Alterations in Adipose Tissue during Critical Illness

An Adaptive and Protective Response?

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***Rationale*: Critical illness is characterized by lean tissue wasting, whereas adipose tissue is preserved. Overweight and obese critically ill patients may have a lower risk of death than lean patients, suggestive of a protective role for adipose tissue during illness.**
***Objectives*: To investigate whether adipose tissue could protectively respond to critical illness by storing potentially toxic metabolites, such as excess circulating glucose and triglycerides.**
***Methods*: We studied adipose tissue morphology and metabolic activity markers in postmortem biopsies of 61 critically ill patients and 20 matched control subjects. Adipose morphology was also studied in *in vivo* biopsies of 27 patients and in a rabbit model of critical illness ($n = 22$).**
***Measurements and Main Results*: Adipose tissue from critically ill patients revealed a higher number and a smaller size of adipocytes and increased preadipocyte marker levels as compared with control subjects. Virtually all adipose biopsies from critically ill patients displayed positive macrophage staining. The animal model demonstrated similar changes. Glucose transporter levels and glucose content were increased. Glucokinase expression was up-regulated, whereas glycogen and glucose-6-phosphate levels were low. Acetyl CoA carboxylase protein and fatty acid synthase activity were increased. Hormone-sensitive lipase activity was not altered, whereas lipoprotein lipase activity was increased. A substantially increased AMP-activated protein kinase activity may play a crucial role.**
***Conclusions*: Postmortem adipose tissue biopsies from critically ill patients displayed a larger number of small adipocytes in response to critical illness, revealing an increased ability to take up circulating glucose and triglycerides. Similar morphologic changes were present *in vivo*. Such changes may render adipose tissue biologically active as a functional storage depot for potentially toxic metabolites, thereby contributing to survival.**

Keywords: intensive care; adipose tissue; lipogenesis; lipolysis

AT A GLANCE COMMENTARY

What This Study Adds to the Field

Adipose tissue is substantially altered during critical illness, with an increased number of newly formed adipocytes, and is infiltrated by macrophages. These adipocytes have an increased ability to store glucose and triglycerides, possibly reducing detrimental effects of high levels of these circulating metabolites.

Critically ill patients requiring prolonged intensive care are characterized by a profound decrease of lean body mass, evoking weakness and impairing rehabilitation, whereas, paradoxically, adipose tissue is preserved (1, 2). Remarkably, overweight (body mass index [BMI], 25–30 kg/m^2) and obese (BMI, 30–40 kg/m^2) critically ill patients have recently been shown to have a lower risk of death than patients with a normal BMI (3–5). Morbid obesity (BMI $\geqslant$ 40 kg/m^2), however, is an independent risk factor for death in the intensive care unit (ICU) (4). These observations suggest that adipose tissue may play a protective role during severe illness in the intensive care setting.

The primary metabolic role of adipose tissue is to store excess energy as triglycerides. This excess energy mainly originates from circulating lipids, which are taken up by adipocytes via action of lipoprotein lipase (LPL). A much smaller part of the stored triglycerides in adipocytes is *de novo* synthesized from circulating carbohydrates through lipogenesis (6). Critically ill patients suffer from dyslipidemia and hyperglycemia, partly due to increased hepatic lipogenesis (7) and gluconeogenesis (8). The severity of these alterations is associated with adverse outcome (9–11). Moreover, elevated circulating glucose (12–14) levels as well as high levels of triglycerides (15–17) can aggravate vital organ dysfunction during critical illness. We hypothesized that adipose tissue can respond to illness by increasing its storage properties for such circulating toxic metabolites, whereby it may enhance the chances of survival. To test this hypothesis, we obtained abdominal subcutaneous and omental adipose tissue biopsies from 61 nonsurviving critically ill patients with a BMI ranging from 16.3 to 31.2 kg/m^2, and compared these with biopsies from 20 matched control subjects undergoing elective abdominal surgery. More specifically, we studied adipose tissue morphology as well as its ability to take up and metabolize glucose and triglycerides. In addition, we studied adipose tissue morphological changes in response to critical illness in *in vivo* subcutaneous limb adipose tissue from 27 critically ill patients, and in the controlled setting of an animal model.

Some of the results of this study have been previously reported in the form of an abstract (18, 19).

METHODS

Patients

Patients were all enrolled in a large prospective, randomized, controlled study on the effects of intensive insulin therapy on outcome of

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critical illness (20). The detailed protocol of the study has been previously published (20). In brief, patients who had been randomly assigned to conventional insulin therapy received insulin only when glucose concentrations exceeded 215 mg/dl, resulting in mean blood glucose of 157 mg/dl (hyperglycemia). Intensive insulin therapy targeted blood glucose levels between 80 and 110 mg/dl, which resulted in mean blood glucose of 110 mg/dl (normoglycemia). Glucose control in each group was maintained according to protocol until ICU discharge or death. Postmortem biopsy samples of abdominal subcutaneous and omental adipose tissue were harvested within minutes after death from 61 long-stay patients of the 266 long-stay patients who died in the ICU, during weekdays, after consent, and when sufficient technical support was available. For postmortem tissue sampling, for academic purposes, each patient or his/her legal representative consented upon admission, via a hospital-wide information and consent procedure that requires active opting-out when not consenting. Opting-out remained possible until time of death. This strategy was approved by the K.U. Leuven Institutional Ethical Review Board (internal identification number ML 1820). The baseline and outcome characteristics of the critically ill patients from whom postmortem adipose tissue biopsies were collected are described in Table 1. From 27 long-stay patients, we collected qualitative *in vivo* subcutaneous adipose tissue biopsies from the vastus lateralis region on Day 15 of their ICU stay. The *in vivo* biopsies were taken after specific written informed consent from the patient or his/her legal representative. The baseline and outcome characteristics of the critically ill patients from whom *in vivo* adipose tissue biopsies were collected are described in the online supplement. All baseline characteristics were comparable for the two insulin treatment groups.

For comparison, we also collected abdominal subcutaneous and omental adipose tissue biopsy samples from demographically matched patients who were not critically ill, and who underwent elective abdominal surgery for restorative rectal resection (ML 2707; *see* online supplement). These patients provided written informed consent prior to the procedure.

The protocols and all consent forms were approved by the K.U. Leuven Institutional Ethical Review Board (internal identification numbers ML 1820 and ML 2707). All tissue samples were snap frozen in liquid nitrogen and stored at −80°C until analysis.

Gene and Protein Expression

Messenger RNA was isolated and cDNA quantified in real time as described previously (12). Data are expressed normalized to glyceraldehyde 3-phosphate dehydrogenase expression and as a fold change of the mean of the control patients. A complete list of primers and probes is provided in the online supplement. Western blots were performed as described in the online supplement.

Glucose and Triglyceride Tissue Content

Spectrophotometric assays were used to quantify the tissue levels of glucose, glucose-6-phosphate, glycogen, and glycerol levels, the latter in Bligh-Dyer extracts of tissues (21).

Fatty Acid Synthase, Lipase, and AMP-activated Protein Kinase Activity Assays

For fatty acid synthase (FAS) activity, reaction mix containing reduced nicotinamide adenine dinucleotide phosphate, acetyl CoA, malonyl CoA, and [2–14C]malonyl CoA was added to tissue homogenates. After incubation and extraction of the fatty acids, incorporation of [2–14C]malonyl CoA was analyzed by scintillation counting. The kinase assay for AMP-activated protein kinase (AMPK) activity has been previously described (22), and was determined by the amount of ^{32}P incorporation into an AMPK substrate. The detailed protocol is described in the online supplement. Total lipase activity was determined with the commercial Confluolip kit (Progen Biotechnik, Heidelberg, Germany).

Circulating Parameters

Serum levels of insulin were measured by ELISA (Invitrogen, Merelbeke, Belgium). Serum triglyceride levels were measured by a spectrophotometric assay, as described previously here. Samples were all run in duplicate.

Rabbit Model of Critical Illness

The experiment was performed in our previously validated rabbit model of prolonged critical illness, which has shown to mimic the characteristic endocrine, biochemical, and immunological disturbances

TABLE 1. CHARACTERISTICS OF PATIENTS FROM WHOM POSTMORTEM BIOPSIES WERE STUDIED

Characteristics	Conventional Insulin (*n* = 33)	Intensive Insulin (*n* = 28)	*P* Value
Male sex, no.	19	17	0.80
Age, yr*	64 ± 17	70 ± 11	0.14
BMI, kg/m²*	23.5 ± 3.6	24.8 ± 3.3	0.12
History of diabetes, no.	4	5	0.53
APACHE-II score on admission†	26 (22–35)	28 (20–32)	0.98
SOFA score on admission†	10 (7–14)	8 (5–12)	0.10
Blood glucose on admission, mg/dl*	167.5 ± 73.7	174.5 ± 75.9	0.71
Days in ICU†	10 (6–21.5)	10 (7–15.5)	0.95
Diagnostic group upon admission			0.69
Cardiovascular	3	2	
Gastrointestinal or liver	4	1	
Hematologic or oncologic	7	3	
Neurologic	1	2	
Renal	1	1	
Respiratory	15	15	
Other	2	4	
Cause of death in ICU			0.37
Severe brain damage	2	0	
Respiratory failure	13	13	
Therapy resistant septic shock/cardiovascular collapse	8	6	
Persistent MOF after septic or SIRS induced shock	10	9	
CRP level on biopsy day, mg/L†	178 (61–235)	152 (73–268)	0.8
Time lag, time of death to biopsy preservation, min*	14.5 ± 8.3	19.5 ± 12.7	0.07

Definition of abbreviations: APACHE = Acute Physiology and Chronic Health Assessment Evaluation; BMI = body mass index; CRP = C-reactive protein; ICU = intensive care unit; SOFA = Sequential Organ Failure Assessment (higher scores indicate more severe illness); MOF = Multiple Organ Failure; SIRS = systemic inflammatory response syndrome.

* Data presented are mean ± SD.

† Data presented are median (interquartile range).

of human critical illness (23). For the purpose of this study, the protocol was slightly modified. We sampled subcutaneous adipose tissue biopsies before randomization and again after 7 days. Rabbits were randomized into two groups: 7 sham-operated animals, and 15 critically ill animals. The detailed protocol is described in the online supplement.

Adipocyte Morphology

For quantification of adipocyte size, sections were stained with hematoxylin and eosin, and adipocyte size was determined by computer image analysis. For macrophage evaluation, sections were stained with a primary anti-macrophage antibody, and counterstained with hematoxylin. Adipose tissue sections were evaluated in a blinded fashion. A detailed protocol is described in the online supplement.

Statistical Analysis

We used analysis of variance and unpaired t test for normally distributed data and the nonparametric Kruskal-Wallis and Mann-Whitney U test when data appeared to be not normally distributed. We used χ^2 test for comparison of proportions. We used Wilcoxon's signed ranked paired analysis for the rabbit data. The significance of correlations was assessed by calculation of the Pearson (r) correlation coefficient. P values of 0.05 or less were considered statistically significant.

RESULTS

Adipocyte Morphology and Macrophage Staining

Adipocyte cell size of both subcutaneous and omental adipose tissue of critically ill patients was smaller than that of healthy control subjects. This was revealed by a smaller median cell area (Figure 1A). The fraction of small adipocytes in postmortem subcutaneous adipose tissue (defined as cell area below the 25th percentile of those in healthy control subjects [400 μm^2]) was increased to 33.4% in biopsies from critically ill patients (P = 0.008). A comparable amount (30.5%, ranging from 21.8 to 43.9%) of small adipocytes was observed in *in vivo* harvested subcutaneous adipose tissue biopsies (n = 27). In omental postmortem adipose tissue, 41.4% of the adipocytes in the critically ill biopsies were small (defined as cell area smaller than the 25th percentile of the healthy control subjects [500 μm^2]), which was significantly more than in control subjects ($P < 0.003$). Total protein content per gram tissue was similar in critically ill and control biopsies (*see* the online supplement). Gene expression levels of perilipin, a protein coating the lipid droplet in adipocytes, was lower in subcutaneous and omental postmortem adipose tissue of critically ill patients than in control subjects (Figure 1B). In contrast, protein levels of the pre-adipocyte marker, preadipocyte factor (Pref)–1, was higher in biopsies from critically ill patients than in control subjects (Figure 1C). We stained sections of adipose tissue for the macrophage marker, CD68 (Figure 1D). In both subcutaneous adipose tissue and omental adipose tissue biopsies of healthy control subjects, two-thirds stained negative, whereas, in one-third of the biopsies, some positive staining was present. This was in sharp contrast with the postmortem biopsies from critically ill patients, where 100% (subcutaneous adipose tissue, $P < 0.0001$) and 95% (omental adipose tissue, $P < 0.0001$) stained positive for CD68. Furthermore, in two-thirds of the biopsies of critically ill patients,

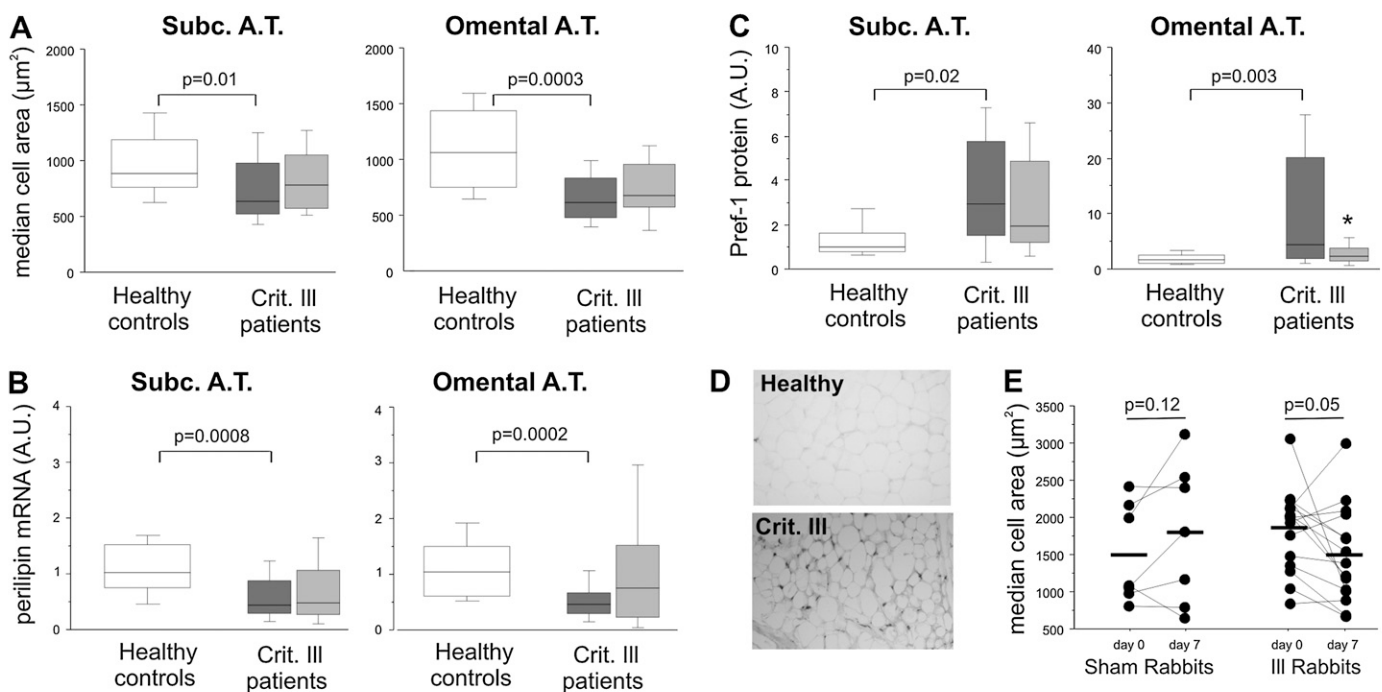

Figure 1. Human adipocyte morphology in critical illness in postmortem biopsies (*A–D*) and paired *in vivo* analysis of adipocyte cell size in a rabbit model of critical illness (*E*). (*A*) Median adipocyte cell area, (*B*) mRNA adipose tissue (A.T.) levels of perilipin, (*C*) protein A.T. levels of preadipocyte factor (Pref)–1, (*D*) CD68 macrophage staining. *Data boxes* represent median and interquartile range; whiskers represent the 10th and the 90th percentiles. *White boxes* represent healthy control subjects undergoing elective surgery (n = 20); *dark gray boxes* represent critically ill patients who received conventional insulin therapy (n = 33); *light gray boxes* represent critically ill patients who received intensive insulin therapy (n = 28). *Lines* and *P* values indicate difference between healthy and conventionally treated critically ill patients. *Different from conventional insulin therapy ($P \leq 0.05$). A.U. = arbitrary units. (*E*) Paired median adipocyte cell area in sham-treated healthy rabbits (n = 7) and critically ill rabbits (n = 15). Subcutaneous A.T. biopsies were taken at Day 0 (before randomization) and after 7 days. At Day 7, A.T. biopsies from two healthy rabbits and eight sick rabbits stained positive for macrophages. A.U. = arbitrary units; Subc. = subcutaneous.

the number of CD68$^+$ cells was high. CD68$^+$ staining was also observed in the 27 *in vivo* subcutaneous limb adipose tissue biopsies of prolonged critically ill patients. All of the 15 *in vivo* biopsies of conventional patients stained positive for macrophages, and, in 73% of these, the number of positively staining cells was high. From the 12 *in vivo* biopsies obtained from patients under intensive insulin treatment, 11 stained positive for macrophages, and, in 50% of these, the number of positively staining cells was high.

We also performed a control experiment in our rabbit model of critical illness, in which we compared 15 ill rabbits with 7 sham-operated rabbits. This model allowed us to sample adipose tissue before and after 7 days of illness in the same animal. We could demonstrate a decrease in median cell size in the ill rabbits compared with the healthy rabbits (Figure 1E), whereas the total weight of the isolated fat pad did not decrease (median weight, 4.1 g for both; $P = 0.9$), indicative of an increased cell number. We also measured an increase in macrophage staining in the biopsies from ill rabbits versus those from healthy rabbits on Day 7 (increase from 29% of biopsies with RAM11$^+$ staining in sham-operated animals to 53% in ill rabbits).

Storage Properties of Adipose Tissue during Critical Illness

We first studied the ability of the adipose tissue to take up and metabolize glucose into fatty acids in patients with prolonged critical illness as compared with control subjects. In postmortem subcutaneous and omental adipose tissue of critically ill patients, mRNA and protein levels of the insulin-independent glucose transporters, GLUT1 and GLUT3, but not the insulin-dependent GLUT4, were strongly elevated (*see* Figures 2A–2B and the online supplement). The tissue content of glucose in postmortem subcutaneous and omental adipose tissue of critically ill patients was higher than in control subjects (Figure 2D), but was lowered by intensive insulin therapy. These differences in the adipose tissue glucose content among the groups mimicked the differences in plasma glucose (Figure 2E).

When glucose enters the cell, it is phosphorylated by the enzyme, hexokinase, to glucose-6-phosphate, which can, in turn,

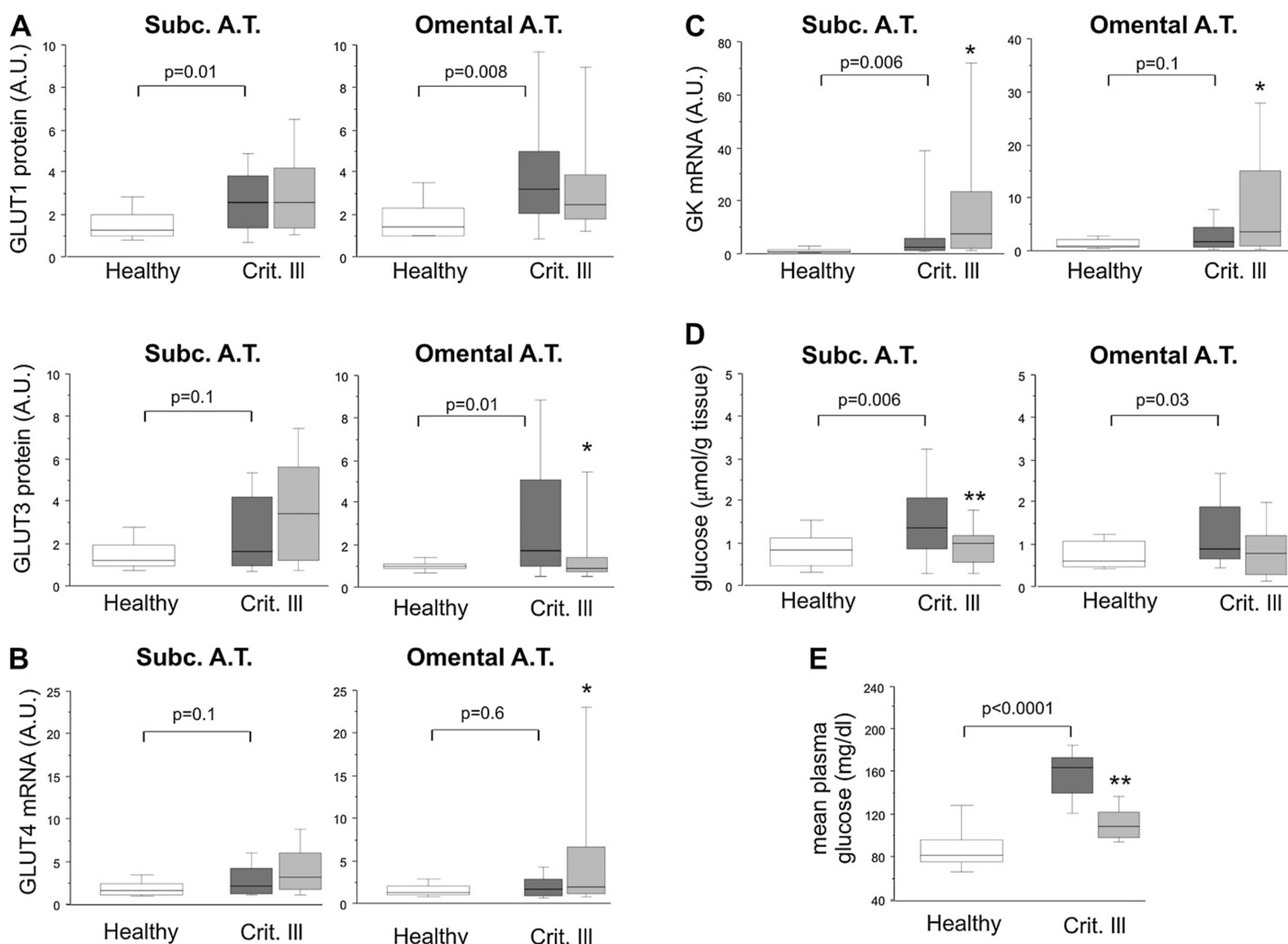

Figure 2. Glucose uptake in postmortem adipose tissue (A.T.) of critically ill patients. Human A.T. levels of (*A*) protein of GLUT1 and GLUT3, (*B*) mRNA of GLUT4, (*C*) mRNA of glucokinase, (*D*) tissue levels of glucose, and (*E*) mean daily plasma glucose levels. *Data boxes* present median and interquartile range; *whiskers* represent the 10th and the 90th percentiles. *White boxes* represent healthy control subjects undergoing elective surgery ($n = 20$); *dark gray boxes* represent critically ill patients who received conventional insulin therapy ($n = 33$); *light gray bars* represent critically ill patients who received intensive insulin therapy ($n = 28$). *Lines* and *P* value indicate difference between healthy and conventionally treated critically ill patients. *Different from conventional insulin therapy ($P \leq 0.05$); **different from conventional insulin therapy ($P \leq 0.01$). A.U. = arbitrary units; Subc. = subcutaneous.

be stored as glycogen, or further metabolized to pyruvate. In healthy control subjects, we documented very low glucokinase gene expression levels compared to the much higher levels in postmortem subcutaneous adipose tissue of critically ill patients (Figure 2C). Hexokinase 1 and 2 expression in the critically ill was not different from control subjects (*see* the online supplement). The tissue levels of glucose-6-phosphate and of glycogen were hardly detectable in postmortem subcutaneous and omental adipose tissue of both critically ill patients and control subjects.

Because, in the adipose tissue biopsies, glucose was not stored as glycogen, we studied lipogenesis as the alternative metabolic destiny of glucose. When glucose is metabolized into fatty acids, the glycolytic end product, pyruvate, is metabolized to acetyl CoA, which is subsequently turned into malonyl CoA by the enzyme, acetyl CoA carboxylase (ACC). In postmortem subcutaneous adipose tissue of critically ill patients, total levels of ACC were indeed several fold higher than in healthy control subjects (Figure 3A). In addition, the enzymatic activity of FAS, another lipogenic enzyme, was strongly up-regulated (Figure 3B). Expression of stearoyl CoA desaturase, involved in the biosynthesis of monounsaturated fatty acids, was also higher in postmortem subcutaneous adipose tissue of critically ill patients (Figure 3C). Similar changes in these lipogenic enzymes were observed in the omental adipose tissue biopsies of critically ill patients (Figures 3A–3C).

The other important metabolic storage process that takes place in adipose tissue is lipolysis. LPL is responsible for hydrolysis and uptake of circulating triglycerides. On the other hand, activated hormone-sensitive lipase (HSL) is the rate-limiting enzyme in the hydrolysis and the release of stored adipose triglycerides back into the bloodstream. Circulating triglyceride levels were increased in critical illness (Figure 4B). In both omental and subcutaneous postmortem adipose tissue homogenates of critically ill patients, levels of activated (Ser563 phosphorylated) HSL remained equal compared with that of healthy control subjects (Figure 3D). In contrast, protein levels of LPL were increased several fold in both omental and subcutaneous adipose tissue homogenates of critically ill patients (Figure 3E). Total lipase activity was also increased several fold in postmortem omental and subcutaneous adipose tissue homogenates of critically ill patients (Figure 3F), and correlated with LPL levels ($r = 0.279$, $P = 0.03$ in omental adipose tissue), but not with phosphorylated HSL.

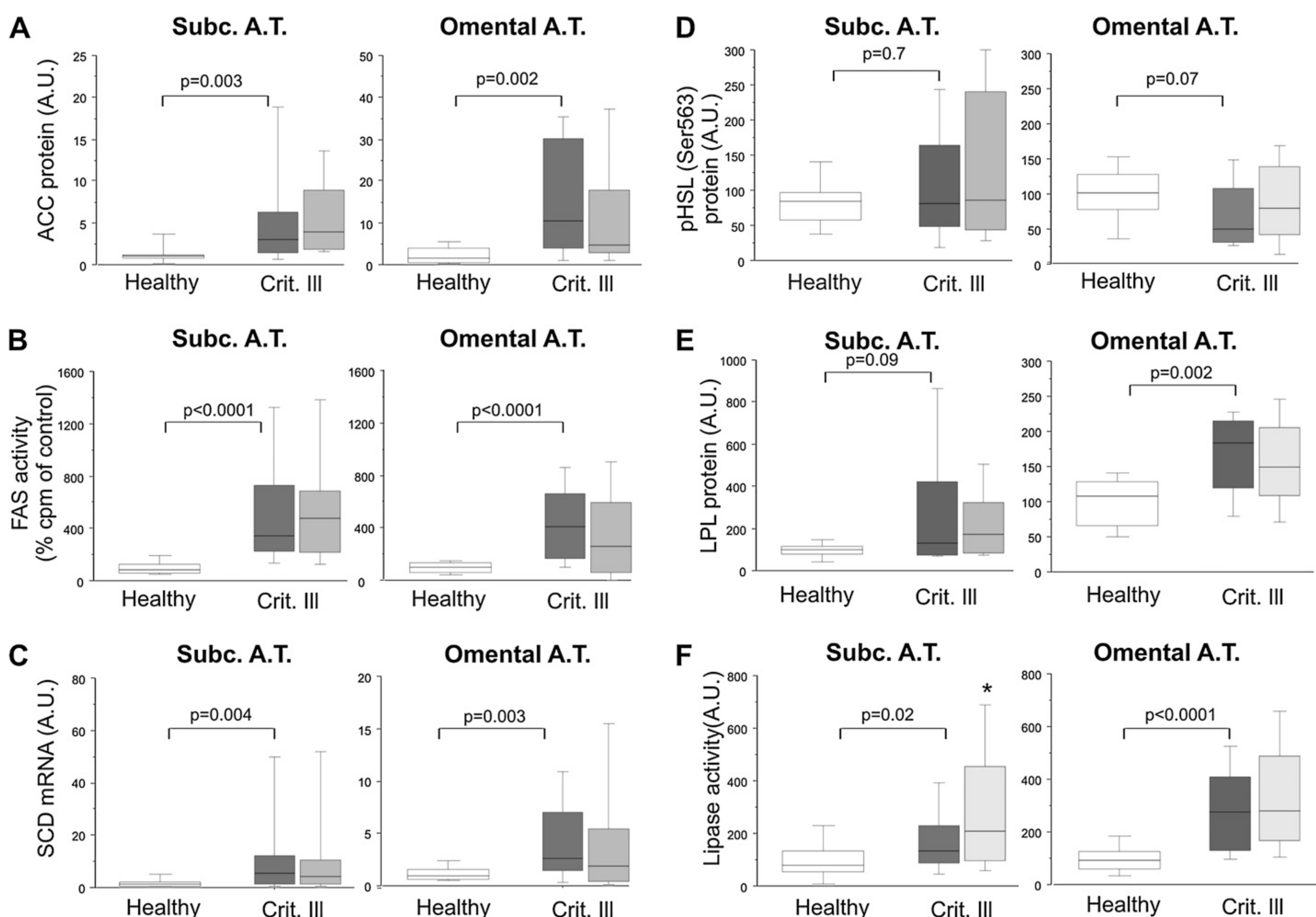

Figure 3. Lipogenesis and lipolysis in postmortem adipose tissue (A.T.) of critically ill patients. Human A.T. levels of (*A*) acetyl CoA carboxylase protein, (*B*) fatty acid synthase activity, (*C*) stearoyl CoA desaturase mRNA, (*D*) phospho(Ser563) hormone-sensitive lipase protein, (*E*) protein of lipoprotein lipase, and (*F*) lipase activity. *Data boxes* present median and interquartile range; *whiskers* represent the 10th and the 90th percentiles. *White boxes* represent healthy control subjects undergoing elective surgery ($n = 20$); *dark gray boxes* represent critically ill patients who received conventional insulin therapy ($n = 33$); *light gray bars* represent critically ill patients who received intensive insulin therapy ($n = 28$). *Line* and *P* value indicate difference between healthy and conventionally treated critically ill patients. *Different from conventional insulin therapy ($P < 0.05$). A.U. = arbitrary units; Subc. = subcutaneous.

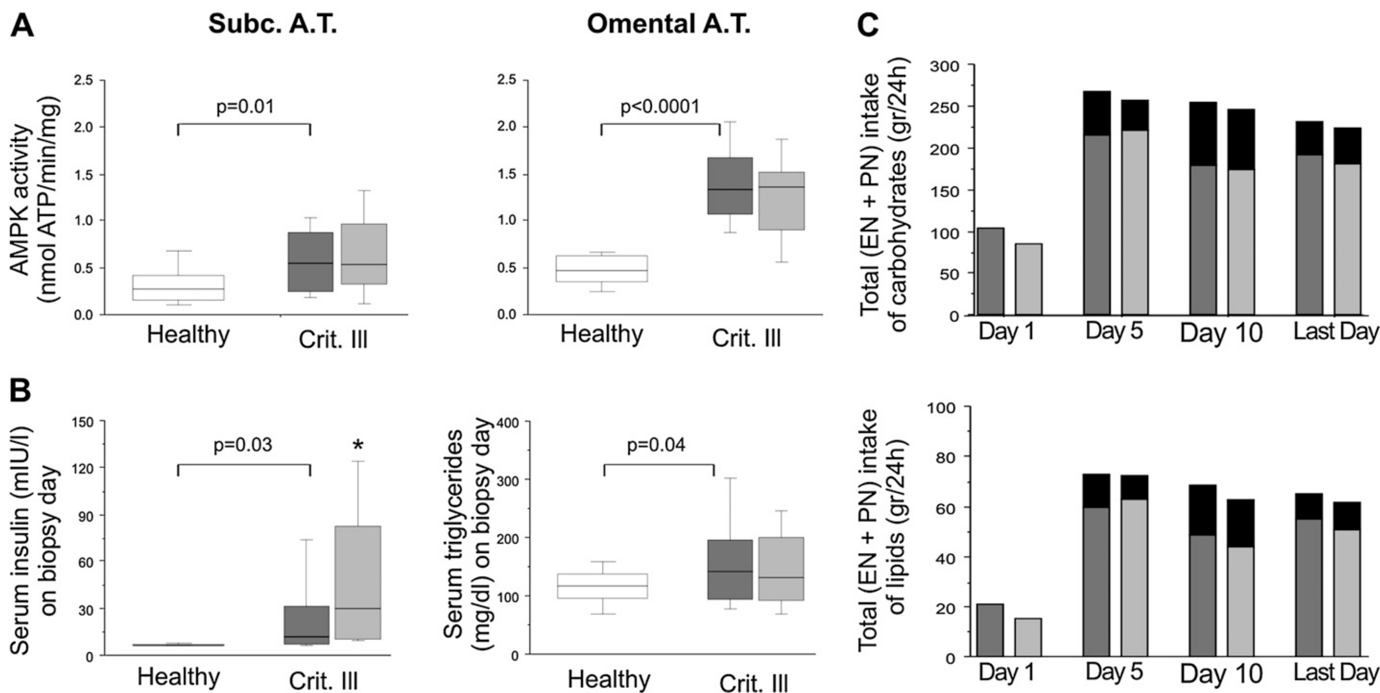

Figure 4. Mechanism behind altered substrate handling in postmortem adipose tissue (A.T.) of critically ill patients. (*A*) Human A.T. activity levels of AMP-activated protein kinase (AMPK), (*B*) serum insulin and serum triglyceride levels on biopsy day, (*C*) daily intake of enteral and parenteral feeding during intensive care unit (ICU) stay. *Data boxes* represent median and interquartile range; *whiskers* represent the 10th and the 90th percentiles. *Open boxes* represent healthy control subjects undergoing elective surgery (n = 20); *light gray boxes* represent critically ill patients who received conventional insulin therapy (n = 33); *dark gray boxes* represent critically ill patients who received intensive insulin therapy (n = 28). *Lines* and P value indicate difference between healthy and conventionally treated critically ill patients. *Different from conventional insulin therapy ($P \leq 0.05$). (*C*) *Gray bars* represent the daily intake of parenteral feeding in critically ill patients who received conventional insulin therapy (*light gray*; n = 33) and intensive insulin therapy (*dark gray*; n = 28), and *black bars* represent the daily enteral intake. The *upper panel* represents the administered carbohydrates; the *lower panel* represents the administered lipids. Data are presented as mean total daily (24-h) intake on day of admission, Day 5, Day 10, and last day of ICU stay. EN = enteral; PN = parenteral; Subc. = subcutaneous.

Impact of Intensive Insulin Therapy on Adipose Tissue Morphology and Studied Metabolic Pathways

Critically ill patients in both the conventional and the intensive insulin treatment arms revealed elevated circulating glucose and insulin levels as compared with control subjects, typical for the insulin resistance associated with severe illness. Blood glucose levels were lower (Figure 2E), as per protocol, and circulating insulin levels were higher in intensive insulin–treated patients, who received higher doses of insulin as compared with patients in the conventional insulin group (Figure 4B).

The intensity of insulin therapy during critical illness, however, only had a relatively small effect on adipocyte morphology and on the studied storage parameters tested. Intensive insulin therapy reduced the incidence of positive macrophage staining in subcutaneous adipose tissue (from 64 to 53% in omental, and from 66 to 27% in subcutaneous adipose tissue). Intensive insulin therapy reduced the glucose content in postmortem adipose tissue, as well as the expression levels of Pref-1 and GLUT-3 in omental adipose tissue (Figures 1C and 2A), elevated the expression levels of GLUT-4 in omental adipose tissue (Figure 2B), and increased the expression levels of glucokinase in postmortem subcutaneous and omental adipose tissue (Figure 2C).

Mechanism behind Altered Substrate Handling

The energy-sensing enzyme, AMPK, usually activated by a high AMP:ATP ratio, was clearly activated in subcutaneous and omental adipose tissue of critically ill patients as compared with control subjects (Figure 4A). AMPK increases glucose uptake and glycolysis, but can also inactivate ACC by phosphorylation. Indeed, protein levels of phosphorylated ACC were increased in postmortem adipose tissue of critically ill patients (in subcutaneous adipose tissue: median [interquartile range], 6.8 [2.8–11.9] in critically ill patients versus 1 [0.4–3.3] in control subjects; $P = 0.0002$; in omental adipose tissue: 5.9 [2.7–12.7] in critically ill patients versus 1.4 [0.7–3.1] in control subjects; $P = 0.04$). The ratio of phosphorylated ACC to total ACC levels was not different between critically ill patients and control subjects ($P = 0.6$ for subcutaneous adipose tissue and $P = 0.5$ for omental adipose tissue).

One might hypothesize that the artificial enteral and parenteral feeding used for critically ill patients may affect lipogenesis and lipolysis. Although the sum of daily enteral and parenteral amounts of carbohydrate and lipids at no time exceeded the required daily amounts (Figure 4C), the ICU patients included in this study all received at least partial parenteral feeding during ICU stay, due to the type and severity of illness (patients from whom biopsies were studied were all nonsurvivors, with high on-admission Acute Physiology and Chronic Health Assessment Evaluation II scores). However, the studied parameters in subcutaneous and omental adipose tissue did not correlate with the daily parenteral or total caloric intake, except for FAS activity in omental adipose tissue, which correlated with the mean total caloric intake ($r^2 = 0.251$) (*see* the online supplement).

The patient population from which we were able to collect postmortem adipose tissue biopsies has a mean BMI of 25,

ranging from 16.3 to 31.2. The tested 61 biopsies were predominantly from normal to overweight patients: 33 (54%) of the patients had a normal BMI, 22 (36%) were overweight, and only 3 (5%) were obese. Only omental median adipocyte cell size showed a biologically relevant correlation with BMI. This suggests that adipocytes in either normal or overweight critically ill patients may act identically.

DISCUSSION

Our data suggest that adipose tissue is substantially altered during critical illness, with an increased number of newly formed adipocytes and macrophage infiltration. The larger number of small adipocytes appear to have an increased ability to take up and metabolize glucose and store triglycerides, a process in which an increased activity of the energy-sensing enzyme, AMPK, could partially play a role. Taking into account the clear association between both hyperglycemia and dislipidemia and mortality observed in critical illness, these alterations may present an adaptive and protective response.

We observed a smaller median adipocyte cell size and an increase in number of small adipocytes in critically ill patients, indicating a higher number of newly formed adipocytes. Although its function is not unequivocally clear, the observed elevation in the preadipocyte marker, Pref-1, also may be indicative of an up-regulated differentiation from mesenchymal stem cells into preadipocytes, and further into full functional adipocytes. However, Pref-1 has not only been suggested to be a potentiator of adipogenesis in mesenchymal cells (24, 25), but also to maintain the undifferentiated state of preadipocytes (26, 27). As we observed a higher number of small-sized adipocytes together with higher levels of Pref-1, our results could be interpreted as increased adipogenesis.

The high number of CD68 positive–stained cells in all studied adipose tissue biopsies of critically ill patients, obtained from both the subcutaneous and the omental site, postmortem and *in vivo*, is striking. CD68 is recognized to be a specific macrophage marker (28). Macrophage accumulation in adipose tissue has been demonstrated in obesity (29, 30), and systemic hypoxia has been associated with increased inflammation in adipose tissue (31). Several reasons for macrophage accumulation in adipose tissue of obese people have been proposed. It has been suggested that hypertrophic or expanding adipocytes may produce chemotactic signals, leading to macrophage recruitment in an attempt to limit adipose tissue expansion (32–34). The other suggested mechanism involves oxidative stress in the endothelium due to increased glucose delivery. Endothelial injury in the adipose tissue could attract inflammatory cells, such as macrophages, further exacerbating local inflammation (35). Why critical illness is associated with increased infiltration of macrophages is not clear. Unlike in otherwise healthy obese people, we observed smaller adipocytes during critical illness. Only rarely did we observe large necrotic adipocytes surrounded by foaming macrophages. The hyperglycemic environment of critical illness, which has been shown to evoke endothelial injury (12), may play a role. Indeed, patients in the conventional insulin arm revealed more pronounced macrophage infiltration than patients in the intensive insulin arm. However, expanding preadipocytes have also been shown to produce chemotactic signals (36). In addition, macrophages may also originate from transdifferentiation of preadipocytes into macrophage-like cells (37). Hence, the macrophage accumulation in critical illness could be, in part, a consequence of increased adipogenesis. Studies on the acute phase of critical illness showed high levels of inflammatory markers, but unaltered macrophage markers (38, 39), suggesting that the macrophage accumulation that we observe is not part of the acute stress response, but part of the complex hypermetabolic condition of prolonged critical illness.

The larger number of small adipocytes appeared to have an increased ability to take up and metabolize glucose into fatty acids and to take up and store circulating triglycerides. Adipose tissue normally takes up glucose through the insulin-dependent glucose transporter, GLUT4 (40). We have now observed that the insulin-independent glucose transporters, GLUT1 and GLUT3, in adipose tissue were highly up-regulated during critical illness. This may evoke an increased influx of glucose into adipose tissue. Indeed, these high-affinity transporters have a K_m below the observed range of blood glucose in the critically ill, so an increase in cell surface expression will affect the rate of glucose uptake into the cells. This increase in glucose transporters might be triggered by metabolic stress, such as cellular hypoxia, but also by hyperglycemia, insulin, or cytokines (41–43). Once transported into the cell, glucose becomes phosphorylated to glucose-6-phophate by hexokinase. We measured an increased expression of the low-affinity glucokinase, but not of the high-affinity hexokinase I and II. In contrast to hexokinase I and II, glucokinase is not inhibited by its end product, glucose-6-phophate (41), whereby it contributes to rapid phosphorylation and trapping of glucose into the cell. We measured only very low levels of glucose-6-phophate and glycogen in the adipose tissue, indicating that glucose-6-phophate is further metabolized. In normal conditions, glucose-6-phophate would be oxidized in the Krebs cycle. The increased activity of the lipogenic enzymes, ACC, FAS, and stearoyl CoA desaturase, in the critically ill, however, may point to an increased metabolization of glucose into fatty acids (44).

Although adipose tissue can synthesize free fatty acids *de novo*, free fatty acids for lipid storage are preferentially provided by LPL-mediated hydrolysis of circulating triglyceride-rich lipoproteins. We could indeed demonstrate that adipose tissue of critically ill patients showed a markedly increased LPL protein level and lipase activity. Furthermore, LPL is an important marker for adipocyte differentiation with increased LPL expression when preadipocytes differentiate (45). Contrary to the uptake of circulating triglycerides, HSL will hydrolyze and release stored triglycerides back into the circulation. In the studied biopsies from critically ill patients, we observed no alteration in the level of activated HSL.

All the studied ICU patients received intravenous feeding during ICU stay. Although the sum of enteral and parenteral feeding components did not exceed the reference daily intake (46), one could expect intravenously administered glucose and lipids to cause increased lipogenesis or lipolysis in adipose tissue. However, we did not observe a correlation between the mean total or intravenous caloric intake and the studied glycolytic, lipogenic, and lipolytic parameters, which appears to exclude a prominent role of artificial feeding in explaining our observations. In addition, it has been shown previously that, as compared with hepatic lipogenesis, human adipose tissue lipogenesis is less responsive to dietary carbohydrates (47, 48).

A likely explanation for the observed shift in metabolization is the hypoxic condition of critical illness. Critically ill patients suffer from reduced oxygen pressure and oxygen blood content, causing cellular hypoxia in different tissues (49). The cellular pathways activated by hypoxic stress, such as the hypoxia-inducible factor and AMPK pathway, shut down energy-consuming pathways that are not acutely needed for cell survival. Activation increases the uptake of glucose and glycolysis, resulting in pyruvate production (50, 51), but, in addition, HSL activity is attenuated (52) and fatty acid uptake is increased (53, 54). We clearly demonstrated increased AMPK activity in adipose tissue of critically ill patients. A lower oxygen availability in adipose tissue during critical illness may, thus, not only stimulate uptake

of glucose, but also impair complete glucose oxidation, with a shift to fatty acid synthesis. On the other hand, this mechanism may attenuate hydrolysis of stored triglycerides, but stimulate uptake of circulating fatty acids. Unfortunately, we were not able to quantify possible hypoxia in the adipose tissue; thus, these conclusions remain speculative. The high levels of circulating insulin that are present in critically ill patients are also a possible trigger for increased metabolization of glucose into fatty acids (11). Insulin action in adipose tissue indeed involves stimulation of glucose uptake, increase of glucokinase expression, and stimulation of FAS expression and activity (41, 55, 56). Insulin has been shown to reduce lipid oxidation by increasing intracellular glucose flux (57). Insulin inhibits HSL activity, but stimulates LPL levels and activity, thereby reducing hydrolysis of stored triglycerides and increasing uptake of circulating triglycerides (58–60). In addition, small-sized adipocytes have been demonstrated to be more insulin sensitive and display increased lipogenesis (61–63). The studied patients were all enrolled in a large, randomized, controlled trial on glycemic control in the medical ICU (20). Patients under intensive insulin therapy received high doses of insulin to maintain normal blood glucose levels, whereas patients who had been randomly assigned to conventional insulin therapy received insulin only when glucose concentrations exceeded 215 mg/dl. Although patients under intensive insulin therapy received much higher doses of insulin throughout the stay in the ICU, circulating insulin levels were not that different. We demonstrated earlier that this is primarily due to a lowered endogenous insulin production in the intensive insulin therapy group, as well as an effect on insulin sensitivity (64, 65). The clearly elevated circulating insulin levels in both insulin therapy groups may explain the minor differences in the metabolic activity of the adipose tissue. A third possible mediator in the observed changes might be the typical uniform suppression of the neuroendocrine axes in prolonged critical illness (66). Indeed, thyroid-stimulating hormone normally stimulates release of free fatty acids from adipose tissue, and growth hormone reduces adipocyte differentiation, triglyceride synthesis, and LPL activity (67), but the pulsatile release of both hormones becomes suppressed in prolonged critical illness. In addition, the inhibition of the melanocortin system can promote lipid uptake and triglyceride synthesis in adipose tissue (68). Thus, a single cause of the altered metabolism in adipose tissue of critically ill patients is not very likely, and a complex interplay of different affected pathways can be inferred.

Two important limitations of this study should be highlighted. The tissue biopsies used for the metabolic study were obtained from nonsurviving critically ill patients only, which may have induced a bias due to the potential confounding effects of agonal hypoxia. However, a previous study on phosphorylated signaling molecules (64), and the observation that the AMPK enzyme in liver samples from the same patients was not affected by illness (unpublished observations), argues against such bias. In addition, in the small set of *in vivo* subcutaneous adipose tissue biopsies from patients with prolonged critical illness, we observed the same morphological changes and macrophage staining pattern as in the postmortem biopsies. Furthermore, we demonstrated a reduction in adipocyte cell size and an increase in macrophage staining in paired *in vivo* adipose tissue biopsies from critically ill rabbits, very comparable to what we observed in the human biopsies. A second limitation of the study is that the tested biopsies were predominantly from normal and overweight patients; thus, we cannot extrapolate the results to obese critically ill patients. Indeed, adipose tissue from non–critically ill obese individuals markedly differs from that of lean subjects (e.g., with regard to inflammation, adipokine expression, and macrophage infiltration). However, when we compared lean subjects with overweight patients, we found no correlation between the tested parameters and BMI, except for cell size, and for this parameter the association was also present in the healthy control subjects. This suggests that adipocytes, in either normal or overweight critically ill patients, appear to act identical in response to the stress of illness, and that it may be merely the larger total fat depot that might explain the lower mortality in overweight patients versus patients with a normal BMI (3–5). However, based on some contradictory studies in which a higher mortality was already observed in obese critically ill patients (69–71), it appears, as yet, unclear at what point a higher BMI switches from being protective to malignant.

In conclusion, adipose tissue is substantially altered during critical illness, with an increased number of newly formed adipocytes and macrophage infiltration observed in *in vivo*– and postmortem-harvested biopsies of patients, as well as in an animal model. The larger number of small adipocytes appears to have an increased ability to take up circulating glucose and triglycerides, although this was only studied in postmortem biopsies. Such changes may render adipose tissue biologically active as a functional storage depot for these possibly toxic metabolites during critical illness, thereby contributing to survival. Hypothetically, this protective reaction may be more pronounced in critically ill patients with a larger fat depot, but whether the observed changes are also present in adipose tissue from obese and morbidly obese patients needs further thorough investigation.

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