0013-7227/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(3):1082–1088
Copyright © 2003 by The Endocrine Society
doi: 10.1210/jc.2002-021478

Intensive Insulin Therapy Exerts Antiinflammatory Effects in Critically Ill Patients and Counteracts the Adverse Effect of Low Mannose-Binding Lectin Levels

TROELS KRARUP HANSEN, STEFFEN THIEL, PIETER JOZEF WOUTERS, JENS SANDAHL CHRISTIANSEN, AND GREET VAN DEN BERGHE

Medical Department M (Endocrinology and Diabetes), Aarhus University Hospital (T.K.H., J.S.C.), and Department of Medical Microbiology and Immunology, University of Aarhus (S.T.), DK-8000 Aarhus, Denmark; and Department of Intensive Care Medicine, University of Leuven (P.J.W., G.V.d.B.), B-3000 Leuven, Belgium

Adverse outcome of critical illness is often caused by systemic inflammation and sepsis. A recent study showed that mortality is significantly reduced by maintenance of normoglycemia using intensive insulin therapy. We examined whether the beneficial effects of intensive insulin therapy involve modulations of mannose-binding lectin (MBL) and C-reactive protein (CRP) levels.

From a study of 1548 patients randomly assigned to either conventional treatment or intensive insulin therapy at an intensive care unit (ICU) we included all 451 patients who needed prolonged intensive care (>5 d). CRP and MBL concentrations were measured on admission, d 5, d 15, and the last day in the ICU.

In all patients, serum MBL concentrations increased with time in the ICU ($P < 0.0001$). This acute phase response was suppressed by intensive insulin therapy at all time points studied ($P < 0.02$). Selectively in patients receiving conventional therapy, MBL concentrations at baseline were almost 3 times higher in survivors than in nonsurvivors ($P = 0.04$). Baseline CRP concentrations were elevated, but decreased with time in ICU ($P < 0.0001$). The decrease in CRP was significantly more pronounced in the intensive insulin-treated patients compared with the conventionally treated patients ($P \leq 0.02$) at all time points. Multivariate logistic regression analysis, corrected for all other determinants of outcome, revealed that the antiinflammatory action on CRP, but not on MBL, largely explained the beneficial effects of intensive insulin therapy on morbidity and mortality.

In conclusion, intensive insulin therapy exerts a powerful antiinflammatory effect during critical illness which at least partially explains improvement in morbidity and mortality. Possible adverse effects of low baseline MBL are overcome by intensive insulin therapy. (*J Clin Endocrinol Metab* 88: 1082–1088, 2003)

MORTALITY AMONG PATIENTS with prolonged critical illness exceeds 20% (1, 2), with most deaths being attributable to sepsis and multiple organ failure. Each year, these conditions affect more than 500,000 patients in the United States alone (3). An increased susceptibility to severe infections during critical illness as well as adverse effects of an excessive systemic inflammatory response on organ function may be operative.

Within the last few years, two large scale studies of different hormone therapies have yielded remarkable results. Intensive insulin therapy to maintain normoglycemia during intensive care resulted in significant reductions in morbidity and mortality (1), whereas high dose GH treatment almost doubled mortality compared with placebo (2). In both studies the effects on mortality were present among patients receiving prolonged (>5 d) intensive care and were predominantly related to sepsis, inflammation, and lethal, multiple organ failure, but the exact mechanisms behind the opposite actions of insulin and GH remain speculative.

Serum levels of acute phase proteins, which are synthesized in the liver, can serve as indicators of the presence and extent of inflammation and tissue necrosis. Some of these proteins also have intrinsic antimicrobial properties and have been linked to innate immunity and host defense. C-Reactive protein (CRP) is the most prominent member of the first group (4), whereas mannose-binding lectin (MBL; also known as mannan-binding lectin) belongs to the latter.

Measurements of CRP concentrations are widely used to help clinicians differentiate between inflammatory and noninflammatory conditions and to monitor the course of a large number of diseases (4). The exact pathophysiological role of CRP in the inflammatory process remains unclear, but may involve binding to ligands such as polysaccharides found on the surface of bacteria and necrotic tissue, and activation of leukocytes and the complement system (5).

MBL plays an important role in innate immunity by recognizing and initiating opsonization of microorganisms (6). As a C-type lectin, MBL can bind specifically to patterns of terminal nonreducing sugars, including *N*-acetylglucosamine, mannose, and fucose. MBL binds to such carbohydrate structures on the surface of microorganisms, upon which it initiates complement activation through association with serine proteases (MBL-associated serine protease-1, -2, and -3) (7–9). This complex activates the complement system at the levels of C4 and C2 in a series of interactions that has been termed the lectin pathway of complement activation. The average serum concentration of MBL in the adult population is between 1000–2000 μg/liter, with very large variations (6). The between-subject differences in serum concentrations are primarily caused by genetic factors, and point

Abbreviations: CRP, C-reactive protein; ICU, intensive care unit; IQR, interquartile range; MBL, mannose-binding lectin.

Downloaded from https://academic.oup.com/jcem/article/88/3/1082/2845194 by guest on 20 August 2022

mutations within exon 1 as well as in the promoter region of the MBL gene occur with high incidence. As a consequence, approximately one third of the population has MBL concentrations below 500 μg/liter, and more than 10% have concentrations below 50 μg/liter (10). Normally within-subject variations in MBL levels are very small (11), but serum concentrations increase during acute phase responses (12) and can be specifically induced by GH administration (11). However, although the genetically determined between-subject variations in basal MBL levels cover several orders of magnitude, the within-subject changes caused by acute phase responses or induced by GH are far smaller. Deficiency of MBL is associated with an increased incidence of infections (13–16), but due to the redundancy of the immune system the increased risk may only be apparent if other coexisting immunological abnormalities are present. In line with this, it was recently reported that low levels of MBL in patients receiving cancer chemotherapy are associated with an increased frequency of febrile neutropenic episodes and severe infections (17, 18). The impact of MBL concentrations on the course of disease in otherwise immunocompetent critically ill patients has not been studied.

We here investigated whether low baseline MBL levels affect outcome in critically ill patients. Furthermore, as both MBL and CRP have pronounced and distinctive interactions with the complement system and leukocyte activity, and excessive inflammation may contribute to organ failure and death, we studied the effect of intensive insulin therapy on these inflammatory markers and documented their impact on outcome.

Subjects and Methods

The samples used for the present study were obtained from a prospective, randomized, controlled study including all patients on mechanical ventilation admitted to the Department of Intensive Care Medicine, University of Leuven (Leuven, Belgium), from February 2000 to January 2001. On admission, patients had been randomly assigned to receive intensive insulin therapy or conventional treatment. The main outcome and the complete study protocol with description of study design, end-point definitions, clinical measures, data collection, and baseline characteristics have been published (1). The institutional review board approved the protocol, and written informed consent was obtained from the closest family member of all patients at intensive care unit (ICU) admission.

Study design

From the total study population of 1548 patients, we included all patients who received intensive care for more than 5 d, which was the group in which the mortality benefit of intensive insulin therapy occurred (1). Upon ICU admission, all patients were randomly assigned to receive either intensive or conventional insulin therapy. Insulin (Actrapid HM, Novo Nordisk, Copenhagen, Denmark) was administered as an insulin infusion (50 IU Actrapid HM in 50 ml 0.9% sodium chloride) using an infusion pump (Perfusor-FM, B. Braun, Melsungen, Germany). In the intensive treatment group the insulin infusion was started if blood glucose levels exceeded 6.1 mmol/liter and was adjusted to keep blood glucose between 4.4–6.1 mmol/liter, whereas in the conventional treatment group the insulin infusion was started at blood glucose levels above 12 mmol/liter and was adjusted to keep blood glucose between 10–11 mmol/liter (Fig. 1).

Bloodstream infection was defined by a blinded investigator as the presence of bacterial pathogens, excluding contamination according to strict criteria (19), in blood cultures obtained when central body temperature steeply rose above 38.5 C. The use of antibiotics was recorded

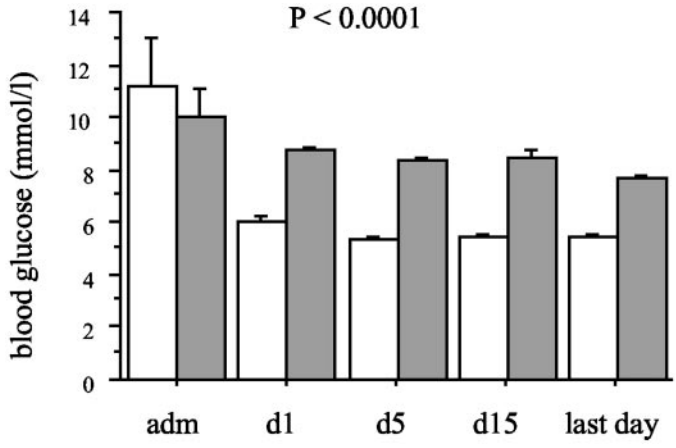

FIG. 1. Blood glucose levels upon admission; on the morning of d 1, 5, and 15; and on the last day of intensive care in the intensive insulin-treated group (□) and the conventionally treated group (▨) of prolonged critically ill patients. Data are the mean ± SEM. As intended, blood glucose levels were significantly different between the two groups ($P < 0.0001$) at all time points after randomization.

as the total number of days on any systemic antibiotic treatment. The number of days during which leukopenia (≤4,000 cells/μl) or leukocytosis (≥12,000 cells/μl) was present and the number of days during which an episode of hypothermia (≤36 C) or hyperthermia (≥38 C) occurred were also analyzed. The incidence of acute renal failure requiring renal replacement therapy was recorded. Weekly electromyography screenings were performed for the diagnosis of critical illness polyneuropathy. The occurrence at any time during ICU stay of a positive electromyography for critical illness polyneuropathy, as defined by an electrophysiologist who was blinded for treatment allocation, was analyzed. The cause of death for all patients who died was established clinically by the attending physician and was confirmed on postmortem examination by a pathologist who was unaware of treatment assignment.

MBL and CRP measurements

Blood samples were drawn within 24 h after admission to the ICU (baseline), and subsequently on d 5 and 15 and/or the last day of intensive care (*i.e.* the day of discharge or death) for determination of serum MBL and CRP. Samples at all time points were available in 203 of 209 patients who stayed in the ICU for more than 15 d, whereas complete sets of samples from baseline, d 5, and last day were available in 439 patients.

Serum MBL concentrations were measured in all available samples by an investigator who was blinded for treatment allocation using an in-house, time-resolved immunofluorometric assay (20). In brief, microtiter wells were coated with mannan, followed by incubation with diluted samples. After washing, europium-labeled monoclonal anti-MBL antibody (131-1, Immunolex, Copenhagen, Denmark) labeled with europium using reagents from Wallac, Inc. (Turku, Finland) was added, and after incubation and washing the amount of bound labeled antibody was assessed by time-resolved fluorometry (Delphia, Wallac, Inc.). Serum CRP concentrations were measured by immunoturbidimetric assay (Roche-Itachi-Modular-P, Roche, Basel, Switzerland). Normal CRP levels in healthy volunteers are below 5 mg/liter.

Statistical analysis

Differences between intervention groups were evaluated by Mann-Whitney U and χ^2 tests. Changes in MBL and CRP concentrations over time during the ICU stay were analyzed by Friedman's test for several related samples. Spearman correlation with two-tailed probability values was used to estimate the strength of association between variables. The impact of randomization schedule and baseline MBL and CRP levels on outcome variables (mortality, acute renal failure, bacteremia, prolonged need for antibiotic treatment, and polyneuropathy) was assessed

Downloaded from https://academic.oup.com/jcem/article/88/3/1082/2845194 by guest on 20 August 2022

by multivariate logistic regression analysis. In addition, multivariate logistic regression analysis was used to assess whether the changes in MBL and CRP over time explained clinical outcome variables. Data are given as medians with interquartile ranges unless specified otherwise, and statistical significance was assumed for $P < 0.05$. All statistical calculations were performed with StatView 5.0.1 for Macintosh (SAS Institute, Inc., Cary, NC).

Results

Clinical patient characteristics and outcome

The 451 patients who required intensive care for more than 5 d (long-stay patients) were included for this analysis. These patients needed intensive care for a median of 20 d [interquartile range (IQR), 9–24], and their mean age at ICU admission was 61 ± 15 yr. The 208 patients who had been randomized to intensive insulin therapy received insulin throughout the ICU stay at a median dose of 70 IU/d (IQR, 47–93), whereas 124 of the 243 patients in the conventional treatment group received insulin for 41% (IQR, 14–92%) of the ICU stay at a median dose of 32 IU/d (IQR, 14–58) on the days insulin was given. There were no significant differences in age, sex, body mass index, reason for intensive care, or severity of illness scores between the 2 treatment groups on admission.

As previously reported (1), 71 of these long-stay patients died during intensive care. The cause of death was multiple organ failure with or without a proven septic focus in 83% of the cases, acute cardiovascular collapse in 13%, and severe brain damage in 4% of the cases. In the intensive insulin therapy group, the mortality during intensive care was significantly lower than in the conventional treatment group (10.6% *vs.* 20.2%; $P = 0.005$) (1). The incidence of acute renal failure requiring replacement therapy was reduced from 24% to 15% ($P = 0.01$) by intensive insulin therapy, and the incidence of critical illness polyneuropathy decreased form 45% to 22% ($P < 0.0001$).

Bacteremia occurred in 15% of intensive insulin-treated, long-stay patients compared with 25% of the conventionally treated patients ($P = 0.01$). Of all positive blood cultures, 34% were polymicrobial, with coagulase-negative staphylococci, enterococcus species, and nonfermenting, Gram-negative bacilli being the most frequently isolated pathogens. There were no significant differences in causative pathogens between the 2 study groups. A total of 445 patients received treatment with antibiotics for a median duration of 10 d (IQR, 6–18). Intensive insulin therapy significantly reduced the duration of antibiotic treatment from a median of 12 d (IQR, 6–21) to 9 d (IQR, 6–16; $P = 0.002$). Intensive insulin therapy also reduced the number of days during which leukopenia or leukocytosis was present from a median of 6 d (IQR, 2–13) to 4 d (IQR, 1–10; $P = 0.02$) and the number of days with hypo- or hyperthermia from a median of 10 d (IQR, 5–16) to 7 d (IQR, 4–11; $P = 0.0004$) independent of its preventive effect on bloodstream infections.

Serum MBL concentrations

Upon ICU admission, the average serum MBL concentration was 1019 μg/liter (median, 726 μg/liter; IQR, 245-1520), which is comparable with the level documented in healthy Danish and British subjects (6, 11). Admission MBL concentrations were identical in both insulin therapy groups [724 μg/liter (IQR, 255-1520) in the intensive insulin-treated group and 820 μg/liter (IQR, 241-1518) in the conventionally treated group; $P = 0.96$]. Also, the number of patients with a baseline MBL level below 500 μg/liter and the number with baseline MBL below 50 μg/liter was equal in both study groups (41% and 9.8%, respectively, in the intensive insulin group and 40% and 8.9%, respectively, in the conventionally treated group; $P = 0.9$ and $P = 0.8$, respectively).

Overall, baseline MBL levels tended to be lower in nonsurvivors (median, 550 μg/liter; IQR, 190-1390) than in survivors (median, 738 μg/liter; IQR, 260-1675; $P = 0.13$). In a subanalysis of the 243 conventionally treated patients, nonsurvivors revealed significantly lower baseline MBL concentrations compared with survivors [387 μg/liter (IQR, 190-1289) *vs.* 897 μg/liter (IQR, 246-1686), respectively; $P = 0.04$; Table 1]. The association between low levels of MBL and increased risk of death was even stronger on d 5 ($P = 0.012$) and on the last day in the ICU ($P = 0.002$). Likewise, the

TABLE 1. Serial measurements of MBL and CRP concentrations in the two treatment groups

		Intensive insulin therapy			Conventional treatment		
		Died in ICU	ICU survivor	*P*	Died in ICU	ICU survivor	*P*
All patients		n = 22	n = 186		n = 49	n = 194	
MBL concentrations [μg/liter, median (IQR)]	Day 1	1170 (170–1593)	705 (268–1520)	0.79	387 (190–1287)	897 (246–1686)	0.045
	Day 5	1295 (269–1960)	1220 (275–2113)	0.97	460 (158–2140)	1321 (346–2706)	0.012
	Last day	1308 (331–3615)	1567 (370–2971)	1.0	990 (240–2408)	1960 (569–3848)	0.002
CRP concentrations [mg/liter, median (IQR)]	Day 1	111 (76–163)	135 (62–217)	0.78	129 (57–229)	137 (75–211)	0.37
	Day 5	185 (108–225)	137 (73–196)	0.055	161 (99–275)	148 (98–217)	0.14
	Last day	167 (113–208)	56 (26–94)	<0.0001	137 (94–175)	66 (34–102)	<0.0001
Patients with bacteremia		n = 4	n = 28		n = 16	n = 43	
MBL concentrations [μg/liter, median (IQR)]	Day 1	1135 (417–1440)	765 (215–1724)	0.89	320 (11–1259)	1035 (335–1825)	0.090
	Day 5	1183 (647–1394)	1053 (184–1814)	0.93	680 (14–2293)	2285 (488–3925)	0.067
	Last day	1479 (597–2979)	1273 (374–2142)	0.76	330 (26–1888)	2550 (700–2550)	0.008
CRP concentrations [mg/liter, median (IQR)]	Day 1	151 (54–161)	121 (66–178)	0.65	138 (61–231)	169 (71–210)	0.64
	Day 5	137 (116–191)	111 (60–201)	0.42	131 (64–224)	147 (105–193)	0.67
	Last day	145 (88–187)	37 (25–86)	0.04	140 (89–158)	54 (28–114)	0.006

Downloaded from https://academic.oup.com/jcem/article/88/3/1082/2845194 by guest on 20 August 2022

fraction of conventionally treated patients with MBL concentrations below 500 μg/liter was 54% among nonsurvivors compared with 36% among survivors ($P = 0.02$).

However, multivariate logistic regression analysis including all patients and corrected for all upon admission risk factors revealed that insulin therapy (adjusted odds ratio, 0.52; 95% confidence interval, 0.28–0.95; $P < 0.04$), but not baseline MBL concentration (per 100 μg/liter MBL added: adjusted odds ratio, 0.990; 95% confidence interval, 0.950–1.020; $P = 0.4$) significantly determined outcome. Also, there was no relation between the cause of death and serum MBL concentrations at any time point (data not shown). Likewise, insulin therapy schedule, but not baseline MBL concentration, significantly determined the incidence of bacteremia, acute renal failure and polyneuropathy, duration of antibiotic treatment, and number of days during which leukopenia/leukocytosis or hypo-/hyperthermia was present, as indicated by multivariate logistic regression analysis.

In all patients regardless of treatment group, MBL concentrations increased significantly with time in intensive care ($P < 0.0001$; Fig. 2). This rise was independent of the baseline MBL concentration and was mostly attributable to the survivors, with the highest relative increases in serum MBL concentrations observed in the conventionally treated survivors. Intensive insulin therapy blunted the rise in serum MBL levels significantly at all time points (Fig. 3). However, the beneficial effect of insulin on mortality and morbidity was not significantly related to the suppressive effect on serum MBL concentrations, as indicated by multivariate logistic regression analysis (Table 2). The suppressive effect of insulin on MBL levels was present even among survivors without bloodstream infections (n = 155 in the intensive treatment group and n = 145 in the conventional treatment group; $P < 0.05$ at all time points; data not shown). Regardless of treatment group, patients who developed bacteremia revealed a lower relative increase in MBL levels on d 15 compared with those who did not develop bacteremia ($P \leq 0.02$).

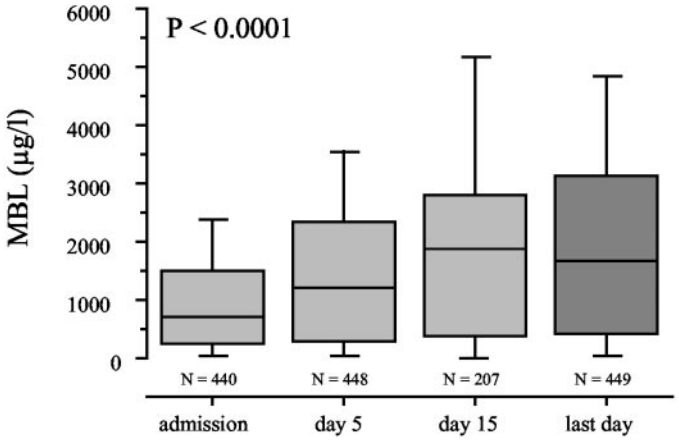

FIG. 2. Serial measurements of MBL concentrations in patients receiving prolonged (>5 d) intensive care. *Bars* represents medians, *boxes* indicate IQRs, and *whiskers* show the 10th and 90th percentiles. *P* values refer to Friedman's test for several related samples.

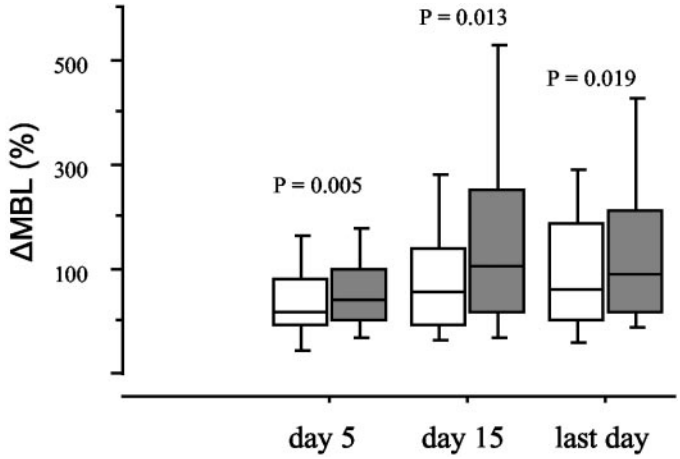

FIG. 3. Relative change in MBL concentrations from d 1 (Δ%) in patients receiving intensive insulin therapy (□) or conventional therapy (▨). *P* values refer to between-group comparison with Mann-Whitney *U* test.

Serum CRP concentrations

Serum CRP concentrations upon ICU admission were equally elevated in both study groups [median 132 μg/liter; interquartile range, 64–217) in the intensive insulin group and median 135 μg/liter (interquartile range, 70–214) in the conventionally treated group; $P = 0.8$] and were equal in survivors and nonsurvivors. It was the insulin therapy group, and not the baseline CRP level, that significantly determined the risk of bacteremia, polyneuropathy, acute renal failure, and mortality, as indicated by multivariate logistic regression analysis. Both a high baseline CRP level and being randomized to the conventional insulin therapy group significantly and independently increased the risk of prolonged (>10 d) antibiotic treatment and of prolonged inflammation, as indicated by multivariate logistic regression analysis.

The acute phase CRP response started to level off after d 5 in both groups (Fig. 4). Intensive insulin therapy significantly suppressed serum CRP concentrations at all time points (Fig. 4), an effect that was also present in those patients who did not develop bacteremia. The suppressive effect of intensive insulin therapy on CRP after d 5 at least partially explained its beneficial effect on acute renal failure and mortality, but not polyneuropathy (Table 2), as indicated by multivariate logistic regression analysis, which is in contrast to the effect of insulin on serum MBL concentrations. Indeed, after correction for other risk factors and compared with a CRP level of less than 50 mg/liter, the relative risk of death increased 41-fold (95% confidence interval, 11–160) for a CRP concentration exceeding 200 mg/liter on the last day of intensive care ($P < 0.0001$; Fig. 5). On d 15 and on the last day, nonsurvivors in both study groups had equally high CRP levels, which were significantly higher than the CRP levels in conventionally and intensively insulin-treated survivors, with the suppressive effect of insulin on CRP levels also present among survivors only (Table 1).

There was a positive correlation between the number of days on which a substantially elevated CRP level (>150 mg/liter) was noted and the number of days with leukope-

Downloaded from https://academic.oup.com/jcem/article/88/3/1082/2845194 by guest on 20 August 2022

TABLE 2. Multivariate logistic regression analysis of the impact of changes in MBL and CRP on the last day of intensive care, corrected for other risk factors of morbidity and mortality, including the randomized insulin therapy group

	OR	95% CI	*P*
ICU mortality			
Other than cardiac surgery	1.377	0.541–3.504	0.5
Age (per added year)	1.026	0.995–1.058	0.1
Delayed admission	0.480	0.198–1.162	0.1
History of diabetes	0.628	0.184–2.148	0.5
Admission hyperglycemia (>11 mmol/liter)	1.799	0.720–4.495	0.2
History of malignancy	1.683	0.690–4.103	0.3
First 24-h APACHE-II (per unit)	1.083	1.009–1.163	0.03
Intensive insulin therapy	0.738	0.347–1.569	0.4
Relative change in MBL (per % added)	1.000	0.998–1.002	0.9
CRP (per 50 mg/liter added)	1.900	1.600–2.540	<0.0001
Acute renal failure			
Other than cardiac surgery	0.912	0.470–1.768	0.8
Age (per added year)	1.003	0.984–1.022	0.8
Delayed admission	0.712	0.385–1.316	0.3
History of diabetes	0.982	0.424–2.275	1.0
Admission hyperglycemia (> 11 mmol/liter)	1.193	0.590–2.413	0.6
History of malignancy	0.788	0.379–1.635	0.5
First 24-h APACHE-II (per unit)	1.127	1.067–1.191	<0.0001
Intensive insulin therapy	0.717	0.416–1.233	0.2
Relative change in MBL (per % added)	1.001	1.000–1.002	0.2
CRP (per 50 mg/liter added)	1.250	1.050–1.400	0.02
Critical illness polyneuropathy			
Other than cardiac surgery	1.171	0.664–2.066	0.6
Age (per added year)	1.005	0.990–1.021	0.5
Delayed admission	1.305	0.799–2.131	0.3
History of diabetes	0.566	0.262–1.222	0.2
Admission hyperglycemia (> 11 mmol/liter)	1.041	0.556–1.951	0.9
History of malignancy	1.039	0.595–1.816	0.9
First 24-h APACHE-II (per unit)	1.052	1.006–1.100	0.03
Intensive insulin therapy	0.363	0.232–0.572	<0.0001
Relative change in MBL (per % added)	1.001	1.000–1.002	0.05
CRP (per 50 mg/liter added)	0.996	0.992–1.000	0.04

APACHE II denotes Acute Physiology and Chronic Health Evaluation and is an indicator of severity of illness, with higher values indicating more severe illness. The prevention of acute renal failure and mortality with intensive insulin therapy appears largely mediated by its effect on CRP, but not on MBL. Insulin-induced prevention of polyneuropathy involves other pathways besides its effect on inflammation. OR, Odds ratio; CI, confidence interval.

nia/leukocytosis ($\rho = 0.43$; $P < 0.0001$) and hypo-/hyperthermia ($\rho = 0.46$; $P < 0.0001$). There were no significant correlations between the relative changes in MBL and CRP from baseline at any time point.

Discussion

Protracted critical illness is associated with substantial metabolic and immunological derangements and a high risk of death (21). Here, we have documented strong antiinflammatory effects of intensive insulin therapy in prolonged critically ill patients, as reflected by reduced duration of leukocytosis/leukopenia and hypo-/hyperthermia, and suppression of the acute phase responses of MBL and CRP, independent of its effect on severe infections. We also found that the beneficial effects of intensive insulin therapy on organ failure and mortality were at least partially linked to the antiinflammatory effects, more specifically to the lowering of circulating CRP concentrations. In addition, we observed that low on-admission concentrations of MBL may predict a poor outcome among patients treated with conventional intensive care, but that the adverse effects of low baseline MBL levels can be overcome by intensive insulin therapy.

More than two thirds of patients admitted to intensive care units develop signs of the systemic inflammatory response syndrome (22), caused by either infection or tissue damage, and a substantial number of these patients progress to shock and multiple organ failure. Stress hyperglycemia is a prominent feature in critically ill patients. Maintaining normoglycemia with insulin significantly prevented multiple organ failure and bloodstream infections, and shortened the duration of antibiotic treatment, the number of days with leukopenia/leukocytosis, and the duration of hypo-/hyperthermia; together these resulted in less lethality. Whether these beneficial effects are attributable to the prevention of hyperglycemia, the increased availability of insulin, or a combination of both remains speculative. Multivariate logistic regression analysis revealed that the antiinflammatory effect of intensive insulin therapy, as indicated by the lowering of circulating CRP, to a large extent explained the prevention of acute renal failure and mortality. Although, theoretically, suppression of the acute phase CRP response with insulin could be a secondary phenomenon to its effect on severe infections and mortality, it was present even among uninfected survivors, which points to a direct antiinflammatory

Downloaded from https://academic.oup.com/jcem/article/88/3/1082/2845194 by guest on 20 August 2022

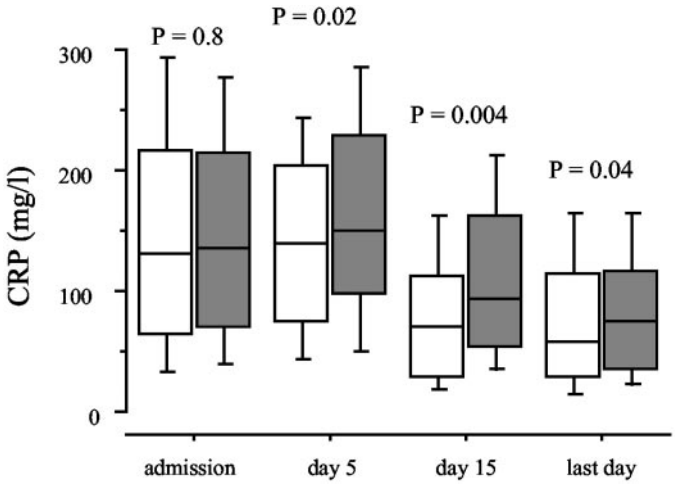

FIG. 4. Serial measurements of CRP concentrations in patients receiving intensive insulin therapy (□) or conventional treatment (▨). *P* values refer to between-group comparison with Mann-Whitney *U* test.

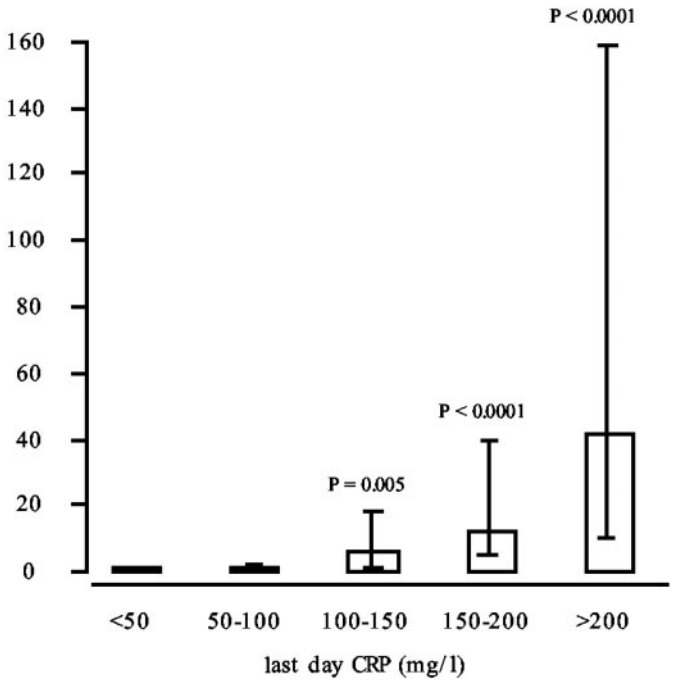

FIG. 5. Relative risk of death with increasing serum CRP concentration on the last of intensive care, adjusted for reason for ICU admission, delayed admission, history of diabetes and malignancy, hyperglycemia upon admission, severity of illness, relative change in MBL level on the last day of intensive care, and randomized insulin therapy group, as estimated by multivariate logistic regression analysis. Data are presented as adjusted odds ratios and 95% confidence intervals.

effect. In rat hepatoma cells, insulin has been shown to inhibit cytokine-induced transcription of acute phase proteins (23). However, in the current study, the direct hepatic effects of insulin cannot with certainty be distinguished from its effects on glycemic control, as both occurred concomitantly. In patients with type II diabetes, treatment with insulin, but not improved glycemic control *per se*, has been shown to reduce circulating CRP (24), which is in favor of a direct effect of insulin on the hepatic acute phase response. Insulin is indeed emerging as a molecule with strong antiinflammatory properties, suppressing the generation of a range of early proinflammatory substances, including TNFα, macrophage migration inhibitory factor, superoxide anions, and intranuclear nuclear factor-κB (25, 26). A recent study, however, showed that short-term hyperinsulinemia *per se* can also induce proinflammatory responses in euglycemic healthy volunteers (27). In addition, hyperglycemia has been shown to exert direct proinflammatory effects in nondiabetic rats (28).

A number of publications have reported a possible association between low levels of MBL and increased risk of infections, particularly in patients who are immunocompromised, such as children with immature antibody repertoire (15, 16), patients with acquired immunodeficiency syndrome (29), or patients with malignancies receiving chemotherapy or stem cell transplantation (17, 18, 30). Only 1 report of unusual and severe infections in 5 adults with no other known immune deficiency suggested that MBL deficiency may confer a life-long risk of infection (31). The current subanalysis of the 243 conventionally treated patients revealed that MBL levels on admission were almost 3 times higher in survivors as in nonsurvivors, in favor of a vulnerability associated with low MBL levels in critically ill patients who are presumably immunocompetent. Intensive insulin therapy, however, not only reduced the risk of severe infections and lethality, but also overruled the increased vulnerability associated with low MBL levels, which may point to a common pathway. Indeed, although a selection bias cannot be totally ruled out, our observations suggest that the conventionally treated, critically ill patients, because of hyperglycemia and/or lack of insulin effect, may be less immunocompetent than the intensive insulin-treated group, and in that situation a low MBL level does seem to predispose to adverse outcome. The association between low levels of MBL and outcome of conventionally treated ICU patients was not restricted to severe MBL deficiency, but was evident even when using a concentration of less than 500 μg/liter as a cut-off level for functional MBL deficiency, as previously suggested by Peterslund *et al.* (18).

MBL is known to activate complement after binding to a broad range of pathogens (32), and it seems plausible that MBL may protect against sepsis and multiple organ failure through early neutralization of invading microorganisms. However, the exact antimicrobial mechanism of MBL probably depends on the nature of the infecting organism and may involve enhanced phagocytosis through deposition of complement factors on the surface of the microorganism or direct destruction through the membrane attack complex (C5b-9) (6). As intensive insulin therapy overcomes the adverse effects of a low baseline MBL level, immune enhancement through similar mechanisms may be operative.

The high prevalence of point mutations in the MBL gene has been interpreted as evidence of some biological advantage associated with low circulating MBL (6). Recent studies have shown that MBL mediates complement activation after endothelial hypoxia, thereby potentially aggravating the resulting postischemic injury (33). Likewise, it has been suggested that CRP directly participates in local inflammatory processes in infarcted tissues during sepsis (34). Consequently, suppression of the acute phase response of CRP and

Downloaded from https://academic.oup.com/jcem/article/88/3/1082/2845194 by guest on 20 August 2022

MBL may partially mediate the advantageous effects of intensive insulin therapy in critically ill patients with ischemic injury and of glucose-insulin-potassium infusion in patients after acute myocardial infarction (35, 36).

In conclusion, we found that intensive insulin therapy suppresses the hepatic acute phase response, as indicated by circulating CRP levels, and that this antiinflammatory property at least partially explains the beneficial effects on organ failure and mortality in surgical critically ill patients. Low levels of MBL may predict a poor outcome in protracted critical illness, but the unfavorable effects of low baseline MBL can be neutralized by intensive insulin therapy.

Acknowledgments

We thank Ilse Milants, Jenny Gielens, An Andries, Myriam Vandenbergh, Vivian Celis, Lisbeth Jensen, and Annette Hansen for expert technical assistance.

Received September 23, 2002. Accepted December 3, 2002.

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

This work was supported by research grants from the Belgian Fund for Scientific Research (G. 0144.00 and G.3C05.95N, to G.V.d.B.), the Research Council of the University of Leuven (OT 99/32, to G.V.d.B.), the Belgian Foundation for Research in Congenital Heart Diseases (to G.V.d.B.), and the Danish Research Council (Grant 960082, to T.K.H.).

S.T. is a cofounder of NatImmune A/S, a biotech company producing recombinant MBL. The current research did not receive support from NatImmune.

J.S.C. is a recipient of an unrestrictive research grant from Novo Nordisk, Denmark.

G.V.d.B. is a holder of an unrestrictive research chair from Novo Nordisk, Denmark.

References

1. **Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R** 2001 Intensive insulin therapy in the critically ill patients. N Engl J Med 19:1359–1367
2. **Takala J, Ruokonen E, Webster NR, Nielsen MS, Zandstra DF, Vundelinckx G, Hinds CJ** 1999 Increased mortality associated with growth hormone treatment in critically ill adults. N Engl J Med 11:785–792
3. **Wheeler AP, Bernard GR** 1999 Treating patients with severe sepsis. N Engl J Med 3:207–214
4. **Gabay C, Kushner I** 1999 Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 6:448–454
5. **Szalai AJ, Agrawal A, Greenhough TJ, Volanakis JE** 1997 C-reactive protein: structural biology, gene expression, and host defense function. Immunol Res 2:127–136
6. **Turner MW, Hamvas RM** 2000 Mannose-binding lectin: structure, function, genetics and disease associations. Rev Immunogenet 3:305–322
7. **Matsushita M, Fujita T** 1992 Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. J Exp Med 6:1497–1502
8. **Thiel S, Vorup-Jensen T, Stover CM, Schwaeble W, Laursen SB, Poulsen K, Willis AC, Eggleton P, Hansen S, Holmskov U, Reid KB, Jensenius JC** 1997 A second serine protease associated with mannan-binding lectin that activates complement. Nature 6624:506–510
9. **Dahl MR, Thiel S, Matsushita M, Fujita T, Willis AC, Christensen T, Vorup-Jensen T, Jensenius JC** 2001 MASP-3 and its association with distinct complexes of the mannan-binding lectin complement activation pathway. Immunity 1:127–135
10. **Steffensen R, Thiel S, Varming K, Jersild C, Jensenius JC** 2000 Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. J Immunol Methods 241:33–42
11. **Hansen TK, Thiel S, Dall R, Rosenfalck AM, Trainer P, Flyvbjerg A, Jorgensen JO, Christiansen JS** 2001 GH strongly affects serum concentrations of mannan-binding lectin: evidence for a new IGF-I independent immunomodulatory effect of GH. J Clin Endocrinol Metab 11:5383–5388
12. **Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC** 1992 The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol 1:31–35
13. **Super M, Thiel S, Lu J, Levinsky RJ, Turner MW** 1989 Association of low levels of mannan-binding protein with a common defect of opsonisation. Lancet 2:1236–1239
14. **Koch A, Melbye M, Sorensen P, Homoe P, Madsen HO, Molbak K, Hansen CH, Andersen LH, Hahn GW, Garred P** 2001 Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA 10:1316–1321
15. **Summerfield JA, Sumiya M, Levin M, Turner MW** 1997 Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. Br Med J 7089:1229–1232
16. **Garred P, Madsen HO, Hofmann B, Svejgaard A** 1995 Increased frequency of homozygosity of abnormal mannan-binding-protein alleles in patients with suspected immunodeficiency. Lancet 8980:941–943
17. **Neth O, Hann I, Turner MW, Klein NJ** 2001 Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. Lancet 357:614–618
18. **Peterslund NA, Koch C, Jensenius JC, Thiel S** 2001 Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. Lancet 358:637–638
19. **Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, Reller LB** 1997 The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 4:584–602
20. **Thiel S, Moller Kristensen M, Jensen L, Jensenius JC** 2002 Assays for the functional activity of the mannan-binding lectin pathway of complement activation. Immunobiology 205:446–454
21. **Van den Berghe G** 2000 Novel insights into the neuroendocrinology of critical illness. Eur J Endocrinol 1:1–13
22. **Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP** 1995 The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study. JAMA 2:117–123
23. **Campos SP, Baumann H** 1992 Insulin is a prominent modulator of the cytokine-stimulated expression of acute-phase plasma protein genes. Mol Cell Biol 4:1789–1797
24. **Yudkin JS, Panahloo A, Stehouwer C, Emeis JJ, Bulmer K, Mohamed-Ali V, Denver AE** 2000 The influence of improved glycaemic control with insulin and sulphonylureas on acute phase and endothelial markers in Type II diabetic subjects. Diabetologia 9:1099–1106
25. **Das UN** 2001 Is insulin an antiinflammatory molecule? Nutrition 5:409–413
26. **Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S** 2001 Insulin inhibits intranuclear nuclear factor κB and stimulates IκB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab 7:3257–3265
27. **Soop M, Duxbury H, Agwunobi AO, Gibson JM, Hopkins SJ, Childs C, Cooper RG, Maycock P, Little RA, Carlson GL** 2002 Euglycemic hyperinsulinemia augments the cytokine and endocrine responses to endotoxin in humans. Am J Physiol 6:E1276–E1285
28. **Kwoun MO, Ling PR, Lydon E, Imrich A, Qu Z, Palombo J, Bistrian BR** 1997 Immunologic effects of acute hyperglycemia in nondiabetic rats. J Parenter Enteral Nutr 2:91–95
29. **Garred P, Madsen HO, Balslev U, Hofmann B, Pedersen C, Gerstoft J, Svejgaard A** 1997 Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. Lancet 349:236–240
30. **Mullighan CG, Heatley S, Doherty K, Szabo F, Grigg A, Hughes TP, Schwarer AP, Szer J, Tait BD, Bik To L, Bardy PG** 2002 Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation. Blood 10:3524–3529
31. **Summerfield JA, Ryder S, Sumiya M, Thursz M, Gorchein A, Monteil MA, Turner MW** 1995 Mannose binding protein gene mutations associated with unusual and severe infections in adults. Lancet 345:886–889
32. **Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW** 2000 Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect Immun 2:688–693
33. **Jordan JE, Montalto MC, Stahl GL** 2001 Inhibition of mannose-binding lectin reduces postischemic myocardial reperfusion injury. Circulation 12:1413–1418
34. **Baidoshvili A, Nijmeijer R, Lagrand WK, Hack CE, Niessen HW** 2002 Localisation of C reactive protein in infarcted tissue sites of multiple organs during sepsis. J Clin Pathol 2:152–153
35. **Diaz R, Paolasso EA, Piegas LS, Tajer CD, Moreno MG, Corvalan R, Isea JE, Romero G** 1998 Metabolic modulation of acute myocardial infarction. The ECLA (Estudios Cardiologicos Latinoamerica) Collaborative Group. Circulation 21:2227–2234
36. **Malmberg K** 1997 Prospective randomised study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus. DIGAMI (Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction) Study Group. Br Med J 7093:1512–1515

Downloaded from https://academic.oup.com/jcem/article/88/3/1082/2845194 by guest on 20 August 2022