included in the model, only age and the APACHE II score remained as independent determinants of nonsurvival.

The effect of intensive insulin therapy on serum LDL ($P = 0.003$) and HDL ($P = 0.016$), but no longer serum triglycerides or the blood glucose-lowering effect and the insulin dose, explained its effect on mortality (Table 3).

A similar analysis was performed for the observed effects on morbidity. As reported previously in the entire study population (1), also in this subset of protracted critically ill patients, intensive insulin therapy reduced the incidence of acute renal failure from 28 to 19% ($P = 0.05$), critical illness polyneuropathy from 52 to 29% ($P < 0.0001$), and bacteremia from 29 to 18% ($P = 0.02$).

For the effect on acute renal failure, the effect of intensive insulin therapy on serum triglycerides [odds ratio (OR) of 1.40 per 100 mg/dl added, 95% confidence interval (CI) 1.01–1.70, $P = 0.033$)] and LDL (OR of 0.32 if LDL ≥ 20 mg/dl, 95% CI 0.16–0.66, $P = 0.002$) remained as independent determinants, together with APACHE II score and age as positive risk factors. In contrast, randomization to intensive insulin therapy as such remained in the multiple regression model as an independent determinant for the prevention of critical illness polyneuropathy (OR of 0.15, 95% CI 0.07–0.33, $P < 0.0001$) and bacteremia (OR of 0.19, 95% CI 0.08–0.47, $P = 0.0004$) together with insulin dose as a positive risk factor. For critical illness polyneuropathy, the elevation of LDL was also an independent contributing factor (OR of 0.49 for LDL ≥ 20 mg/dl, 95% CI 0.27–0.91).

Discussion

Critical illness constitutes a serious derangement of metabolism, hallmarked by protein breakdown, hyperglycemia, and an altered serum lipid profile (26). Intensive insulin therapy, targeting normoglycemia during critical illness, clearly improved survival and decreased morbidity (1). However, pathways through which insulin exerted its clinical benefits in critically ill patients remained incompletely understood.

The disposal of glucose under intensive insulin therapy during prolonged critical illness has been the first burning question to be answered. The failure of insulin therapy to suppress the gene expression of PEPCK, the rate-limiting enzyme of gluconeogenesis, in the liver has previously been

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TABLE 3. Multivariate logistic regression analysis for the effect of intensive insulin therapy on intensive care mortality

	OR	95% CI	*P*
Age (per added year)	1.06	1.03–1.09	0.0002
APACHE II first 24 h (per 1 added)	1.09	1.02–1.16	0.012
Admission blood glucose > 11 mM	0.83	0.32–2.11	0.69
Positive history of diabetes	0.84	0.26–2.76	0.78
Positive history of malignancy	1.48	0.67–3.27	0.33
Noncardiac surgery	0.89	0.41–1.91	0.76
Insulin dose on d 8 (per U/d added)	1.00	0.99–1.01	0.77
Blood glucose on d 8 (per 1 mM added)	0.99	0.83–1.17	0.87
CRP on d 8 (per 50 mg/liter added)	1.20	0.50–1.40	0.087
Triglycerides on d 8 (per 100 mg/dl added)	1.10	0.70–1.50	0.60
HDL cholesterol on d 8 ≥ 15 mg/dl	0.34	0.14–0.82	0.016
LDL cholesterol on d 8 ≥ 20 mg/dl	0.30	0.14–0.66	0.003
Intensive insulin therapy	0.83	0.29–2.43	0.74

The effect of intensive insulin therapy on serum LDL and HDL cholesterol, but not on serum triglycerides or blood glucose levels, could possibly explain the effect of intensive insulin therapy on intensive care mortality.

described by our group (2). The unresponsiveness of the liver to insulinization during protracted critical illness was further confirmed by the lack of GK mRNA induction in the intensively insulin treated patients. The liver is characterized by free glucose uptake because GLUT-2 enables high capacity, facilitated diffusion of glucose into the hepatocyte. Therefore, hepatic glucose uptake is regulated on its phosphorylation step by GK (27). In other models GK is stimulated by insulin at the transcriptional level (28). Together with GK, GLUT-4 and HXK-II are the other major contributors to insulin-stimulated glucose disposal. The latter two proteins are responsible for the uptake of glucose in the peripheral insulin-responsive compartment, notably skeletal muscle and adipose tissue. This study in prolonged critically ill patients showed that steady-state mRNA levels of GLUT-4 and HXK-II in skeletal muscle are increased by intensive insulin therapy, which suggests a stimulation of peripheral glucose uptake. However, only glucose turnover studies would be able to give a reflection of the glucose kinetics.

Such a study (29), using a well-designed canine model of critical illness, recently endorsed our findings to a great extent. Donmoyer *et al.* (29) revealed that the presence of an infection decreased hepatic glucose uptake, which was unresponsive to insulin treatment. In contrast, peripheral glucose uptake did respond to insulin infusion. Unlike our findings, a suppression of hepatic glucose production was reported. Inhibition of glycogenolysis rather than diminished hepatic uptake of gluconeogenic amino acids and gluconeogenesis by insulin therapy appeared to be the determining factor.

Another important feature of critical illness is a strongly deranged serum lipid profile. The increased serum triglyceride levels together with decreased serum LDL and HDL levels resemble the dyslipidemia of the diabetic patient. This study of 363 long-stay intensive care patients revealed, for the first time, that intensive insulin therapy partially reversed the alterations in the serum lipid profile, which possibly contributed to the observed reduced mortality of critical illness.

Foremost, intensive insulin therapy prevented the rise in serum triglycerides during full nutritional support. Manifest hypertriglyceridemia occurs frequently in the critically ill, particularly when hyperalimentation is used in which critically ill patients receive about 35–40 kcal/kg of total parenteral nutrition (30). This currently obsolete practice also induced severe hyperglycemia, azotemia, hepatic steatosis, fat-overload syndrome, and hypertonic dehydration without improved patient outcome (31). However, even in our study in which patients were receiving less than 25 kcal/kg·d, moderate hypertriglyceridemia appeared. This rise in serum triglycerides was almost completely abolished by the intensive insulin therapy. The exact role of circulating triglycerides during critical illness seems ambiguous.

On the one hand, elevated levels of triglycerides have been described to reflect the severity of critical illness (32), whereas on the other hand, triglyceride-rich lipoproteins (VLDL and chylomicrons) have been shown to alter endotoxin processing and prevent death in experimental animal models (33, 34).

Second, intensive insulin therapy elevated the circulating levels of HDL and LDL cholesterol as compared with those measured in the conventionally treated patients, although the levels remained lower than those of healthy subjects (80–150 mg/dl for LDL and 35–60 mg/dl for HDL).

Apart from the importance of these lipoproteins for transportation of lipid components (cholesterol, triglycerides, phospholipids, lipid-soluble vitamins), reports have emerged over the years showing that lipoproteins may play a significant role in the binding and processing of endotoxins (35, 33). Hence, low levels of LDL and HDL may result in a defective scavenging of endotoxin in the circulation. Therefore, the use of HDL infusions has been proposed to temper the response to endotoxinemia in humans (8, 36).

To obtain an indication of the site of insulin action on lipid metabolism in the critically ill, SREBP-1c, a key regulatory transcription factor of lipogenesis (37), and LPL, the rate-limiting enzyme in the hydrolysis of triglycerides (23), were examined in tissues obtained from patients who died during the study. Although the former is principally expressed in the liver, the latter is crucial in the uptake of free fatty acids for energy provision in skeletal muscle. However, the lack of suppression of serum triglycerides by insulin in the nonsurvivors was reflected in unchanged SREBP-1c and LPL gene expression levels. This is the consequence of the inevitable selection bias toward the more seriously ill patients because biopsies were taken only in nonsurvivors for ethical reasons. Hence, an effect in survivors cannot be excluded.

Additonal *in vivo* studies are needed to clarify the effects of intensive insulin therapy on lipid metabolism in the critically ill patient.

Although intensive insulin therapy may not have fully restored a physiological serum lipid profile, the improvement of the deranged lipidemia explained a significant part

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of its beneficial effect on mortality and organ failure, surpassing the effect of glycemic control and insulin dose in the multivariate logistic regression analysis. The mechanisms involved in the link between esterified cholesterol, or its carrying particles, and outcome of prolonged clinical illness remain to be elucidated. Nevertheless, one could speculate on a role for LDL and HDL as scavengers for endotoxin or as transporters for cholesterol as an essential substrate for the integrity of cell membranes (38).

An explanation for the prevention of critical illness polyneuropathy and bacteremia by intensive insulin therapy is still lacking because the randomization for intensive insulin therapy *per se*, and not blood glucose or lipid control, remained decisive in the regression model. The fact that insulin dose remained an independent positive risk factor may point to the association between severity of illness and degree of insulin resistance. It was also intriguing to observe that the effect of intensive insulin therapy on inflammation (39), reflected by a lowering of the serum CRP concentrations, was no longer independently related to the outcome benefit when the changes in lipid metabolism were taken into account. This may suggest a link between the antiinflammatory effect of intensive insulin therapy and its amelioration of the lipid profile.

In conclusion, intensive insulin therapy exerted its glucose-lowering effect in prolonged critically ill patients mainly through stimulation of skeletal muscle glucose uptake rather than through affecting hepatic glucose handling. Concomitantly, intensive insulin therapy lowered serum triglyceride levels and increased circulating HDL and LDL cholesterol. The improved lipid control achieved by insulin-titrated maintenance of normoglycemia, rather than the glucose control *per se*, statistically explained at least part of the improved morbidity and mortality of prolonged critical illness.

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