0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(7):3105–3113

doi: 10.1210/jc.2003-032102

Regulation of the Somatotropic Axis by Intensive Insulin Therapy during Protracted Critical Illness

DIETER MESOTTEN, PIETER J. WOUTERS, ROBIN P. PEETERS, KEVIN V. HARDMAN, JEFF M. HOLLY, ROBERT C. BAXTER, AND GREET VAN DEN BERGHE

Department of Intensive Care Medicine, University Hospital Gasthuisberg, Catholic University Leuven (D.M., P.J.W., G.V.d.B.), B-3000 Leuven, Belgium; Department of Internal Medicine, Erasmus University Medical Center (R.P.P.), 3015 GE Rotterdam, The Netherlands; Department of Surgery, Bristol Royal Infirmary (J.M.H.), Bristol, United Kingdom BS2 8HW; and Kolling Institute of Medical Research, University of Sydney, Royal North Shore Hospital (K.V.H., R.C.B.), St. Leonards, New South Wales 2065, Australia

The catabolic state of critical illness has been linked to the suppressed somatotropic GH-IGF-binding protein (IGFBP) axis. In critically ill patients it has been demonstrated that, compared with the conventional approach, which only recommended insulin therapy when blood glucose levels exceeded 12 mmol/liter, strict maintenance of blood glucose levels below 6.1 mmol/liter with intensive insulin therapy almost halved intensive care mortality, acute renal failure, critical illness polyneuropathy, and bloodstream infections. Poor blood glucose control in diabetes mellitus has also been associated with low serum IGF-I levels, which can be increased by insulin therapy. We hypothesized that intensive insulin therapy would improve the IGF-I axis, possibly contributing to the clinical correlates of anabolism. Therefore, 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 the somatotropic axis. Contrary to expectation, intensive insulin therapy suppressed serum IGF-I, IGFBP-3, and acid-labile subunit concentrations. This effect was independent of survival of the critically ill patient. Concomitantly, serum GH levels were increased by intensive insulin therapy. The suppression of IGF-I in association with the increased GH levels suggests GH resistance induced by intensive insulin therapy, which was reflected by the decreased serum GH-binding protein levels. Intensive insulin therapy did not affect IGFBP-3 proteolysis, which was markedly higher in protracted critically ill patients compared with healthy controls. Also, intensive insulin therapy did not suppress the urea/creatinine ratio, a clinical correlate of catabolism. In conclusion, our data suggest that intensive insulin therapy surprisingly suppressed the somatotropic axis despite its beneficial effects on patient outcome. GH resistance accompanied this suppression of the IGF-I axis. To what extent and through which mechanisms the changes in the GH-IGF-IGFBP axis contributed to the survival benefit under intensive insulin therapy remain elusive. (*J Clin Endocrinol Metab* 89: 3105–3113, 2004)

PROTRACTED CRITICAL ILLNESS involves a serious metabolic derangement (1). The clinical syndrome is depicted by wasting of lean body mass despite adequate nutritional support, with a relative preservation of adipose tissue (2). Protein hypercatabolism gives rise to functionally important complications, such as prolonged mechanical ventilation and immobilization, organ dysfunction, impaired tissue repair, and atrophy of the intestinal mucosa, which together result in prolonged intensive care support with, conceivably, a higher cost burden. To a certain extent, this catabolic state of critical illness has been linked to the suppressed somatotropic GH-IGF-IGF-binding protein (IGFBP) axis (3, 4).

In the acute phase of critical illness, circulating levels of GH are promptly elevated, with a large number of GH pulses superimposed on elevated interpulse GH concentrations (3). In contrast, protracted critical illness, defined as an intensive care stay of more than 7 d, reveals a high frequency, low amplitude GH secretory pattern with much lower interpulse levels, resulting in low, normal, or only moderately elevated mean GH concentrations (5). Acute as well as protracted critical illnesses result in low circulating IGF-I, IGFBP-3, and acid-labile subunit (ALS) levels (6). In the circulation, these proteins form a ternary complex to extend the half-life of IGF-I and regulate its hypoglycemic potential (7). The binary IGF-I-IGFBP-3 complex that results from dissociation of ALS can rapidly traverse the capillary endothelial barrier and leave the circulation (8). Bioavailability of IGF-I may also be increased by proteolysis of IGFBP-3. This IGFBP-3 proteolytic activity has been described in different conditions, such as pregnancy (9) and critical illness (10–13). Increased proteolytic activity has also been linked to insulin resistance, such as in noninsulin-dependent diabetes mellitus (14), in which the increased IGF-I bioavailability may stimulate peripheral glucose uptake and hence contribute to the glucoregulation (7, 15). Whereas IGFBP-3 is produced by many cell types, circulating IGF-I and ALS are almost exclusively liver derived (16, 17). The synthesis of the latter proteins is strongly stimulated by GH at the transcriptional level (18). Hence, serum IGF-I and ALS concentrations are often used as markers of the peripheral GH effect (19, 20). In acute critical illness, an elevated mean GH level coinciding with a low IGF-I level has been regarded as evidence of GH resistance, which may be related to decreased GH receptor ex-

Abbreviations: ALS, Acid-labile subunit; GHBP, GH-binding protein; ICU, intensive care unit; IGFBP, IGF-binding protein; LD, last day of intensive care unit stay; UCR, urea/creatinine ratio.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

pression (21) or postreceptor signaling defects. Low IGF-I levels during the chronic phase of critical illness are less likely to be caused by GH resistance because they are not accompanied by elevated GH secretion and correlate positively with pulsatile GH secretion.

Intensive insulin therapy, aimed at maintaining normoglycemia (4.5–6.1 mmol/liter or 80–110 mg/dl) during intensive care stay was shown to reduce morbidity and mortality by 40% compared with the conventional approach, in which insulin therapy is started only when blood glucose levels exceed 12 mmol/liter (220 mg/dl) (22). The benefits from intensive insulin therapy also included a reduction of acute renal failure requiring dialysis, septicemia, critical illness polyneuropathy, prolonged ventilatory support, and intensive care stay.

In insulin-dependent diabetes, serum IGF-I levels are decreased during poor blood glucose control. These lowered IGF-I concentrations can be normalized by insulin therapy (23). Moreover, insulin, commonly regarded as the storage hormone, has anabolic properties.

Therefore, we hypothesized that in analogy with insulinization in diabetes mellitus, intensive insulin therapy would improve the IGF-I axis, thereby inducing anabolism and explaining certain clinical correlates thereof, such as facilitated weaning from mechanical ventilation and earlier discharge from the intensive care unit (ICU).

Subjects and Methods

Subjects

The subjects in this study (n = 363) were part of a large randomized controlled trial of intensive insulin therapy in intensive care patients (n = 1548), of which the treatment protocol and major clinical outcomes have been published in detail previously (22, 24). 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 was obtained. On ICU admission, patients were randomly assigned to either intensive or conventional insulin treatment. Assignments to the treatment groups were made with the use of sealed envelopes, with stratification according to the type of critical illness, and were balanced with the use of permuted blocks of 10. In the conventional group, continuous insulin infusion [50 IU Actrapid HM (Novo Nordisk, Denmark) in 50 ml 0.9% NaCl using a Perfusor-FM pump (B. Braun, Melsungen, Germany)] was started only when the blood glucose level exceeded 11.9 mmol/liter (215 mg/dl) and was adjusted to maintain glycemia between 10 and 11.1 mmol/liter (180 and 200 mg/dl). In the intensive insulin group, insulin infusion was started when blood glucose levels exceeded 6.1 mmol/liter (110 mg/dl) and was adjusted to maintain normoglycemia (4.4–6.1 mmol/liter or 80–110 mg/dl). The maximal insulin dose was arbitrarily set at 50 IU/h. The protocol was approved by the institutional review board of Catholic University (Leuven, Belgium).

For the current analysis of the effect of intensive insulin therapy on the somatotropic axis, 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.

Also in this subgroup of prolonged critically ill patients, intensive insulin therapy reduced mortality, septicemia, and critical illness polyneuropathy and tended to prevent acute renal failure requiring dialysis (Table 2). Postmortem liver biopsies (from the left lower quadrant) and skeletal muscle biopsies (from the right musculus rectus abdominis) were taken from 74 of the 98 patients who died in the ICU during the study, either acutely or after a long ICU stay. Of those 98 patients, 63 died after an ICU stay of at least 7 d, reducing the number of biopsies from prolonged critically ill patients to 47. Of these, only the liver and skeletal muscle samples that revealed acceptable mRNA upon quality assessment, as previously described (25), were analyzed.

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

Insulin treatment	Conventional	Intensive
No. of patients	206	157
Male gender, no. (%)	136 (66)	110 (70)
Age (yr; mean ± SEM)	61 ± 16	61 ± 15
On-admission APACHE-II [median (IQR)]	12 (9–15)	12 (7–16)
Preadmission diabetes, no. (%)	18 (9)	17 (11)
On-admission hyperglycemia >11 mM, no. (%)	29 (14)	20 (13)
On-admission blood glucose level (mM)	8.2 ± 3.2	8.1 ± 3.0
On-admission IGF-I (μg/liter)	65.7 ± 2.0	72.7 ± 2.4
On-admission IGFBP-3 (mg/liter)	1.5 ± 0.04	1.5 ± 0.05
On-admission ALS (mg/liter)	7.9 ± 0.3	8.1 ± 0.3
On-admission GH (μg/liter)	5.8 ± 0.9	5.7 ± 0.9
On-admission GHBP (μg/liter)	1.22 ± 0.06	1.20 ± 0.07

IQR, Interquartile range.

Serum analyses

Blood was sampled on admission, on d 1 and 8, and on the last day (LD) of ICU stay. After acid-ethanol extraction, concentrations of total serum IGF-I were measured by RIA in the presence of excess of IGF-II (25 ng/tube) (26). Serum concentrations of IGFBP-3 and ALS were determined by RIA as previously described (27, 28). The serum GH levels determined with a human GH immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). The detection limit was 0.2 μg/liter. Serum GH-binding protein (GHBP) concentrations were measured by ELISA (Diagnostics System Laboratory, Webster, TX). Normal values were: IGF-I, 126 ± 4 μg/liter; IGFBP-3, 3.7 ± 0.1 mg/liter; ALS, 15.0 ± 0.4 mg/liter; and GHBP, 1.8 ± 0.1 μg/liter (as determined in our age- and gender-matched reference group).

Proteolysed IGFBP-3 was assessed by immunoblotting after electrophoresis of 2.5 μl serum on a 12% polyacrylamide gel. The density of the protein bands was quantified by scanning densitometry and analyzed using Molecular Analyst software (Bio-Rad Laboratories, Hercules, CA). The ratio of fragmented IGFBP-3 over total IGFBP-3 was quantitated. We have previously demonstrated that this technique correlates well with an *in vitro* protease assay (r = 0.73) (29).

The serum urea/creatinine ratio (UCR) was used as a marker of overall protein degradation, which is appropriate in the absence of prerenal kidney failure and with a normal, constant protein and fluid intake, as was present in this study (30). The UCR of our matched controls was 29.0 ± 1.0 (range, 21.6–38.3).

RNA isolation and real-time PCR

RNA isolation and the generation of cDNA and external standards were performed as previously described (25). To avoid amplification of genomic DNA, primer pairs were designed to span an intron (Table 3). 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 (Applied Biosystems, Foster City, CA). The reaction mixtures for IGF-I and GH receptor contained 1× Platinum Quantitative PCR-Supermix-UDG (Invitrogen, Carlsbad, CA), 200 nM forward primer, 200 nM reverse primer, 200 nM TaqMan probe, and 2 mM $MgCl_2$, made up to a total volume of 25 μl with sterile water. The real-time PCR protocol was 10 min at 95 C, and 40 cycles of 15 sec at 95 C and 1 min at 60 C.

To assess PCR specificity, samples without reverse transcriptase were included. Gene expression was corrected for between-well loading variation by expressing data as a ratio of 18S ribosomal RNA or glyceraldehyde-3-phosphate dehydrogenase gene expression, measured using the TaqMan ribosomal RNA kit (Applied Biosystems). All samples were analyzed in duplicate, and the percent coefficient of variation was calculated. Individual samples with a copy number coefficient of variation greater than 20% were reanalyzed. All patient samples were analyzed in two runs. A separate run was performed for repeat samples.

TABLE 2. Outcome of all patients with an ICU stay of more than 1 wk

	Conventional therapy (n = 206)	Intensive therapy (n = 157)	*P* value
Death during ICU stay, no. (%)	44 (21)	19 (12)	0.021
Acute renal failure, no. (%)	57 (28)	30 (19)	0.058
Septicemia, no. (%)	59 (29)	28 (18)	0.017
Critical illness polyneuropathy, no. (%)	107 (52)	45 (29)	<0.0001

TABLE 3. Primer sets used to measure IGF-I and GH receptor (GH-R) gene expression by real-time PCR

Primer	Sequence
IGF-I forward	5′-CTTCAGTTCGTGTGTGGAGACAG-3′
IGF-I reverse	5′-GCCCTCCGACTGCTGGA-3′
IGF-I probe	5′-CTTTTATTTCAACAAGCCCAC-3′
GH-R forward	5′-TTTGGGCTAACAGTGATGCT-3′
GH-R reverse	5′-TCACTGTGGAATTCGGGTTT-3′
GH-R probe	5′-TGCCCCCAGTTCCAGTTCCA-3′

[a] The primer sets for IGF-I (S 85346) and GH receptor (NM 000163) were designed to be intron-spanning to avoid measuring genomic DNA contamination.

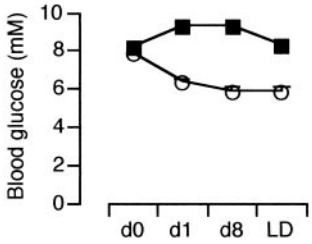

FIG. 1. Intensive insulin therapy lowered blood glucose levels in patients with an ICU stay longer than 7 d. ■, Conventional insulin therapy; ○, intensive insulin therapy. Samples were taken on d 0 (admission day; n = 363), d 1 (n = 343), d 8 (n = 344), and LD (n = 337), with a median stay of 16 d. Repeated measures ANOVA was used to determine the significance of differences.

Statistical analysis

Statistical analyses were performed using StatView 5.0.1 for Macintosh (SAS Institute, Cary, NC). Data are represented as the mean ± SEM or median (interquartile range), and statistical significance was set at $P < 0.05$. Differences between treatment groups were analyzed by two-tailed Mann-Whitney U test when numbers in each group where insufficient to perform a two-tailed unpaired *t* test. Changes in serum protein concentrations over time were evaluated by factorial and repeated measures ANOVA with Fisher's least significant difference test. The Bonferroni correction for multiple testing was used where necessary. Pearson product-moment correlation coefficients (r) and determination coefficients (r^2) were calculated for quantifying the relationship between variables.

Results

Intensive insulin therapy lowered blood glucose levels in patients with an ICU stay longer than 7 d

The two study groups, conventionally and intensively insulin-treated patients, were comparable for gender, age, history of diabetes, prevalence of hyperglycemia on admission, and severity of illness, as reflected by on-admission acute physiology and chronic health evaluation II (APACHE-II) score (Table 1). On-admission blood glucose levels did not differ between the two treatment groups (Fig. 1). From d 1 onward, intensive insulin therapy significantly suppressed blood glucose levels compared with those using the conventional treatment schedule ($P < 0.0001$, by repeated measures ANOVA) in both survivors and nonsurvivors. Also on LD, 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 those in the conventionally treated patients (9.0 ± 0.5 mmol/liter; $P < 0.0001$). Blood glucose control with intensive insulin therapy positively affected outcome in these protracted, critically ill patients (Table 2).

Intensive insulin therapy suppressed IGF-I, IGFBP-3, and ALS

Although baseline serum IGFBP-3, ALS, and GH concentrations did not differ between the two treatment groups, serum IGF-I concentrations were slightly, although significantly, higher in intensive insulin-treated patients compared with conventionally treated patients ($P = 0.03$). As baseline IGF-I, IGFBP-3, and ALS concentrations were highly variable within the treatment groups, the relative (percent) changes from baseline were analyzed (Fig. 2). The serum concentrations of all three proteins increased over time in both treatment groups ($P < 0.0001$). Nevertheless, the increase was significantly less in intensive insulin-treated patients ($P < 0.002$).

In the conventionally treated patients, LD compared with on-admission concentrations of serum IGF-I, IGFBP-3, and ALS were 48 ± 7%, 35 ± 5%, and 41 ± 8% higher, respectively, whereas in the intensive insulin treatment group the corresponding increases were 24 ± 5%, 14 ± 5%, and 20 ± 7%, respectively. Although serum concentrations of the ternary complex proteins increased by up to 48% on LD in the conventional treatment group, they remained lower than levels measured in our reference group (89 ± 3 *vs.* 126 ± 4 μg/liter for IGF-I, 1.8 ± 0.05 *vs.* 3.7 ± 0.1 mg/liter for IGFBP-3, 111 ± 5 *vs.* 150 ± 5 nmol/liter for ALS).

Suppressive effect of intensive insulin therapy on the somatotropic axis was independent of survival

In survivors as well as nonsurvivors, the rise in IGF-I (Fig. 3A), IGFBP-3 (Fig. 3B), and ALS (Fig. 3C) serum concentrations from admission to LD was lower in the intensive insulin treatment group compared with the conventionally treated patients. However, in nonsurvivors the increases in IGF-I ($P = 0.006$), IGFBP-3 ($P = 0.002$), and ALS ($P = 0.04$) were less than those in survivors. Two-factor ANOVA revealed a clear suppressive effect of nonsurvival ($P = 0.003$) and intensive insulin therapy ($P = 0.02$), with no interaction between these two factors ($P = 0.8$), on IGF-I on LD. Similar effects were present for IGFBP-3

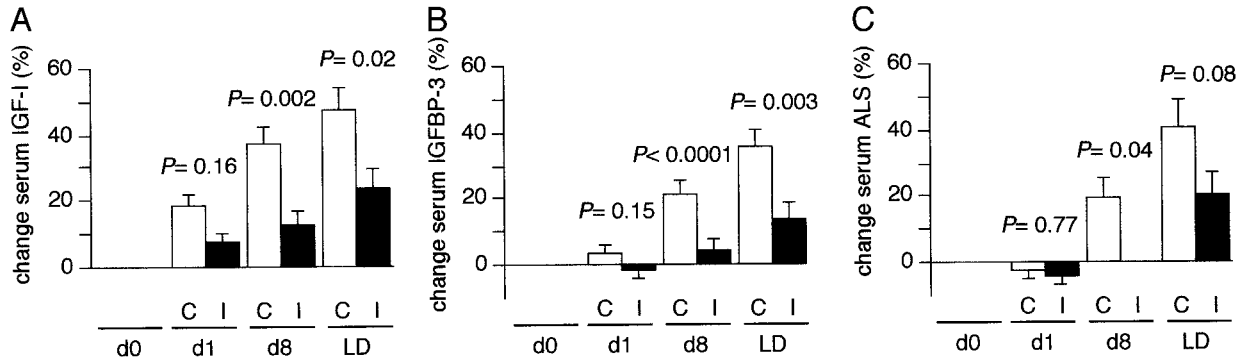

FIG. 2. Intensive insulin therapy suppressed serum IGF-I, IGFBP-3, and ALS. The percent changes from baseline in IGF-I (A), IGFBP-3 (B), and ALS (C) are shown (mean ± SEM). C, Conventional insulin therapy; I, intensive insulin therapy. Bonferroni correction for multiple testing was used to determine the significance of differences.

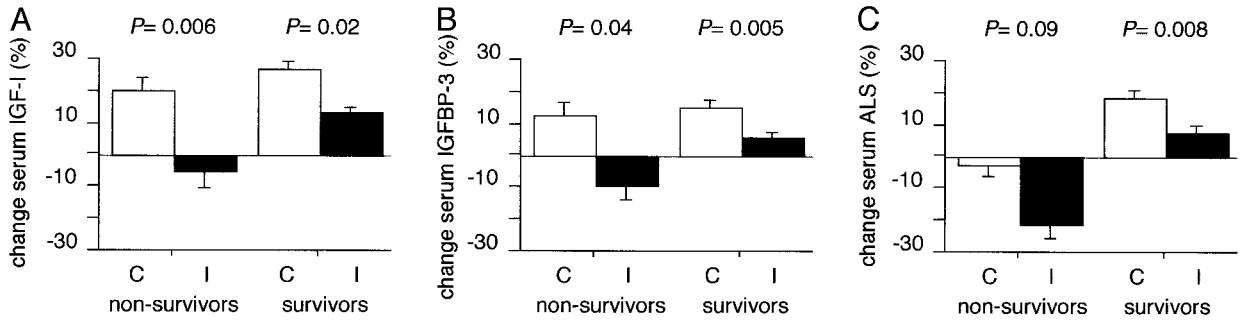

FIG. 3. The suppressive effect of intensive insulin therapy on the somatotropic axis was independent of survival. The percent changes from baseline in IGF-I (A), IGFBP-3 (B), and ALS (C) are shown (mean ± SEM). n = 39, Nonsurvivors and conventional insulin therapy (C); n = 16, nonsurvivors and intensive insulin therapy (I); n = 143, survivors and C; n = 128, survivors and I.

($P = 0.009$, $P = 0.007$, and $P = 0.6$, respectively) and ALS ($P < 0.0001$, $P = 0.07$, and $P = 0.9$).

Intensive insulin therapy did not affect the fraction of proteolysed IGFBP-3

Compared with control subjects, in whom 53.2% (interquartile range, 49.3–55.7) of IGFBP-3 immunoreactivity corresponded in electrophoretic mobility to proteolysed forms, the apparent fraction of proteolysed over total IGFBP-3 on d 8 of intensive care in our critically ill study population was 92% (interquartile range, 81.5–97.2; $P < 0.0001$; Fig. 4A). However, the ratio of fragmented over total IGFBP-3 on d 8 did not differ between patients in the conventional (n = 192) and intensive insulin treatment groups (n = 150; $P = 0.99$; Fig. 4B). Likewise, the fraction of proteolysed IGFBP-3 in nonsurvivors (n = 58) was not different compared with that in survivors (n = 284; $P = 0.54$; Fig. 4C).

Because of the suppressive effect of intensive insulin therapy on serum concentrations of total IGFBP-3, the absolute circulating level of IGFBP-3 proteolysed, as calculated from the product of the fraction of IGFBP-3 proteolysed and the serum concentration of total IGFBP-3, was also lower in the intensive insulin treatment group (1.27 ± 0.05 mg/liter) compared with the conventional treatment group (1.48 ± 0.05 mg/liter; $P = 0.003$). In contrast, the amount of intact IGFBP-3 was not different between the treatment groups.

Intensive insulin therapy decreased hepatic IGF-I gene expression, which correlated with serum IGF-I

Intensive insulin therapy decreased hepatic IGF-I mRNA levels to 50% ($P = 0.047$; Fig. 5A). The assumption that serum IGF-I levels measured on LD would reflect hepatic IGF-I gene expression in postmortem liver samples was supported by the positive correlation between these measurements ($r = 0.40$; $P = 0.02$; Fig. 5B). In contrast, skeletal muscle IGF-I gene expression was not affected by intensive insulin therapy (Fig. 5C) and did not correlate with serum IGF-I concentrations on LD ($r = 0.06$; $P = 0.70$; Fig. 5D).

Intensive insulin therapy increased GH secretion

Serum GH concentrations showed a temporal effect regardless of treatment group ($P < 0.0001$). Although levels on d 1 were higher (7.7 ± 0.6 μg/liter), those measured on d 8 (3.7 ± 0.2 μg/liter) and LD (3.5 ± 0.2 μg/liter) were lower than on-admission GH concentrations (5.8 ± 0.7 μg/liter; Fig. 6A). Moreover, on d 8 and LD, GH levels were significantly higher in intensive insulin-treated patients compared with those in the conventional treatment group.

As a surrogate indicator of GH resistance, the GH/IGF-I ratio was calculated, while recognizing the different serum dynamics of the two analytes. Paralleling the increased GH concentrations from d 8 onward, the GH/IGF-I ratio also

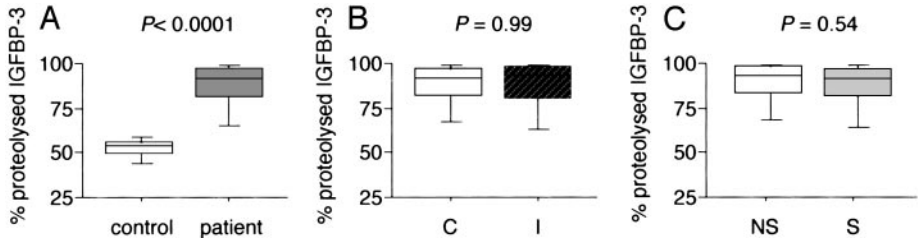

FIG. 4. The fraction of proteolysed IGFBP-3 was not affected by intensive insulin therapy. The ratio of fragmented to total IGFBP-3 on d 8 is shown. A, Controls (n = 20) *vs.* patients (n = 342); B, conventional (C; n = 192) *vs.* intensive insulin therapy (I; n = 150); C, nonsurvivors (NS) (n = 58) *vs.* survivors (S; n = 284).

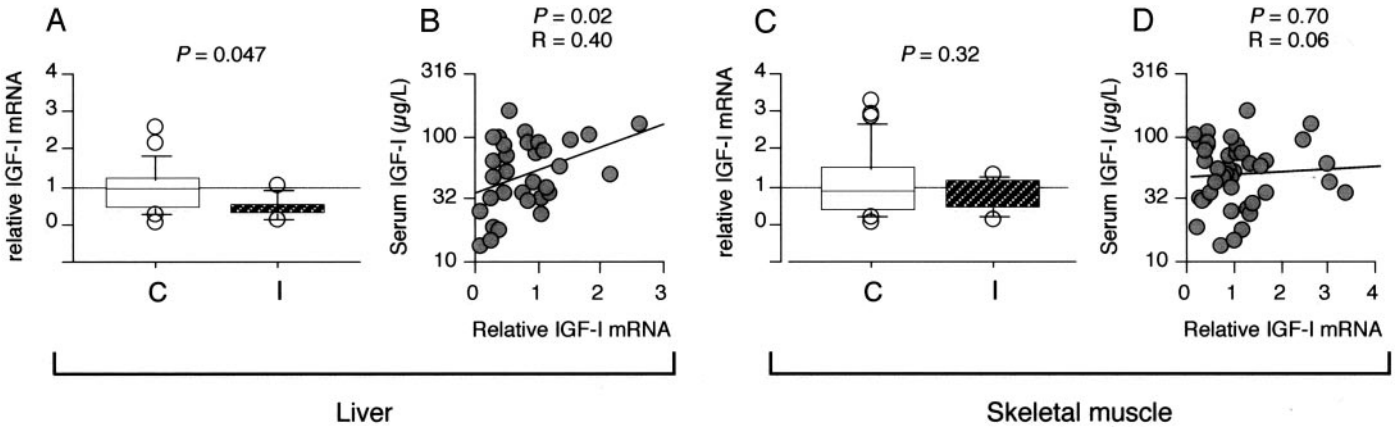

FIG. 5. Intensive insulin therapy decreased hepatic IGF-I gene expression in nonsurvivors, which correlated with LD serum IGF-I. A, Real-time PCR quantification of hepatic IGF-I gene expression for the two insulin treatment regimens with conventional (C) insulin therapy (n = 24) or intensive (I) insulin therapy (n = 8). B, Regression analysis of LD serum IGF-I *vs.* hepatic IGF-I mRNA (n = 32). C, Real-time PCR quantification of skeletal muscle IGF-I gene expression for C (n = 31) or I (n = 11). D, Regression analysis of LD serum IGF-I *vs.* skeletal muscle IGF-I mRNA (n = 42).

increased, suggesting the induction of GH resistance by intensive insulin therapy during protracted critical illness (Fig. 6B). Two-factor ANOVA revealed a clear effect (higher GH/IGF-I ratio and, thus, more presumed GH resistance) of nonsurvival ($P < 0.0001$) and intensive insulin therapy ($P = 0.036$), but with no interaction between these two factors ($P = 0.7$). Thus, the aggravating effect of intensive insulin therapy on GH resistance was present in survivors and nonsurvivors (Fig. 6C).

Intensive insulin therapy suppressed serum GHBP, but did not alter GH receptor mRNA expression

To examine whether the aggravated GH resistance by intensive insulin therapy was associated with suppressed GH receptor expression, serum GHBP concentrations and mRNA levels of GH receptor in postmortem samples were determined. Although there was no difference in GHBP concentrations between admission and d 1, intensive insulin therapy markedly suppressed GHBP levels on d 8 ($P < 0.0001$; Fig. 7A). Three-way ANOVA (for randomized insulin treatment assignment, outcome, and time) revealed that randomization ($P = 0.019$) and time ($P = 0.001$) were significantly associated with serum GHBP levels, without an effect of nonsurvival ($P = 0.76$). However, neither hepatic (Fig. 7B) nor skeletal muscle (Fig. 7C) GH receptor mRNA levels were significantly altered by intensive insulin therapy. Day 8 serum GHBP levels did not correlate with GH receptor in liver ($P = 0.63$) or muscle ($P = 0.25$).

Tissue responses

Averaged for the two treatment groups, the UCR increased from 41.9 ± 1.1 on d 1 to 69.8 ± 1.3 and 69.1 ± 1.5 on d 8 and LD, respectively ($P < 0.0001$). There was no effect of intensive insulin therapy on the UCR at any time. Nonsurvivors had higher UCR levels than survivors (68.3 *vs.* 58.6; $P < 0.0001$).

Discussion

Critical illness is accompanied by elevated net protein catabolism, which is only partially, if at all, counteracted by protein synthesis, together with hyperglycemia and hypertriglyceridemia (31–33). There is some evidence that a deranged GH/IGF/IGFBP axis may play a pivotal role in these metabolic changes during critical illness (3, 4). We observed that intensive insulin therapy, which was previously found to improve the morbidity and mortality of critical illness (22), surprisingly suppressed hepatic IGF-I gene expression, reduced circulating levels of the ternary IGF-I complex, and increased serum GH levels, and this occurred independently of its effects on clinical outcome of the critical illness.

Throughout their ICU stay, critically ill patients had invariably low levels of IGF-I, IGFBP-3, and ALS, which form the 150-kDa ternary complex. Concomitantly, we previously showed that serum IGFBP-1 levels are strongly elevated (25). This has been confirmed in other studies of acute and prolonged critical illness (34–36). Here we showed that the components of the ternary complex gradually increased in the serum of conventionally treated patients, but remained below levels in age-matched controls. Although intensive in-

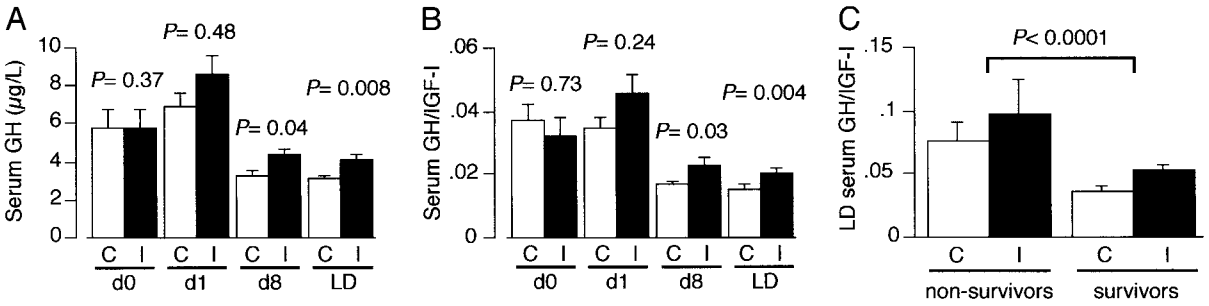

FIG. 6. Intensive insulin therapy increased GH secretion, indicating GH resistance. A, Serum GH levels (mean ± SEM). B, GH/IGF-I ratio (mean ± SEM). Samples were taken on d 0 (admission day; n = 338), d 1 (n = 359), d 8 (n = 356), and LD (n = 353). C, Two-way ANOVA of the LD GH/IGF-I ratio (mean ± SEM) in nonsurvivors (n = 59) and survivors (n = 286; C, conventional insulin therapy; I, intensive insulin therapy). Bonferroni correction for multiple testing was used to determine the significance of differences.

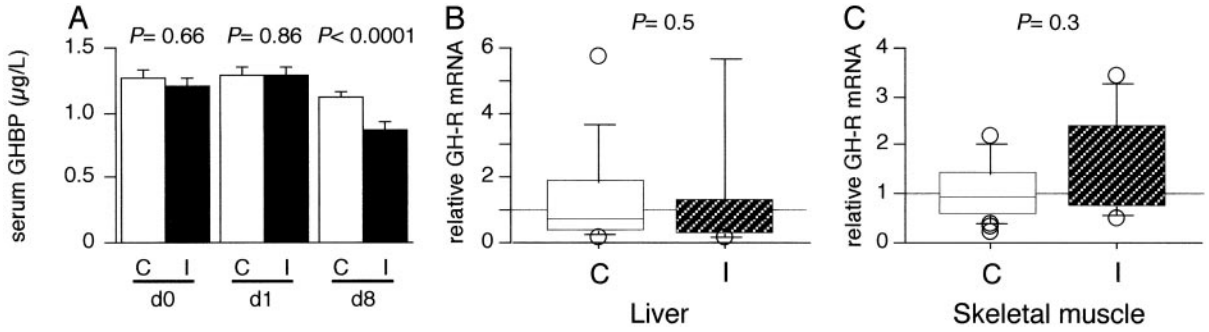

FIG. 7. Intensive insulin therapy suppressed GHBP levels, but did not alter GH receptor mRNA expression. A, Serum GHBP levels (mean ± SEM). Samples were taken on d 0 (n = 291), d 1 (n = 238), and d 8 (n = 300). B, Real-time PCR quantification of hepatic GH receptor gene expression in nonsurvivors for the two insulin treatment regimens with conventional (C) insulin treatment (n = 25) or intensive (I) insulin treatment (n = 12). C, Real-time PCR quantification of skeletal muscle GH receptor gene expression for C (n = 32) or I (n = 13). Bonferroni correction for multiple testing was used to determine the significance of differences.

sulin therapy lost its suppressive effect on IGFBP-1 during critical illness (25), the increases in IGF-I, IGFBP-3, and ALS with time in ICU were mitigated by intensive insulin therapy. These observations are consistent with the previous preliminary findings of suppressed IGF-I and IGFBP-3 levels after prolonged euglycemic hyperinsulinemia in severely burn-injured patients (37).

The suppressive effect of intensive insulin therapy on the ternary complex proteins was present both in patients who survived and in those who died during intensive care, with even lower IGF-I, IGFBP-3, and ALS levels in nonsurvivors. The link between failure of IGF-I recovery and adverse outcome has previously been reported (13, 38). It is therefore intriguing that intensive insulin therapy, which improved survival, suppressed the somatotropic axis. Also, in liver biopsies obtained from nonsurvivors, but not in skeletal muscle samples, intensive insulin therapy decreased steady state IGF-I mRNA, probably explaining at least part of the suppressive effect on circulating IGF-I. In diabetes mellitus, serum ternary complex components are also decreased, with the effects of insulinization being variable (39, 40). In adult type I diabetic patients, insulin infusion increased serum IGF-I levels, whereas it could not correct the low serum IGFBP-3 concentrations (23). In pediatric type I diabetic patients, 1 month of insulin therapy increased serum IGF-I as well as IGFBP-3 and ALS levels (41, 42). In type II diabetes mellitus changes in the IGF-IGFBP axis are less pronounced and often absent (43).

Another important factor in the bioavailability of IGF-I is proteolysis of its carrier protein IGFBP-3. In accordance with previous studies, the ratio of fragmented over total IGFBP-3 was about 75% higher in prolonged critically ill patients compared with that in healthy individuals. In postoperative patients, insulin infusion increased IGFBP-3 proteolytic activity (44, 45). In our study, however, intensive insulin therapy did not affect the fraction of proteolysed IGFBP-3. In fact, because of the suppressive effect of intensive insulin therapy on circulating total IGFBP-3, the circulating amount of proteolysed IGFBP-3 was actually lower in this group. A potential stimulation of IGFBP-3 proteolysis, however, may have been masked by the high baseline proteolytic activity in the current study population of very sick patients. This would also explain similar proteolytic activity in survivors and nonsurvivors.

As the suppressive effect of insulin on IGF-I gene expression and serum concentrations is totally contraintuitive, it is difficult to explain by direct actions of insulin. Hence, indirect effects of intensive insulin therapy via paradoxical hormonal changes or interference with cytokines or chemokines could be hypothesized. Further research is required to clarify which pathways are involved.

In line with the lack of an anabolic effect of intensive insulin therapy on the IGF-I/IGFBP system, it did not detectably reduce protein hypercatabolism, as urea generation remained unaltered (46). Despite this finding, intensive insulin therapy did protect organ function and allowed earlier

discharge from the ICU (22). The lack of an anticatabolic effect of insulin during prolonged critical illness is in line with the previously reported unchanged phosphoenolpyruvate carboxykinase mRNA levels during this therapy (25). Protein breakdown indeed provides the substrates for hepatic gluconeogenesis, of which phosphoenolpyruvate carboxykinase is the rate-limiting enzyme (47). Hyperglycemia has previously been reported to relate to muscle protein catabolism (48), and in contrast to critically ill patients, prolonged euglycemic hyperinsulinemia muscle in burn-injured patients appears to exert anabolic action on skeletal muscle and lean body mass (37, 49). Different doses of insulin and glucose infusion as well as different degrees of severity of illness and organ failure presumably play a role.

Another intriguing observation was the increase in circulating GH levels with intensive insulin therapy and, because of the concomitantly lower IGF-I levels, the apparent induction of GH resistance. Again, this is in contrast to previous reports in patients with diabetes, in whom the excessive GH secretion is lowered by initiation of insulin therapy (15). The apparent insulin-induced GH resistance in the critically ill was accompanied by lowered serum GHBP levels. In general, GH receptor expression and GH effect are positively correlated with serum GHBP levels. GHBP, the extracellular domain of the GH receptor, complexes about half of the serum GH, prolonging its half-life, and may also modulate GH bioactivity through competition with the GH receptor for GH (50). In the critically ill, GHBP was higher upon admission and on d 1 compared with d 8 of the disease, but remained below the levels in the matched control samples at all times. This is in contrast to previous findings reporting high normal levels of GHBP from 2 wk of critical illness onward (36).

Although coinciding with lower GHBP levels, the intensive insulin therapy-induced constellation of GH resistance was not paralleled by a decrease in GH receptor mRNA, suggesting that the effect may have been posttranslational (*e.g.* altered GH receptor turnover) or may have occurred at the postreceptor level. Whatever mechanism explains the reduced IGF-I gene expression with intensive insulin therapy, the reduced serum IGF-I levels are likely to lessen feedback inhibition at the pituitary somatotropes (51), thereby elevating GH secretion. An exaggerated GH response to GHRH in type I diabetic patients has been reported and is in line with this reasoning (52). This GH hyperresponsiveness to GHRH can be even further accentuated by acute insulinization (53).

A provocative teleological interpretation of the current data may encompass maintenance of GH resistance as a protective response to illness and stress. This hypothesis is supported by the observation that GH receptor knockout mice, which are extremely GH resistant, live longer than wild-type mice (54). Also, streptozotocin-treated mice overexpressing GH antagonist or after GH antagonist G120K-polyethylene glycol administration were protected from the development of diabetic glomerulosclerosis (55, 56), paralleling the many diabetes-like complications of critical illness (57). This may be related to the increased insulin sensitivity in GH receptor knockout mice (58), pointing to a constellation of reduced GH and increased insulin effects as a mechanism providing a survival benefit (59, 60). Conversely, to attenuate the catabolic state of critical illness, GH therapy has been tried in postoperative, trauma, burns, and septic patients (61, 62). However, the only large, randomized, controlled trial of high dose GH treatment in protracted critically ill patients unexpectedly doubled mortality and worsened morbidity, despite increased IGF-I and IGFBP-3 levels together with an improved nitrogen balance (63). As a side-effect of GH treatment, pronounced hyperglycemia and insulin resistance ensued. Hence, our current data corroborate such an interaction of reduced GH and increased insulin effects playing a role in the outcome benefit of intensive insulin therapy during critical illness. Nevertheless, we cannot exclude that intensive insulin therapy, by aggravating GH resistance and lowering IGF-I generation, at a certain point may have had adverse effects despite its ability to save lives, reduce infections, and improve organ function. If this were the case, such adverse effects of intensive insulin therapy on metabolism could be interpreted as a small "price to pay" for these important clinical benefits.

In conclusion, intensive insulin therapy suppressed the somatotropic axis with lowered IGF-I, IGFBP-3, and ALS levels. The concomitant increase in serum GH concentrations and the suppression of circulating GHBP levels suggest induction of GH resistance with intensive insulin therapy. These changes in the GH/IGF-I axis are contraintuitive and surprising. Future studies of the effect of intensive insulin therapy on cytokines, chemokines, and other hormonal axes will shed new light on the potential mechanisms involved.

Acknowledgments

We thank Willy Coopmans, Frank Vanderhoydonc, and Erik Van Herck for their valuable technical assistance. The GH-R primers and probe were willingly provided by Leo Hofland (Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands).

Received December 10, 2003. Accepted March 20, 2004.

Address all correspondence and requests for reprints to: Dr. Greet Van den Berghe, Department of Intensive Care Medicine, University Hospital Gasthuisberg, B-3000 Leuven, Belgium. E-mail: greta.vandenberghe@med.kuleuven.ac.be.

This work was supported by the Fund for Scientific Research (Flanders, Belgium; G.0144.00 and G.0278.03), the Research Council of the Catholic University of Leuven (OT 03/56), and the Belgian Foundation for Congenital Heart Disease.

D.M. is a research assistant (aspirant) for the Fund for Scientific Research (Flanders, Belgium).

G.V.d.B. holds an unrestrictive Catholic University of Leuven Novo Nordisk Chair of Research and a Fundamental Clinical Research Investigator (G.3C05.95N) for the Fund for Scientific Research (Flanders, Belgium).

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