Epidural blockade and the catabolic response to surgery:

An integrated analysis of perioperative protein and glucose metabolism using stable isotope kinetics in the fasted and fed state

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Abstract

The present project investigated the effect of epidural blockade with local anesthetic on the catabolic stress response during and immediately after abdominal surgery in fasting patients and during infusion of glucose at 2 $mg \cdot kg^{-1} \cdot min^{-1}$. The kinetics of glucose and protein metabolism were assessed by the stable isotope tracers [6,6-$^{2}H_2$]glucose and L-[1-^{13}C]-leucine.

Epidural blockade was associated with a lower plasma glucose concentration and glucose production when compared to control subjects in the fasted state. Whole body protein breakdown, amino acid oxidation and protein synthesis were suppressed during surgery, and epidural blockade had no modifying effect on perioperative protein metabolism.

The suppression of endogenous glucose production by exogenous glucose was more pronounced in the presence of epidural blockade. Perioperative protein metabolism, however, was not influenced by epidural blockade during glucose infusion.

Although epidural blockade suppressed glucose metabolism both in the fasted state and during glucose administration, it failed to exert a modifying effect on perioperative protein metabolism.

Résumé

Ce projet a étudié l'effet d'un bloc péridural avec agents anesthésiques locaux sur la réponse catabolique au stress durant et immédiatement après une chirurgie abdominale chez des patients à jeun recevant une infusion de glucose à 2 $mg \cdot kg^{-1} \cdot min^{-1}$. La cinétique du métabolisme protéique et du glucose a été évaluée à l'aide des isotopes stables [6,6-$^{2}H_2$]glucose et L-[1-^{13}C]leucine.

Lors de comparaisons avec le groupe de contrôle à jeun, le fait d'avoir reçu un bloc péridural a été associé à une plus faible concentration plasmique de glucose et une diminution de la production de glucose. Cependant, la présence d'une péridurale n'a pas modifié la suppression périopératoire de la sauvegarde protéique, de l'oxydation des acides aminés et de la synthèse protéique. La suppression de la production endogène de glucose par le glucose exogène a été plus prononcée en présence d'une péridurale. Par contre, le métabolisme protéique périopératoire n'a pas été influencé par le bloc péridural durant l'infusion de glucose.

Quoique la présence d'une péridurale ait supprimé le métabolisme du glucose à la fois chez les patients à jeun ainsi que ceux ayant reçu une infusion de glucose, on n'a pu lui attribuer d'effet sur le métabolisme protéique périopératoire.

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List of abbreviations

APE: atom percent excess

FFA: free fatty acids

GCMS: gas chromatography-mass spectrometry

IRMS: isotope ratio mass spectrometry

R_a: rate of appearance

Contribution of Authors

Two manuscripts entitled "*Epidural blockade modifies perioperative glucose production without affecting protein catabolism*" and "*Perioperative glucose infusion and the catabolic response to surgery: effect of epidural blockade*" are included as a part of this thesis. The following describes the contribution of the authors to the manuscripts:

I was involved in developing the study design of both manuscripts. In collaboration with Dr. Carli and Dr. Schricker I recruited the study patients and performed the experiments in the surgical patients (anesthetic care and stable isotope infusions). I collected the samples and prepared them for analysis. GC/MS analysis was performed by Dr. Wykes with the help of Michelle Mackenzie, and by Dr. Orval Mamer.

I also performed the calculations and the statistical analysis of the data. As primary author I was responsible for writing the papers and creating figures and tables. Dr. Carli, Dr. Wykes and Dr. Schricker provided direction and expertise to the study and assisted with the manuscripts.

1. Introduction

Surgical tissue trauma is associated with stereotypical alterations in carbohydrate and protein metabolism often referred to as the catabolic response. There is strong evidence that perioperative pain management and nutritional support influence the metabolic and endocrine response to surgical stress (Carli et al, 2001). The combination of epidural blockade with local anesthetic and general anesthesia blunts the hyperglycemic response to surgery, attenuates the postoperative increase in muscle protein losses and preserves whole body nitrogen balance, when compared to general anesthesia alone (Brandt et al, 1978; Carli et al, 1997; Christensen et al, 1986; Vedrinne et al, 1989). The anticatabolic properties of intravenous glucose in fasting subjects (Gamble, 1946) as well as in surgical patients have also long been recognized (Lund et al, 1986b; Nordenström et al, 1983; Obata et al, 1993; Wolfe, 1993).

Although the effects of epidural blockade and intravenous glucose upon the catabolic responses after the operation have been widely studied, little is known about their influence during the acute phase of the stress response.

Therefore, the aim of the proposed project was to investigate the individual and combined metabolic effect of epidural blockade and intravenous glucose on the catabolic stress response during and immediately after surgery. In the first study perioperative kinetics of glucose and protein metabolism were characterized using stable isotope tracers ([6,6-$^{2}H_{2}$]glucose, L-[1-^{13}C]-leucine) in fasting patients receiving general anesthesia with or without epidural blockade. The second study investigated the effect of epidural blockade on the catabolic responses to surgery in patients receiving intravenous glucose at 2 $mg \cdot kg^{-1} \cdot min^{-1}$ throughout the perioperative period.

2. Literature Review

2.1 The metabolic response to surgery

Surgical trauma commonly leads to a variety of metabolic, endocrine and immunological changes often grouped together as the catabolic stress response (Weissman, 1990). This stress response has a unique characteristic of a generalized and stereotypical pattern of reactions with limited specificity to the aetiology of the initiating event.

The endocrine stress response is characterized by increased secretion of pituitary hormones and activation of the sympathoadrenergic system, resulting in altered protein homeostasis, hypermetabolism, altered carbohydrate metabolism, sodium and water retention and increased lipolysis (Weissman, 1990; Wilmore, 2000). Although the immunologic changes associated with surgical trauma are very complex and have not been fully understood so far, increased levels of the pro-inflammatory cytokines, interleukin IL-1 and IL-6 and tumor necrosis factor (TNF), could been identified as major regulators of the immunologic stress response (Weissman, 1990).

2.1.1 Changes in protein metabolism

More than half a century ago Cuthbertson observed that bone fractures are accompanied by a large increase in urinary nitrogen excretion, thereby establishing negative nitrogen balance as a metabolic hallmark of trauma (Cuthbertson, 1942). The cumulative nitrogen losses after elective abdominal surgery range between 40 and 80 g of nitrogen and complications that delay the use of the gastrointestinal tract may result in nitrogen losses of up to 150 g (Kinney et al, 1983). The clinical relevance of this catabolic pattern is obvious considering the fact that 1 g of nitrogen is equivalent to 30 g of hydrated lean tissue. Therefore, a loss of 50 g nitrogen, as observed after uncomplicated

cholecystectomy, is equivalent to 1500 g of lean tissue. The loss of lean tissue accounts for 50-65 % of the patient`s total weight loss over a three week period after major injury (Hill et al, 1993; Kinney, 1978).

The principal underlying defect responsible for these net protein losses appears to be an accelerated rate of proteolysis along with an insufficient increase in protein synthesis (Carli et al, 1991; Harrison et al, 1989; Shaw et al, 1989). Endogenous amino acid oxidation and amino acid release from the muscle after abdominal surgery have been shown to increase by 90 % and 30 %, respectively, while whole body protein synthesis increases by 10 % only (Carli et al, 1991). The magnitude of this alteration is substantial considering the fact that muscle tissue represents approximately 45 % of body weight and contributes as much as 20 % to total body protein synthesis (Kinney et al, 1983).

From a teleological point of view these catabolic changes probably have evolved to confer a maximum chance of survival due to the increased supply of energy-generating substrates, i.e. glucose, fatty acids and amino acids. If prolonged or aggravated by infectious or surgical complications it may, however, lead to devastating nutritional consequences causing erosion of body cell mass and physiological reserve capacity. Therefore the degree of protein catabolism is of utmost clinical relevance as the length of time for return of normal physiologic function after discharge from the hospital is related to the extent of loss of lean body mass during hospitalization (Christensen et al, 1982). Because protein represents both structural and functional body components, erosion of lean tissue also may lead to devastating consequences such as compromized immune function (Chandra et al, 1983), delayed wound healing (Windsor, 1988a), and diminished muscle strength (Windsor, 1988b) resulting in prolonged convalescence and increased morbidity (Windsor, 1988 c, Wilmore, 2000).

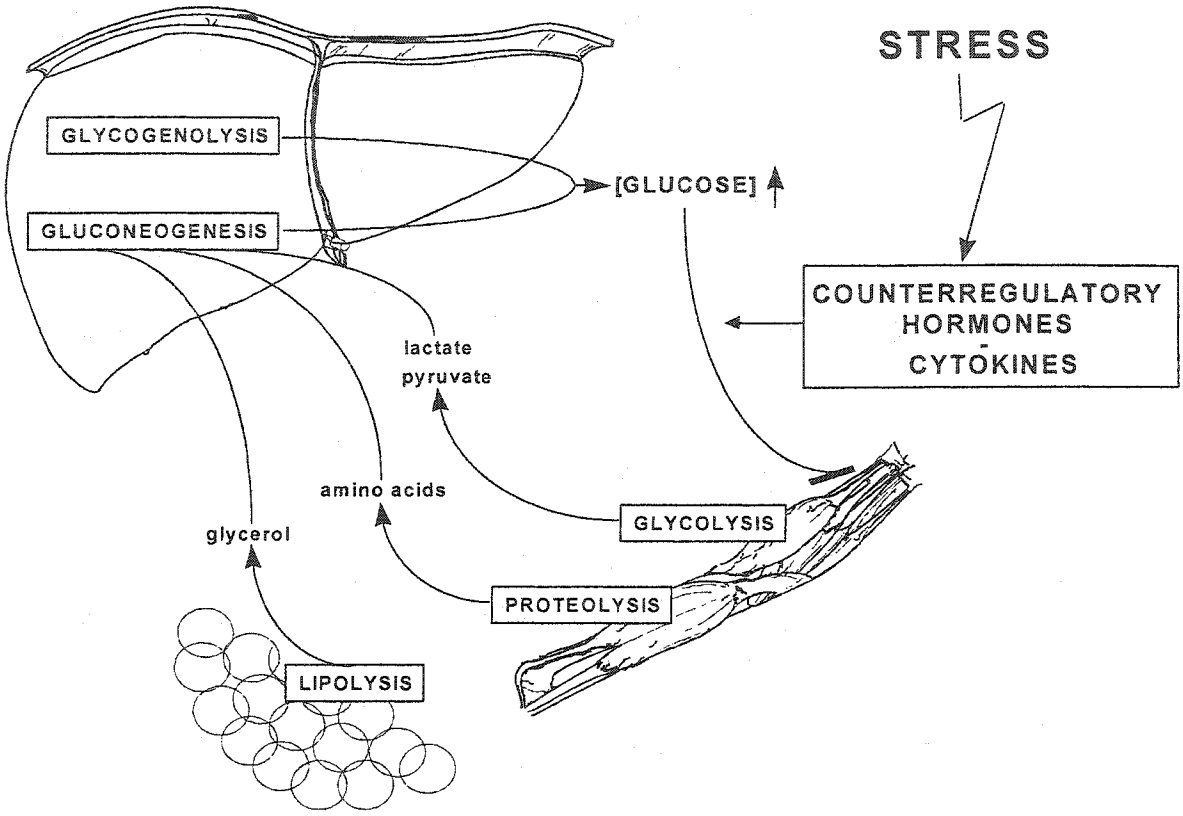

Figure 1.1: The metabolic response to stress.

2.1.2 Changes in glucose metabolism

The alterations in protein metabolism are accompanied by stereotypical changes in glucose metabolism, which are stimulated glucose production and impaired glucose utilization resulting in hyperglycemia (figure 1.1) (Schricker et al, 1998). In healthy volunteers after 14 hours fasting glycogenolysis contributes approximately 55 % of total glucose output, the remaining 45 % being derived from gluconeogenesis (Landau et al, 1996). As a result of increased concentrations of the counterregulatory hormones cortisol, catecholamines and glucagon, a direct consequence of surgery, and the fasting-induced depletion of glycogen stores, hepatic gluconeogenesis is stimulated to a greater degree in

the surgical patient (Chandramouli et al, 1997; Gump et al, 1975).

Gluconeogenesis in the liver is a highly oxygen-consuming pathway accounting for 50 % of hepatic oxygen consumption in the postabsorptive state (Jungas et al, 1992). Because muscle protein is broken down to supply amino acids serving as important precursors for the *de novo* glucose synthesis in the liver, gluconeogenesis has been proposed to occupy a central position in catabolic pathways causing much of the protein losses as seen after surgery (Wolfe, 1993).

Recent studies demonstrated a significant correlation between the rates of glucose production and protein breakdown during and two days after abdominal surgery (figure 1.2) providing evidence of this interdependence of perioperative glucose and protein metabolism in humans (Schricker et al, 2000).

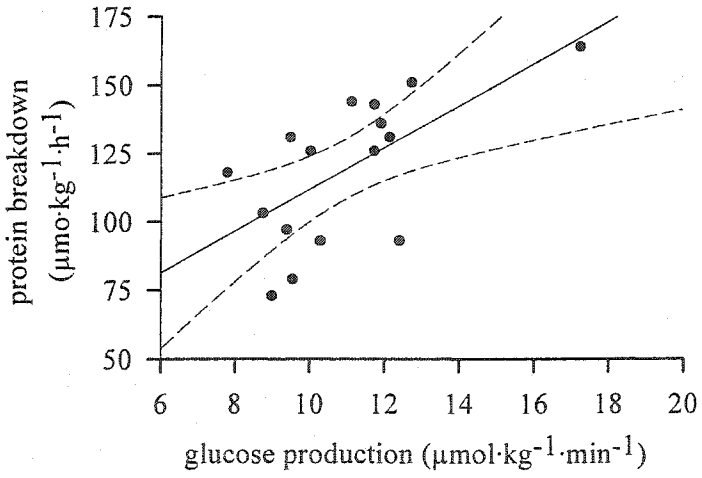

Figure 1.2: Relation between protein breakdown and glucose production in patients two days after abdominal surgery ($r=0.639$, $P<0.05$). (Schricker et al, 2000a)

It was therefore hypothesized that by reducing accelerated gluconeogenesis the muscle amino acid release will also be suppressed resulting in a better preservation of whole body protein. Animal studies demonstrating an inverse correlation between

gluconeogenesis and the protein synthesizing capacity of the liver further document this relationship between glucose and protein metabolism (Ayuso et al, 1986; Dahn et al, 1982).

Thus, any modification of hepatic gluconeogenesis by anesthetic or nutritive interventions is presumably highly significant with regard to the energy balance of the liver and protein catabolism.

2.1.3 Hormonal changes

The biochemical factors initiating, regulating and sustaining the hyperglycemic response to surgery have not all yet been identified. However, much of the observed metabolic profile can be explained by specific hormonal alterations known as the classic neuroendocrine response (Weissman, 1990). The endocrine milieu after surgical trauma is characterized by elevations in plasma levels of the counterregulatory hormones cortisol, glucagon, epinephrine and norepinephrine (Frayn, 1986). Infusion studies in volunteers demonstrated that increased circulating concentrations of these hormones can mimic the typical changes in carbohydrate metabolism. The combined infusion into normal subjects of hydrocortisone, glucagon and epinephrine stimulated gluconeogenesis and decreased glucose clearance resulting in hyperglycemia (Bessey et al, 1984). It was proposed that these hormones act synergistically, i.e. the effect was more pronounced when all these hormones were infused simultaneously than when applied individually or in group of two. All of these hormones exert catabolic effects, either directly (Simmons et al, 1984; Shaw et al, 1988b) or indirectly by inhibiting insulin secretion and / or counteracting the peripheral action of insulin leading to the impairment of tissue insulin sensitivity (Deibert et al, 1980; Bessey et al, 1983). Marked insulin resistance is present after routine surgical procedures, even in the absence of sepsis or other complications, and may persist up to 20

days thereafter (Black et al, 1982; Brandi et al, 1990). The impact of insulin resistance upon carbohydrate and lipid metabolism, i.e. hyperglycemia, stimulated glucose production and lipolysis, has been well documented and gave rise to the term "diabetes of injury" (Frayn, 1986).

There is also indirect evidence that the nitrogen-preserving anabolic effects of insulin are affected; exogenous provision of insulin has been demonstrated to reduce protein losses after elective surgery and burn injury, mediated through enhanced protein synthesis and attenuated protein oxidation, as determined by arteriovenous flux and tracer infusion studies (Sakurai et al, 1995; Woolfson et al, 1979). In addition, insulin restrains amino acid release from endogenous proteins in a dose-dependent manner (Tessari et al, 1986) and decreases leucine oxidation, both *in vitro* (Hutson et al, 1978; Odessey et al, 1972) and *in vivo* (Tessari et al, 1986). Thus, restoration of insulin action seems to be a key factor for the normalization of protein economy after surgical trauma.

2.2 Modulation of the metabolic response to surgery

Numerous strategies of modifying the metabolic response to surgery have been investigated in the past, such as the effect of anesthesia (type and dose of anesthetics) and analgesia (regional techniques, e.g. epidural blockade), the surgical approach (laparoscopic vs. open), provision of nutrition (with a special role of specific nutrients such as glutamine) and even hormonal interventions (insulin, growth hormone) (Carli et al, 2001; Kehlet, 1998). Furthermore, maintenance of normothermia as well as early postoperative mobilization have been found do be effective measures of modulating the catabolic stress response (Carli et al, 1989).

This thesis will address the specific effects of epidural blockade with local anesthetic and

intravenous glucose administration on the catabolic response to surgery. Therefore, the anticatabolic properties of these two measures will be here reviewed.

2.2.1 Concept of combined epidural blockade and general anesthesia

Most studies investigating the modifying effect of epidural blockade with local anesthetic on the surgical stress response compared patients receiving either combined epidural blockade and general anesthesia or general anesthesia alone. When applying this combined anesthetic technique, lower doses of general anesthetics are required than in patients receiving general anesthesia alone. For postoperative analgesia, the epidural blockade can be continued over several days, whereas patients after general anesthesia commonly receive intravenous opioids, such as morphine.

2.2.2 Effect of epidural blockade on the metabolic response to surgery

The central role of the peripheral and central nervous system in mediating the metabolic alterations during and after surgical trauma has been demonstrated indirectly by studies upon the stress response in patients undergoing surgery under regional anesthesia. Neuraxial block of afferent sensory and efferent signals with epidural local anesthetics, i.e. epidural blockade extending from T4 to S5 dermatomes has been shown to be most effective to suppress the catabolic responses to surgery. In contrast epidural administration of opioids has not been proven as effective despite an equivalent satisfactory pain relief (Kehlet, 1998; Liu, 1995). These observations led to the contention that intra- and postoperative pain relief *per se* does not ameliorate the pathophysiological responses after surgery and does not positively effect clinical outcome (Rosenberg et al, 1999).

2.2.2.1 Effect of epidural blockade on perioperative protein metabolism

There is ample evidence that combined general anesthesia and epidural blockade with local anesthetics initiated before and maintained after surgery attenuates the postoperative loss of body nitrogen and the urinary excretion of 3-methyl-histidine, when compared to general anesthesia alone, followed by intravenous analgesia with opioids (Brandt et al, 1978; Vedrinne et al, 1989; Christensen et al, 1986). This protein-sparing effect of neural blockade could be the result of decreased protein breakdown and oxidation or increased protein synthesis. The obvious shortcoming of nitrogen balance measurements is that the contribution from dynamic changes in protein synthesis and degradation cannot be separated. In a previous study, nociceptive blockade with local anesthetics blunted the postoperative rise in whole body protein breakdown and leucine oxidation with minimal changes in protein synthesis (Carli et al, 1997). The amino acids made available from protein breakdown have been demonstrated to be oxidized to a limited extent and incorporated into protein synthesis at whole body and tissue (muscle) level. This finding was in accordance with the results of a recent study showing that epidural analgesia with bupivacaine decreased the release of amino acids from muscle two days after major upper abdominal surgery (Barratt et al, 1999).

In a recent investigation the effects of epidural blockade with bupivacaine upon glucose and protein metabolism were investigated two days after abdominal surgery in the postabsorptive state and during feeding with glucose (4 $mg \cdot kg^{-1} \cdot min^{-1}$) (Schricker et al, 2000). While one group received general anesthesia combined with epidural blockade during and after surgery, the control group received general anesthesia alone, followed by patient controlled analgesia with intravenous morphine. Perioperative epidural blockade did not alter glucose production and whole body protein metabolism in the fasted state

when compared to the control group. However, epidural blockade facilitated the uptake and oxidative utilization of glucose infused at 4 $mg \cdot kg^{-1} \cdot min^{-1}$. This was accompanied by a decrease in protein oxidation indicating a favourable shift from a protein dominated to a more glucose orientated oxidative substrate utilization. A significant inverse correlation between the changes in glucose utilization and protein oxidation could be observed during glucose infusion (figure 1.3). It was therefore concluded that the nitrogen-sparing properties of epidural blockade require adequate energy supply.

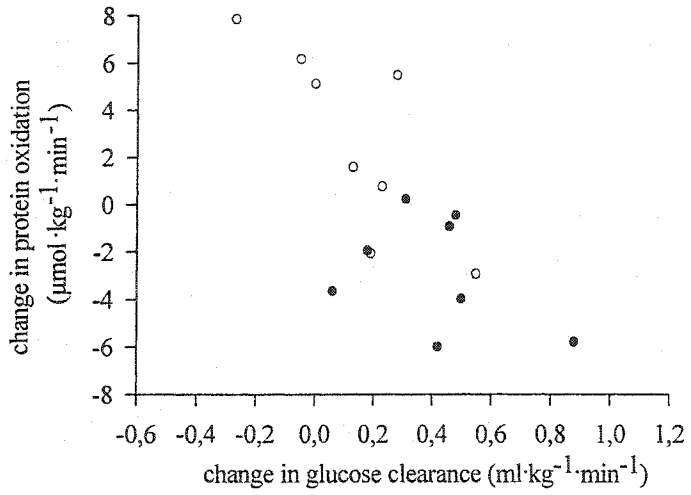

Figure 1.3: Relation between change in leucine oxidation and glucose clearance during dextrose infusion in patients two days after abdominal surgery. (o: PCA, •: epidural blockade, r = -0.735, p=0.002). (Schricker et al, 2000a).

Patients in this study were investigated on the second day after surgery. The effect of epidural blockade in combination with intravenous glucose administration upon protein catabolism during the acute phase of the surgical stress response, however, has not yet been studied.

2.2.2.2 Effect of epidural blockade on perioperative glucose metabolism

It has long been recognized that the combination of epidural blockade and general anesthesia inhibits or prevents the hyperglycemic response during operations on the lower part of the body, when compared to general anesthesia alone (colorectal surgery, gynecological laparotomy, prostatectomy, hip surgery and distal orthopedic procedures) (Buckley et al, 1982; Engquist et al, 1977; Kehlet et al, 1979; Kehlet, 1998; Nakamura et al, 1991).

The mechanisms involved in this modifying effect of neural blockade on perioperative glucose homeostasis and tolerance have not been fully clarified. Two biochemical mechanisms have been proposed to be of importance: First, the inhibition of the cortisol and catecholamine response to surgery may be a causative factor, since any reduction in the plasma levels of these counterregulatory hormones might improve glucose utilization. Intravenous glucose tolerance tests carried out during inguinal herniotomy or transabdominal hysterectomy performed under neural blockade were normal, while glucose tolerance was impaired during inhalational anesthesia (Jensen et al, 1980; Houghton et al, 1978). Second, blocking of the hepatic glucose release mediated through the abolished adrenaline response or blockade of efferent sympathetic neural pathways to the liver may be relevant.

Only few studies investigated the effect of epidural blockade with local anesthetics on the dynamics of perioperative glucose metabolism, i.e. glucose production and utilization. In patients undergoing open cholecystectomy the attenuated hyperglycemic response during combined general anesthesia and epidural blockade with bupivacaine, rather than during general anesthesia alone, could only partly be explained by a lower intraoperative glucose release from the splanchnic region (calculated from the splanchnic blood flow and the

arterio-hepatic venous difference of glucose) (Lund et al, 1986a). A suppressive effect of epidural blockade with local anesthetics on endogenous glucose production was also observed by Shaw, who studied surgical patients between one and five days after various surgical interventions (Shaw et al, 1987). This inhibitory influence of epidural blockade on postoperative glucose production was accompanied by a decrease in urea synthesis, an indirect parameter of protein catabolism.

Further investigations are necessary to elucidate the mechanisms of how epidural blockade prevents the hyperglycemic response that occurs during and immediately after abdominal surgery in patients receiving general anesthesia alone. Considering the established relationship between perioperative glucose and protein metabolism, any modification of glucose production by epidural blockade theoretically should have an impact on protein catabolism (Schricker et al, 2000). However, no data are currently available on the potential relationship between perioperative glucose and protein kinetics.

2.2.2.3 Effect of epidural blockade on the hormonal response

Neuraxial block of afferent and efferent signals with epidural local anesthetics, i.e. epidural blockade, blunts the neuroendocrine response to surgery, especially during operations below the umbilicus, as reflected in lower plasma concentrations of the counterregulatory hormones cortisol, glucagon and catecholamines (Kehlet, 1998). It is also well established that the attenuation of these anti-insulinergic hormones is associated with reduced insulin resistance after surgery (Uchida et al, 1988).

However, complete suppression of the stress response requires complete sympathetic and sensory blockade of the surgical site, such as can be provided with extensive epidural blockade with local anesthetic from T4 to S5 dermatome. Less extensive blockade attenuates the stress response only incompletely because of incomplete suppression of

sympathetic and somatic signals.

2.2.3 Nutrition in the surgical patient

The major goal of nutritional management of surgical patients is to promote wound healing and resistance to infection while preventing persistent loss of functional and structural proteins. The latter point is of utmost clinical relevance because survival of critically ill surgical patients has been shown to be inversely correlated with the loss of lean body mass (Kinney et al, 1983b). Furthermore, the length of time for return to normal physiologic function after discharge from the hospital is related to the extent of muscle catabolism that occurred during hospitalization (Wilmore, 1999). The principal underlying defect appears to be an accelerated rate of proteolysis, along with an insufficient increase in the rate of protein synthesis to fully restore protein balance (Carli et al, 1991).

2.2.3.1 Glucose administration

Intravenous administration of hypocaloric glucose, (glucose infused at a dose below the patient`s energy requirements), represent the primary way of feeding patients after abdominal surgery in North America. One important mechanism, whereby glucose exerts its nitrogen-sparing effect is the suppression of gluconeoegenesis, which reduces the need for gluconeogenic amino acids derived from muscle protein breakdown. The rationale of hypocaloric glucose infusion is based on findings by Gamble 50 years ago, who showed that 150g of glucose per day, quantitatively consistent with the obligatory need of the brain for glucose, causes optimal protein sparing under postabsorptive conditions (Gamble, 1946). However, there is evidence that the anticatabolic properties of hypocaloric feeding strategies observed in normal subjects do not apply to patients after surgical trauma. *Post*operative glucose infusion at 2 $mg \cdot kg^{-1} \cdot min^{-1}$, equivalent to 200 g

glucose per day, does not exert any effect upon negative nitrogen balance (Greenberg et al, 1976; Askanazi et al, 1981; Nordenström et al, 1983) and does not prevent the alterations in muscle and plasma amino acid concentrations after elective surgery (Askanazi et al, 1980). Hypocaloric glucose infusion also does not influence the enhanced whole body protein breakdown and amino acid oxidation after major trauma (Birkhahn et al, 1980) and does not attenuate the decrease in whole body protein synthesis after orthopedic operations (Crane et al, 1977).

Conflicting data have been reported in the literature regarding the impact of intraoperative glucose administration on protein metabolism. In one study, glucose infused at 10 g/h induced an increase in insulin secretion, accompanied by diminished plasma concentrations of branched-chain amino acids, especially leucine and isoleucine, during gastrectomy indicating a decrease in muscle proteolysis (Obata et al, 1993). In contrast another study did not report any change in the plasma concentrations of amino acids during open cholecystectomy when glucose was infused at 1.1 mmol/min (Lund et al, 1986b). In a study by Sieber, glucose infusion at 3 $mg \cdot kg^{-1} \cdot min^{-1}$ also did not produce any protein sparing effect as reflected by an unchanged nitrogen balance during and immediately after neurosurgical procedures (Sieber et al, 1989).

None of the studies investigating the anticatabolic action of various feeding strategies was controlled for the analgesic technique applied during and after surgery, albeit the preserving influence of epidural analgesia on protein and glucose homeostasis has long been established. Thus, the combined metabolic effect of epidural blockade and intravenous glucose during the acute phase of the surgical stress response remained unknown.

2.2.3.2. Insulin

The limited effectiveness of postoperative nutritional therapy alone has led to the investigation of various pharmacological approaches. The use of naturally occurring hormones such as insulin to promote protein anabolism is appealing because insulin is the body`s most important endocrine regulatory factor mediating the anabolic response to carbohydrate intake. Combined infusions of insulin and glucose have been demonstrated to significantly ameliorate protein catabolism in surgical and injured patients (Sakurai et al, 1995; Woolfson et al, 1979; Brandi et al, 1990). In order to overcome insulin resistance, a prominent feature of the endocrine response to surgery, insulin, however, has to be applied in high doses (Sakurai et al, 1995). At the same time provision of excessive amounts of glucose is required to maintain normoglycemia. This issue raises potential metabolic concerns, because excessive carbohydrate and caloric intake might cause fatty infiltration of the liver (Hall et al, 1984) and significantly stimulate carbon dioxide production (Askanazi et al, 1981).

Elective surgery is routinely performed after overnight fast to ensure an empty stomach and to minimize the risks of aspiration, when general anesthesia is induced. The restriction of food before elective surgery is usually set from midnight on the day of operation. Fasting periods before colorectal surgery, however, usually amount to a much longer time (up to 40 hours), because bowel preparation on the preoperative day does not allow any oral food intake. Therefore, fasting periods before abdominal operations are often long enough to substantially deplete carbohydrate reserves and change the metabolic situation of the patient (Nygren et al, 1997). The rationale for fasting patients before surgery has been questioned recently, as it could be shown that perioperative glucose infusion improved insulin sensitivity shortly after surgery. Glucose administered

at 5 $mg \cdot kg^{-1} \cdot min^{-1}$ the night before open cholecystectomy (Ljungvist et al, 1994) and glucose infused at 4 $mg \cdot kg^{-1} \cdot min^{-1}$ during total hip replacement (Nygren et al, 1998) normalized impaired postoperative insulin sensitivity as assessed by a hyperinsulinemic normoglycemic clamp technique.

Although early postoperative restoration of insulin function represents a key factor for the normalization of impaired glucose homeostasis, it remained unclear if the improvement of insulin sensitivity also exerts a positive influence upon perioperative protein catabolism.

In summary, epidural blockade as well as the administration of glucose have been shown to have anticatabolic properties in the surgical patient, strongly depending on when and how these measures are performed. An integrated analysis of their individual and combined effects on the kinetics of glucose and protein metabolism during surgery has not yet been performed.

Therefore, two consecutive studies were performed: the first study (I) was designed to examine the effects of epidural blockade with bupivacaine (from T4 to S5 dermatome) on the kinetics of protein and glucose metabolism in the absence of perioperative glucose infusion.

The second study (II) investigated the combined effect of epidural blockade and intravenous glucose at 2 $mg \cdot kg^{-1} \cdot min^{-1}$ on the catabolic response to surgery from before to two hours after abdominal surgery.

3. Hypotheses and objectives

3.1 Hypotheses

The purpose of this project was to investigate the effect of epidural blockade on protein and glucose metabolism during the acute phase of the stress response in the fasted state and during intravenous glucose administration. Therefore, two consecutive studies were performed.

In the first study (I), it was hypothesized that epidural blockade together with general anesthesia compared to general anesthesia alone inhibits the hyperglycemic response to surgery through the inhibition of endogenous glucose production. It was further determined if the modification of glucose production by epidural blockade has an impact on perioperative protein catabolism.

The second study (II) tested the hypothesis that the suppression of endogenous glucose production by intravenous glucose at 2 $mg \cdot kg^{-1} \cdot min^{-1}$ is more pronounced in patients with epidural blockade than in patients receiving general anesthesia alone. It was also examined if a greater decrease in glucose production would lead to a more accentuated inhibition of protein catabolism, i.e. proteolysis and protein oxidation.

3.2 Objectives

The objectives of the proposed study were

- to examine the effects of epidural blockade with local anesthetic on perioperative glucose and protein kinetics in the fasted state.
- to investigate the modifying effect of epidural blockade on glucose and protein metabolism during intravenous administration of glucose 2 $mg \cdot kg^{-1} \cdot min^{-1}$.

In order to gain insight into dynamic biochemical pathways, stable isotope tracers are applied to assess dynamic changes in glucose and protein metabolism, i.e. glucose production, glucose utilization, protein breakdown, protein oxidation and synthesis.

4. Methodology

4.1 Metabolic assessment by stable isotope tracer technique

Clinical decisions in endocrinology are based most frequently on alterations in the plasma levels of circulating substrates. Whilst generally adequate for clinical management purposes, observations based on metabolite plasma levels alone allow no insight into the dynamic biochemical events that produced the observed value. Since, for instance, an increased blood glucose concentration might be the consequence of accelerated glucose release, diminished glucose uptake or both, inferences made from static observations can be misleading.

The turnover rates of glucose and amino acids can be followed *in vivo* by using stable isotope tracer dilution technique. The basic principle of these tracer measurements is the simple concept of tracer dilution. Substrates, which have been labelled by stable, non-radioactive isotopes (^{2}H, ^{13}C) (i.e. [6,6-$^{2}H_2$]glucose and L-[1-^{13}C]-leucine), are administered as a primed continuous infusion until steady state conditions are achieved. The time required to reach isotopic steady state in plasma and expired air can be reduced to approximately two hours by administering appropriate priming doses of [6,6-$^{2}H_2$]glucose and L-[1-^{13}C]-leucine. The turnover rates can then be calculated from the measurements of isotopic enrichments in plasma, as measured by gas chromatography-mass spectrometry.

4.1.1 Protein metabolism

4.1.1.1 Nitrogen balance

The degree of protein catabolism in surgical patients is frequently characterized by the calculation of net nitrogen balances, that is the difference between the nutritive nitrogen

input and the excretion of nitrogen. While the nitrogen content in urine and total daily renal nitrogen excretion can be reliably measured under clinical conditions, nitrogen losses through drains, wound secretion and faeces cannot be quantified. Another shortcoming of nitrogen balance measurements is that the contribution from alterations in protein synthesis and in protein degradation cannot be separated.

4.1.1.2 L-[1-^{13}C]leucine

In vivo turnover of an individual amino acid can be determined by measuring the dilution of a continuously infused labelled amino acid at isotopic steady state. To characterize the kinetics of protein metabolism in vivo (i.e. protein breakdown, amino acid oxidation and protein synthesis), an isotope dilution technique has been developed using a primed continuous infusion of labelled L-[1-^{13}C]leucine (Matthews et al, 1980). There are several reasons why the leucine tracer system remains the single most applied measure of whole-body amino acid kinetics: in contrast to other essential amino acids that are metabolized primarily in the liver, leucine is catabolized primarily in the muscle, the body`s principal protein reservoir (Odessey et al, 1972; Young, 1970). Furthermore, leucine has a regulatory effect on the other two branched-chain amino acids and on muscle protein synthesis and degradation, plays an important role in oxidative metabolism, and represents a principal source of the alpha-amino nitrogen of gluconeogenic amino acids released from muscle (Adibi, 1976; Hambraus et al, 1976; Odessey et al, 1976).

The flux of leucine represents the total movement of leucine into and from the plasma pool. Under steady state conditions leucine flux (Q) is defined by the equation: Q = S + O = B + I, where S is the rate at which leucine is incorporated into body protein, O is the rate of leucine oxidation, B is the rate at which unlabeled leucine enters the free amino acid pool from endogenous protein breakdown, and I is the rate of leucine intake

including tracer and diet. In the postabsorptive state, the sole source of the essential amino acid leucine for protein synthesis and oxidation is that derived from the breakdown of endogenous proteins. When studies are conducted in the absence of dietary amino acid intake, leucine flux is equal to leucine breakdown.

The ^{13}C of an L-[1-^{13}C]leucine tracer is quantitatively released at the first irreversible step of leucine catabolism. Before the oxidized carbon label is recovered as $^{13}CO_2$ in exhaled air, it must pass through the body bicarbonate pool. In order to account for the fraction of $^{13}CO_2$ released by L-[1-^{13}C]leucine oxidation but not released from the body bicarbonate pool into expired air, correction factors of 0.76 for the fasted state and 0.81 for the fed state were used in the calculation of oxidation (Matthews et al, 1980). The rate at which leucine is incorporated into body protein (protein synthesis) can then be calculated by subtracting leucine oxidation from the total leucine flux.

4.1.2 Glucose metabolism

The kinetics of whole body glucose metabolism, i.e. endogenous glucose production and glucose clearance can be assessed by primed continuous infusion of the deuterated tracer [6,6-$^2H_2$]glucose (Kalhan, 1996). Whole body glucose production is, depending on the metabolic state, composed to a varying extent of glycogenolysis and gluconeogenesis. The use of [6,6-$^2H_2$]glucose does not allow to differentiate between the two metabolic pathways. Patients undergoing colorectal surgery usually take their last meal in the evening two days before the operation. Thus, patients entering the protocol of out studies were fasting for approximately 36 hours, when preoperative endogenous glucose production was measured. According to the results of a recent study in healthy volunteers gluconeogenesis accounted for almost all of glucose production after 42 hours of fasting (Landau et al, 1996; Chandramouli et al, 1997). Thus, the endogenous glucose production

rates measured on the day of colorectal surgery are presumably equivalent to gluconeogenesis.

In the physiologic steady state, the rate of endogenous glucose production equals whole body glucose uptake. Because glucose uptake increases proportionally as blood glucose concentrations rise, changes in whole body glucose uptake do not necessarily reflect corresponding changes in the tissue ability to take up glucose. This may be because most glucose is taken up in non-insulin-sensitive tissues, and the rate of uptake is to a large extent determined by the diffusion gradient for glucose. Thus, the rate of glucose uptake has to be corrected for the prevailing plasma glucose concentration. The resulting value, the glucose clearance rate calculated by dividing endogenous glucose production by the corresponding plasma glucose concentration, represents an index of the ability of tissues to take up glucose (Wolfe, 1993). In contrast to glucose clamp techniques and glucose tolerance tests, which require the administration of high amounts of exogenous glucose and insulin with potential impact on glucose metabolism *per se*, isotope dilution techniques infusing only small amounts of isotope tracers do not affect the metabolic milieu itself.

4.1.3 Indirect calorimetry

Oxygen consumption (VO_2) and carbon dioxide production (VCO_2, for the calculation of leucine oxidation rate) were measured before and after surgery by indirect calorimetry using the open system indirect calorimetry device Deltatrac Metabolic Monitor (Datex Instrumentarium, Helsinki, Finland). VCO_2 was also determined during the operation (70 min after skin incision). The values of VO_2 and VCO_2 and the calculated respiratory quotient (RQ) represent an average of the data obtained over a period of 20 min on each occasion, with a coefficient of variation <10%.

EPIDURAL BLOCKADE MODIFIES PERIOPERATIVE GLUCOSE PRODUCTION WITHOUT AFFECTING PROTEIN CATABOLISM

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5.1 Abstract

Background: Epidural blockade with local anesthetic has been shown to blunt the increase in plasma glucose concentration during and after abdominal surgery. The aim of the study was to test the hypothesis that epidural blockade inhibits this hyperglycemic response by attenuating endogenous glucose production. We further examined if the modification of glucose production by epidural blockade has an impact on perioperative protein catabolism.

Methods: Sixteen patients undergoing colorectal surgery received either general anesthesia and epidural blockade with local anesthetic (n=8) or general anesthesia alone (control, n=8). Glucose and protein kinetics were assessed by stable isotope tracer technique ([6,6-$^{2}H_2$]glucose, L-[1-^{13}C]leucine) during and two hours after surgery. Plasma concentrations of glucose, lactate, free fatty acids (FFA), cortisol, glucagon and insulin were also determined.

Results: Epidural blockade blunted the perioperative increase in the plasma concentration of glucose, cortisol and glucagon when compared to the control group (P<0.05). Plasma concentrations of lactate, FFA and insulin did not change. Intra- and postoperative glucose production was lower in patients with epidural blockade than in control subjects (Intraoperative: epidural blockade 8.2±1.9 vs. control 10.7±1.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, P<0.05; postoperative: EDA 8.5±1.8 vs. control 10.5±1.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, P<0.05), while glucose clearance decreased to a comparable extent in both groups (P<0.05). Protein breakdown (P<0.05), protein synthesis (P<0.05) and amino acid oxidation (P>0.05) decreased with both anesthetic techniques.

Conclusions: Epidural blockade attenuates the hyperglycemic response to surgery through modification of glucose production. The perioperative suppression of protein metabolism was not influenced by epidural blockade.

5.2 Introduction

Surgical tissue trauma is associated with stereotypical alterations in carbohydrate and protein metabolism often referred to as the catabolic response (Weissman, 1990). A characteristic feature of impaired carbohydrate homeostasis in the context of surgery is hyperglycemia, a consequence of stimulated glucose production and impaired glucose utilization (Mizock, 1995). Numerous studies demonstrate that the hyperglycemic response to surgery can be influenced by the anesthetic technique (Schricker et al, 1998). Neuraxial block of afferent and efferent signals with epidural local anesthetics, i.e. epidural blockade, has been shown to attenuate the increase in the plasma glucose concentration during abdominal surgery, most likely mediated through its inhibitory action upon the hypothalamopituitary-adrenal stress response (Kehlet, 1998). Because measurements of plasma glucose concentrations alone do not allow to distinguish between changes in the production and utilization of glucose, the dynamic biochemical changes responsible for this modifying effect of epidural blockade remained unclear. Studies carrying out intravenous glucose tolerance tests during pelvic procedures suggest that epidural blockade is associated with improved whole body glucose uptake (Houghton et al, 1978; Jensen et al, 1980). In contrast more recent findings provide evidence that epidural blockade exerts a suppressive effect on hepatic glucose release without affecting tissue glucose utilization (Lund et al, 1986a; Shaw et al, 1987).

Gluconeogenesis accounts for more than 90% of total glucose production under perioperative conditions, due to long preoperative fasting periods with subsequent exhaustion of endogenous glycogen stores and the surgical stress induced release of gluconeogenic catecholamines, glucagon and cortisol (Chandramouli et al, 1997; Gump et

al, 1975). Gluconeogenic amino acids released during muscle proteolysis become the major source of precursors for *de novo* glucose synthesis in surgical patients (Wolfe, 1993). Therefore, it has been proposed that any inhibition of gluconeogenesis by anesthetic or pharmacological interventions will cause a decrease in protein breakdown leading to a better preservation of whole body protein (Wolfe, 1993; Schricker et al, 2000a). The validity of this assumption is underscored by the results of previous studies showing that maintenance of perioperative glucose homeostasis by epidural blockade is followed by improved protein economy, as reflected by a significant reduction of urinary nitrogen excretion, protein breakdown and amino acid oxidation (Brandt et al, 1978; Carli et al, 1991; Vedrinne et al, 1989). Although the impact of epidural blockade upon postoperative protein economy appears to be well characterized, its potential role in modifying protein catabolism during the acute phase of surgical trauma has not been addressed.

The purpose of the present protocol was to test the hypothesis that epidural blockade with local anesthetic blunts the hyperglycemic response to surgery through attenuation of endogenous glucose production. It was further attempted to determine if the modification of glucose production is associated with changes in protein metabolism, e.g. protein breakdown, protein synthesis and amino acid oxidation. To gain an integrated insight into the catabolic responses to surgery perioperative glucose and protein kinetics were assessed by a stable isotope dilution technique using primed continuous infusions of [6,6-$^{2}H_{2}$]glucose and L-[1-^{13}C]leucine.

5.3 Methods

With the approval of the Ethics Committee of the Royal Victoria Hospital 16 patients undergoing elective colorectal surgery for non-metastatic carcinoma were admitted to the study. Written informed consent was obtained from all patients. All patients had a Body Mass Index (BMI) between 20 and 27 $kg \cdot m^{-2}$ and maintained their body weight over the preceding three months (<5% weight loss). Exclusion criteria were any cardiac, hepatic, renal, endocrine or metabolic disorders, ingestion of any medication known to affect metabolism and history of severe sciatica or back surgery which contraindicates the use of epidural catheters. We further excluded patients with a plasma albumin concentration <35 g/l, with anemia (hemoglobin <10 $g \cdot dl^{-1}$) and patients who received treatment with chemotherapy during six months prior to the date of surgery.

Anesthesia

Patients were randomly assigned to receive either a combination of epidural blockade with bupivacaine and general anesthesia (EDA group) or general anesthesia alone (control group).

In the EDA group an epidural catheter was inserted at a thoracic level between T 9 and T 11 before the operation. Afferent neural blockade was established with 0.5% bupivacaine to achieve a bilateral sensory block from T 4 to S 5 as judged from perception of pinprick and maintained with intermittent boluses of bupivacaine 0.5%. General anesthesia in all patients was induced by 5 mg/kg thiopentone and 1.5 μg/kg fentanyl in the EDA group or 5 μg/kg fentanyl in the control group, respectively. Endotracheal intubation was facilitated with 0.6 mg/kg rocuronium and patients` lungs were ventilated with 30% oxygen in air to maintain normocapnia. No nitrous oxide was used since it has the same

molecular weight as $^{13}CO_2$ and thus would interfere with the isotope ratio measurement of expired $^{13}CO_2$. General anesthesia in the control group was maintained using desflurane at endtidal concentrations as required to keep heart rate within 20% of preoperative values. In the EDA group desflurane was administered at endtidal concentrations of approximately 3 Vol% in order to achieve tolerance of the endotracheal tube and to prevent awareness. The degree of muscle relaxation was monitored using train-of-four ratio and supplemental doses of rocuronium were applied as needed for complete surgical muscle relaxation. Fluid was given as NaCl 0.9% solution at a rate of 10 $ml \cdot kg^{-1} \cdot h^{-1}$ intraoperatively and 6 $ml \cdot kg^{-1} \cdot h^{-1}$ thereafter. During surgery the patients were covered with a warming blanket to maintain normothermia. For pain control after surgery patients in the control group received morphine i.v.. In the epidural group, postoperative analgesia was performed with epidural bupivacaine 0.25% as required to maintain sensory blockade from T8 to L3. Hemodynamic monitoring was performed using a three lead electrocardiogram monitor and radial artery catheterization for continuous blood pressure measurement.

Study protocol

The rates of appearance of glucose (R_a glucose, endogenous glucose production) and leucine (R_a leucine) were determined before, during and two hours after surgery by stable isotope tracer technique using primed continuous infusions of [6,6-$^2H_2$]glucose and L-[1-^{13}C]leucine (Cambridge Isotope Laboratories, Cambridge, MA). Sterile solutions of the isotopes were prepared by the hospital pharmacy. The bottles were heat sterilized at 121°C for 15 min and kept refrigerated at 4°C until administration. Before the infusion

study, each set of solutions was confirmed to be free of pyrogens (LAL Pyrogent Test, Whittaker Bioproducts, Walkersville, MD).

All tests were performed on the day of surgery beginning between 07:00 and 08:00 after fasting for approximately 36 hours. Due to bowel preparation as required for colorectal procedures, patients received only clear fluids until midnight the day preceding the operation. A catheter was placed in a superficial vein in the dorsum of the hand and kept patent with saline 0.9% (2 $ml \cdot kg^{-1} \cdot h^{-1}$). A superficial vein of the contralateral arm was cannulated to provide access for the infusion of [6,6-$^2H_2$]glucose and L-[1-^{13}C]leucine. Blood and expired air samples were taken to determine baseline enrichments. Thereafter, priming doses of $NaH^{13}CO_3$ 1 μmol/kg, L-[1-^{13}C]leucine 4 μmol/kg and [6,6-$^2H_2$]glucose 22 μmol/kg were administered followed immediately by continuous infusions of [6,6-$^2H_2$]glucose 0.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and L-[1-^{13}C]leucine 0.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively (Schricker et al, 2000a). Isotope infusion was uninterrupted throughout the entire study period. Expired breath and blood samples for the determination of isotopic enrichments as well as for the measurement of metabolic substrates (glucose, lactate, free fatty acids (FFA)) and hormones (insulin, glucagon, cortisol) were collected as indicated in figure 1. Breath samples were collected through a mouthpiece in a 3-l bag and transferred immediately to 10-ml vacutainers until analysis. During controlled ventilation, expired gases were collected by a one-way valve into a 5-l bag. Blood samples were immediately transferred to a heparinized tube, centrifuged at 4 °C (3,000 g, 15 min) and the obtained plasma was stored at -70°C until analysis.

Gaseous Exchange

Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured before and after surgery by indirect calorimetry using the open system indirect calorimetry device Deltatrac Metabolic Monitor (Datex Instrumentarium, Helsinki, Finland). VCO_2 was also determined during the operation (70 min after skin incision). The values of VO_2 and VCO_2 and the calculated respiratory quotient (RQ) represent an average of the data obtained over a period of 20 min on each occasion, with a coefficient of variation < 10%.

Analyses

After derivatization of plasma glucose to its pentaacetate compound, the [6,6-$^2H_2$]glucose enrichment was quantified by gas chromatography-mass spectrometry using electron-impact ionization (Schricker et al, 1996). Plasma α-[1-^{13}C]ketoisocaproate (α-[1-^{13}C]KIC) enrichment was analyzed by electron-impact selected-ion monitoring gas chromatography-mass spectrometry as described earlier, except that t-butyldimethylsylyl rather than trimethylsylyl derivatives were prepared (Mamer et al, 1988). Expired $^{13}CO_2$ enrichment for the calculation of leucine oxidation was analyzed by isotope ratio mass spectrometry (Analytical Precision AP2, 003, Manchester, UK).

Plasma concentrations of glucose were quantified using a glucose analyzer 2 (Beckman Instruments, Fullerton, CA) based on a glucose oxidase method. The plasma lactate assay was based on lactate oxidase using the synchron CX 7 system (Beckman Instruments, Fullerton, CA). Circulating concentrations of FFA were measured by an enzymatic assay (Boehringer Mannheim, Laval, Quebec, Canada). Plasma cortisol, insulin and glucagon

concentrations were analyzed by means of double antibody radioimmunoassays (Amersham International, Amersham, Bucks, UK).

Calculations

During physiologic and isotopic steady state, the rate of appearance of unlabeled substrate can be derived from the plasma isotope enrichment (APE = atom percent excess) calculated by: $R_a = I \cdot (APE_{inf} / APE_{pl} - 1)$, where APE_{inf} is the tracer enrichment in the infusate, APE_{pl} is the tracer enrichment in plasma and I is the infusion rate of the labeled tracer. The APE values used for the calculation of the rate of appearance were the average of four (pre- and postoperative) and five (intraoperative) APE measurements. Steady state conditions were assumed when the coefficient of variation (CV) of the APE values at isotopic plateau was < 5%.

During steady state conditions, leucine flux is defined by the equation: Q = S + O = B + I, where S is the rate of leucine uptake for protein synthesis, O is the rate of leucine oxidation, B is the rate at which leucine enters the free amino acid pool from endogenous protein breakdown and I is the rate of leucine intake, including tracer and diet. Therefore, under postabsorptive conditions when there is no exogenous leucine entering the plasma pool, the only source of leucine is that derived from endogenous protein breakdown and consequently, the rate of leucine breakdown equals leucine flux (Matthews et al, 1980). Plasma α-[1-^{13}C]KIC enrichment was used for calculating both flux and oxidation of leucine, because it has been demonstrated to reflect the intracellular precursor pool enrichment more precisely than leucine itself (Schwenk et al, 1985). In the calculation of leucine oxidation, a correction factor of 0.76 was used to account for the fraction of $^{13}CO_2$

released from leucine but retained within slow turnover rate pools of the body (Matthews et al, 1980).

Under steady state conditions, whole body glucose uptake equals the rate of endogenous glucose production. However, glucose uptake rises with increasing blood glucose concentration because most glucose uptake occurs in non-insulin sensitive tissues and consequently depends on the diffusion gradient of glucose. Therefore, whole body glucose uptake is not an accurate measure of the tissue`s ability to take up glucose. The fractional glucose clearance rate represents an index of the tissue`s capacity to take up glucose. The plasma clearance rate of glucose was calculated as the R_a glucose divided by the corresponding plasma glucose concentration. During surgery, the average of two plasma glucose concentration measurements at 60 and 100 min after skin incision was used for the calculation.

Statistical Analysis

The sample size calculation was based on the results of a previous report demonstrating a 50% reduction of splanchnic glucose release by epidural blockade (Lund et al, 1986a). For an expected difference in glucose production of 25% between the groups (power 80%, $\alpha = 5\%$), a total of 16 patients was calculated to be sufficient. Differences between the groups were analyzed using the Mann Whitney U-test. Within-group comparison of variables was made by analysis of variance for repeated measures with *post-hoc* analysis by Student-Newman-Keuls-test. The relationships between the R_a leucine and R_a glucose were evaluated by the correlation coefficient. A probability of $P<0.05$ was considered to be significant. Data are presented as means ± SD.

5.4 Results

The two groups were comparable with regard to patients` age, height and weight, gender and ASA classification (table 1). There were no differences between the groups in duration of surgery, estimated blood loss and the total amount of cristalloid fluid administered throughout the study period.

During EDA heart rate and mean arterial blood pressure decreased and remained lower than in the control group ($P<0.05$, table 2). The end-tidal desflurane concentration was lower in the EDA group than in the control group at 60 min (EDA 2.9 ± 0.4 Vol%, control 5.5 ± 1.2 Vol%, $P<0.05$) and 100 min after skin incision (EDA 2.9 ± 0.3 Vol%, control 5.5 ± 1.2 Vol%, $P<0.05$).

In both groups, plasma glucose concentration increased during and after surgery ($P<0.05$, table 3) revealing higher values in the control group than in the EDA group ($P<0.05$). In all patients, an isotopic plateau of [6,6-$^2H_2$]glucose, α-[1-^{13}C]KIC and expired $^{13}CO_2$ was achieved (CV <5%, figure 2). Intra- and postoperative R_a glucose were lower in the EDA group than in control subjects ($P<0.05$). Plasma glucose clearance decreased with both anesthetic techniques ($P<0.05$) without showing a difference between the two groups. R_a leucine and protein synthesis decreased during and after surgery ($P<0.05$) to a similar extent in both groups. Leucine oxidation intraoperatively decreased in all patients, followed by an increase after surgery ($P>0.05$). A correlation between the R_a leucine and R_a glucose was observed in the EDA group ($r = 0.504$, $P<0.05$, figure 3A), while no correlation could be detected in the control group ($r = -0.198$, $P>0.05$, figure 3B).

Whole body oxygen consumption, carbon dioxide production and the respiratory quotient did not change during the study period (table 4).

Plasma lactate and FFA concentrations remained unaltered with both anesthetic techniques (table 5). There was no perioperative change in plasma insulin concentration. Plasma cortisol concentrations increased throughout the study in both groups ($P<0.05$) with lower values during EDA when compared to the control group ($P<0.05$). Intraoperative plasma glucagon levels were lower in patients receiving EDA than in the control group ($P<0.05$).

5.5 Discussion

Considerable attention has been given to the preservation of glucose homeostasis in surgical patients because acute hyperglycemia, a typical feature of the metabolic response to surgery, has been demonstrated to significantly compromize immune function (Rassias, et al, 1999) and to contribute to poor clinical outcome after cardiopulmonary resuscitation (Longstreth et al, 1984) and cerebral ischemia (Pulsinelli et al, 1983) in humans. It has long been recognized that epidural blockade with local anesthetic, established before and maintained after the operation, prevents or blunts the hyperglycemic response to surgery (Kehlet, 1998; Lund et al, 1986a; Engquist et al, 1977). It remained unclear, however, if this inhibitory effect of epidural blockade was a consequence of a decrease in glucose production, an increase in glucose utilization or a combination of both. The major finding of the present study is that patients receiving epidural local anesthetic showed lower intra- and postoperative R_a glucose than subjects in the control group. The absolute rate of glucose production during epidural blockade was identical to values recently reported during hip surgery performed under intrathecal neuraxial blockade (Nygren et al, 1998). Glucose clearance in our study, an indicator of whole body glucose uptake, decreased to a comparable extent with both anesthetics, lending support to the contention that epidural blockade attenuates the hyperglycemic response by modifying glucose production without affecting endogenous tissue glucose utilization. This conclusion corroborates the previous observation of a suppressive effect of epidural local anesthetics on glucose production in parenterally fed patients studied between one and five days after various surgical interventions (Shaw et al, 1987a). The present results are also in accordance with a recent investigation describing an inhibitory influence of epidural blockade on

splanchnic glucose release during cholecystectomy (Lund et al, 1986a). Because splanchnic glucose production, calculated from the splanchnic blood flow and the arteriohepatic venous plasma concentration difference of glucose, does not account for the metabolic activity, i.e. glucose uptake by the gut, this parameter does not represent an accurate measure of whole body glucose production. The fact that all subjects entering the latter study's protocol were continuously given intravenous dextrose further limits the validity of its conclusions.

Whole body glucose production is composed to a varying degree of glycogenolysis and gluconeogenesis. The use of [6,6-$^2H_2$]glucose, as in the present protocol, does not allow differentiation between the two biochemical pathways. It seems conceivable, however, that gluconeogenesis is activated in patients undergoing colorectal procedures, as a consequence of the long preoperative fasting period (due to bowel preparation) and concurrent depletion of hepatic glycogen stores (Gump et al, 1975). After forty-two hours of fasting, gluconeogenesis contributes to over 90% of the glucose produced in normal subjects (Chandramouli et al, 1997). In surgical patients the rate of this process is further enhanced by the over-production of counterregulatory hormones such as catecholamines, cortisol and glucagon, which all stimulate gluconeogenesis, either directly or indirectly by counteracting the action of insulin (Bessey et al, 1984). Gluconeogenesis in the liver is a highly oxygen-consuming process accounting for 50% of hepatic oxygen consumption under postabsorptive conditions (Jungas et al, 1992). Thus, the decrease in glucose production as seen during epidural blockade assumes clinical relevance with regard to the energy balance of the liver. Furthermore, gluconeogenesis has been proposed to occupy a central position in catabolic pathways because muscle protein is being hydrolyzed to supply glucose precursors *via* the glucoplastic amino acids (Wolfe, 1993). Hence, it is

generally believed that by reducing accelerated gluconeogenesis, amino acids derived from muscle protein breakdown are being directed into anabolic pathways resulting in less protein loss (Schricker et al, 2000a). Confirming this link between perioperative glucose and protein metabolism, we observed a weak, however significant correlation between the R_a leucine and R_a glucose in presence of epidural blockade (Schricker et al, 2000a; Schricker et al, 2001). In contrast, no correlation between the two metabolic pathways was detected in the control group emphasizing the notion that there are more factors than need for gluconeogenic precursors that regulate protein breakdown and gluconeogenesis during surgery. Whole body protein breakdown, synthesis and amino acid oxidation intraoperatively decreased in all patients, independent of the anesthetic technique employed. This depression of protein metabolism is in line with previous studies showing similar decreases in protein breakdown (Schricker et al, 2001; Carli et al, 1990; Rennie et al, 1985) and diminished rates of intraoperative protein synthesis in the whole body as well as in specific organ tissues (liver, muscle) (Barle et al, 1999; Essen et al, 1992). It was also concluded that the effect of anesthetics on protein catabolism during the acute phase of surgical tissue trauma is small (Schricker et al, 2001).

Because the present protocol was not designed to elucidate underlying mechanisms, we can only speculate about potential endocrine, biochemical and hemodynamic factors that may have been responsible for the metabolic effects of epidural blockade and surgery in our study.

Much of the catabolic responses to surgical trauma has been ascribed to alterations in the endocrine milieu, in particular to the elevated plasma concentrations of counterregulatory hormones, all of which promote hyperglycemia by stimulating glucose production and decreasing glucose utilization. In accordance with previous reports epidural blockade

attenuated the neuroendocrine response during surgery as reflected by lower plasma cortisol and glucagon concentrations when compared to the control group (Kehlet, 1998; Lund et al, 1986a; Kehlet et al, 1979). Although plasma catecholamine levels were not determined in the present protocol, the hemodynamic pattern in the EDA group characterized by a diminished heart rate and arterial blood pressure, strongly suggests efferent blockade of sympathoadrenergic fibres, which has been frequently described during thoracic epidural blockade (Kehlet, 1998; Engquist et al, 1977). While the modification of the endocrine responses by epidural local anesthetic fits well with the observed alterations in glucose metabolism, it fails to explain the intraoperative changes in protein metabolism, which occurred independent of the type of anesthesia. Considering the well known catabolic action of cortisol, the 30% decrease in protein breakdown and amino acid oxidation in presence of increased cortisol levels appears to be paradoxical. It has to be noted, however, that the catabolic effects of corticosteroids are unlikely to take effect within two to four hours. Cortisol administration has been shown to have only little influence on nitrogen loss, protein breakdown and amino acid oxidation during the first 24 hours in healthy subjects (Gelfand et al, 1984). Because insulin represents the key endocrine regulator of protein metabolism, alterations in the perioperative plasma concentration of insulin gain metabolic importance. High spinal anesthesia (T2) after single shot intrathecal application of local anesthetic has been shown to impair the insulin response to an intravenous dextrose load, while basal plasma insulin concentrations were not affected (Halter et al, 1980). In agreement with previous studies conducted in healthy subjects and surgical patients receiving thoracic-lumbar dermatome blockade, plasma insulin levels did not change in our subjects, neither in the epidural nor in the control group (Hakanson et al, 1982; Schricker, 2000b). Thus, alterations in the insulin system

seem an unlikely cause for the intraoperative decrease in protein catabolism. It is interesting to note that hyperglycemia *per se*, as seen during surgery, has been associated with a decrease in protein breakdown independent of the action of insulin (Wolfe et al, 1986).

Animal studies have shown that both local ischemia and hypoxia exert suppressive effects on muscle protein metabolism (MacLennan et al, 1989). Even though patients were hemodynamically stable, well hydrated and oxygenated during surgery, it cannot be ruled out entirely that reduced peripheral muscle perfusion and subsequent regional depression of protein metabolism contributed to the overall effect in the whole body.

We conclude that epidural blockade attenuates the hyperglycemic response to abdominal surgery through the modification of glucose production without affecting glucose utilization. The depression of protein metabolism during and immediately after surgery occurs independent of the anesthetic technique employed.

5.6 Appendix

Table 5.1 Patient Characteristics

	Control	EDA
Number (n)	8	8
Age (years)	60 ± 16	64 ± 13
Height (cm)	164 ± 9	164 ± 6
Weight (kg)	66 ± 15	67 ± 15
Gender (male / female)	6 / 2	7 / 1
ASA (I / II / III)	2 / 6 / 0	2 / 5 / 1
Type of surgery		
Hemicolectomy / Colectomy	5	2
Sigmoid Resection	1	3
Anterior Resection	1	3
Ileocolic Resection	1	0
Duration of Surgery (min)	127 ± 29	118 ± 19
Estimated blood loss (ml)	181 ± 80	263 ± 138
Cristalloids (ml)	2938 ± 417	3063 ± 320

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade.

ASA = American Society of Anesthesiologists` classification .

Table 5.2 Hemodynamics, SpO_2

	Before surgery	During surgery I	During surgery II	After surgery
Heart rate (beats/min)				
Control	79 ± 10	75 ± 11	75 ± 10	77 ± 9
EDA	71 ± 11	$58 \pm 6^{+*}$	$60 \pm 7^{+*}$	$65 \pm 9^{*}$
Mean arterial pressure (mmHg)				
Control	88 ± 13	88 ± 7	89 ± 6	91 ± 14
EDA	94 ± 17	$67 \pm 8^{+*}$	$69 \pm 6^{+*}$	$76 \pm 9^{+*}$
SpO_2 (%)				
Control	98 ± 1	99 ± 1	100 ± 1	99 ± 1
EDA	97 ± 1	99 ± 1	99 ± 1	98 ± 1

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade.

SpO_2 = transcutaneous oxygen saturation.

I = 60 min, II = 100 min after skin incision.

$^{+}$ = $P<0.05$ vs. before surgery. * = $P<0.05$ vs. control.

Table 5.3 Glucose and Leucine Metabolism

	Before surgery	During surgery	After surgery
Glucose (mmol/l)			
Control	4.8 ± 0.7	7.0 ± 0.9^{+}	7.3 ± 1.1^{+}
EDA	5.0 ± 0.7	5.7 ± 0.5^{+*}	6.2 ± 0.4^{+*}
R_a Glucose ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
Control	9.9 ± 1.3	10.7 ± 1.4	10.5 ± 1.2
EDA	9.6 ± 2.0	8.2 ± 1.9*	8.5 ± 1.8*
Glucose Clearance ($ml \cdot kg^{-1} \cdot min^{-1}$)			
Control	2.1 ± 0.4	1.5 ± 0.3^{+}	1.5 ± 0.2^{+}
EDA	1.9 ± 0.3	1.4 ± 0.3^{+}	1.3 ± 0.2^{+}
R_a Leucine ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)			
Control	101 ± 15	78 ± 15^{+}	84 ± 17^{+}
EDA	97 ± 18	72 ± 13^{+}	74 ± 14^{+}
Leucine oxidation ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)			
Control	13 ± 4	10 ± 2	14 ± 4
EDA	13 ± 6	10 ± 7	12 ± 7
Protein synthesis ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)			
Control	88 ± 12	68 ± 15^{+}	70 ± 15^{+}
EDA	84 ± 20	62 ± 14^{+}	62 ± 15^{+}

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade. R_a = rate of appearance.
$^{+}$ = $P<0.05$ vs. before surgery. * = $P<0.05$ vs. control.

Table 5.4 Gaseous Exchange

	Before surgery	During surgery	After surgery
VO_2 (ml/min)			
Control	199 ± 57	-	210 ± 57
EDA	196 ± 23	-	221 ± 55
VCO_2 (ml/min)			
Control	157 ± 43	150 ± 30	160 ± 41
EDA	152 ± 22	137 ± 39	167 ± 38
RQ			
Control	0.79 ± 0.02	-	0.79 ± 0.05
EDA	0.77 ± 0.04	-	0.76 ± 0.05

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade.

VO_2 = whole body oxygen consumption. VCO_2 = whole body carbon dioxide production. RQ = respiratory quotient.

Table 5.5 Plasma Concentrations of Metabolites and Hormones

	Before surgery	During surgery I	During surgery II	After surgery
Lactate (mmol/l)				
Control	0.9 ± 0.3	0.8 ± 0.3	0.9 ± 0.4	0.7 ± 0.3
EDA	0.9 ± 0.2	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.4
FFA (μmol/l)				
Control	696 ± 164	722 ± 239	719 ± 173	664 ± 179
EDA	690 ± 196	644 ± 196	643 ± 196	598 ± 115
Insulin (pmol/l)				
Control	65 ± 25	69 ± 22	77 ± 24	89 ± 41
EDA	82 ± 33	56 ± 20	69 ± 24	84 ± 34
Cortisol (nmol/l)				
Control	308 ± 134	823 ± 121^{+}	882 ± 122^{+}	1040 ± 202^{+}
EDA	306 ± 138	640 ± 203^{+*}	643 ± 192^{+*}	919 ± 251^{+}
Glucagon (pmol/l)				
Control	17 ± 4	16 ± 4	17 ± 4	19 ± 4
EDA	15 ± 4	12 ± 4*	13 ± 4*	17 ± 6

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade.

I = 60 min, II = 100 min after skin incision.

FFA = free fatty acids.

$^{+}$ = $P<0.05$ vs. before surgery. * = $P<0.05$ vs. control.

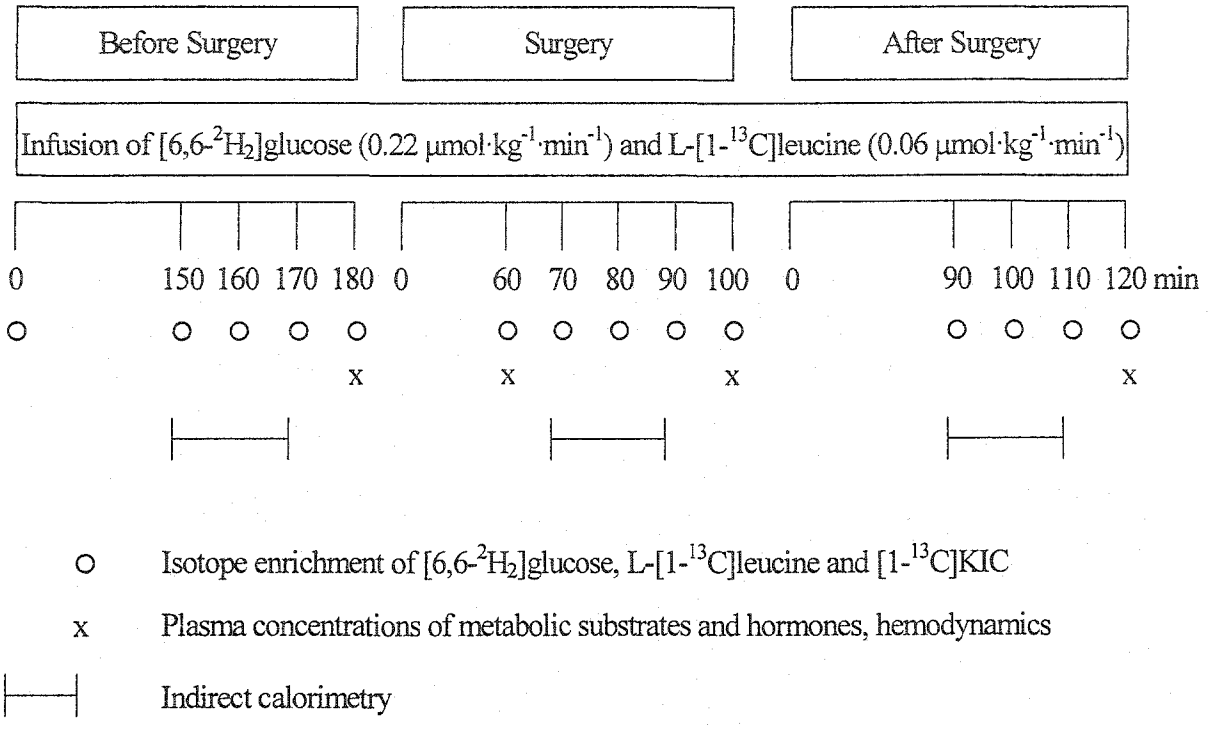


Figure 5.1. Study protocol

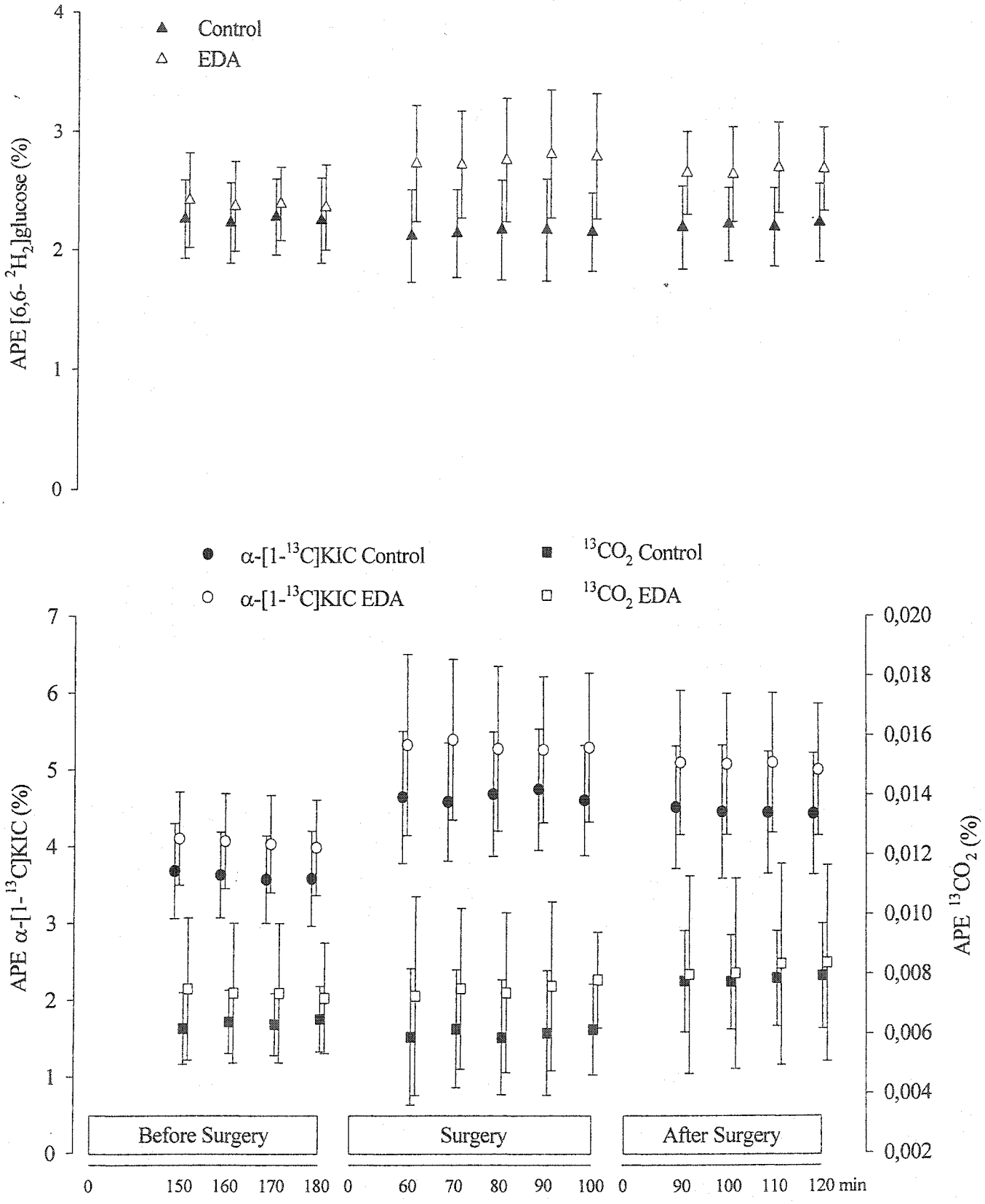
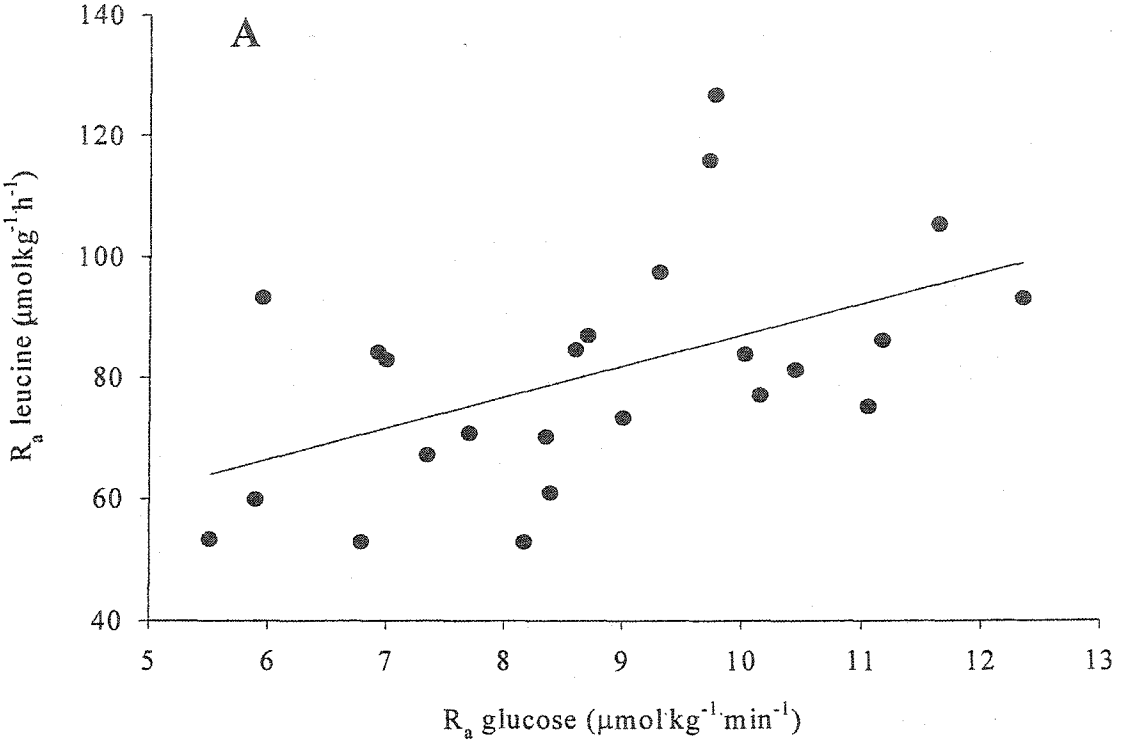


Figure 5.2. Isotopic enrichments (APE = atom percent excess) of [6,6-$^2H_2$]glucose, α-[1-^{13}C]KIC and $^{13}CO_2$.



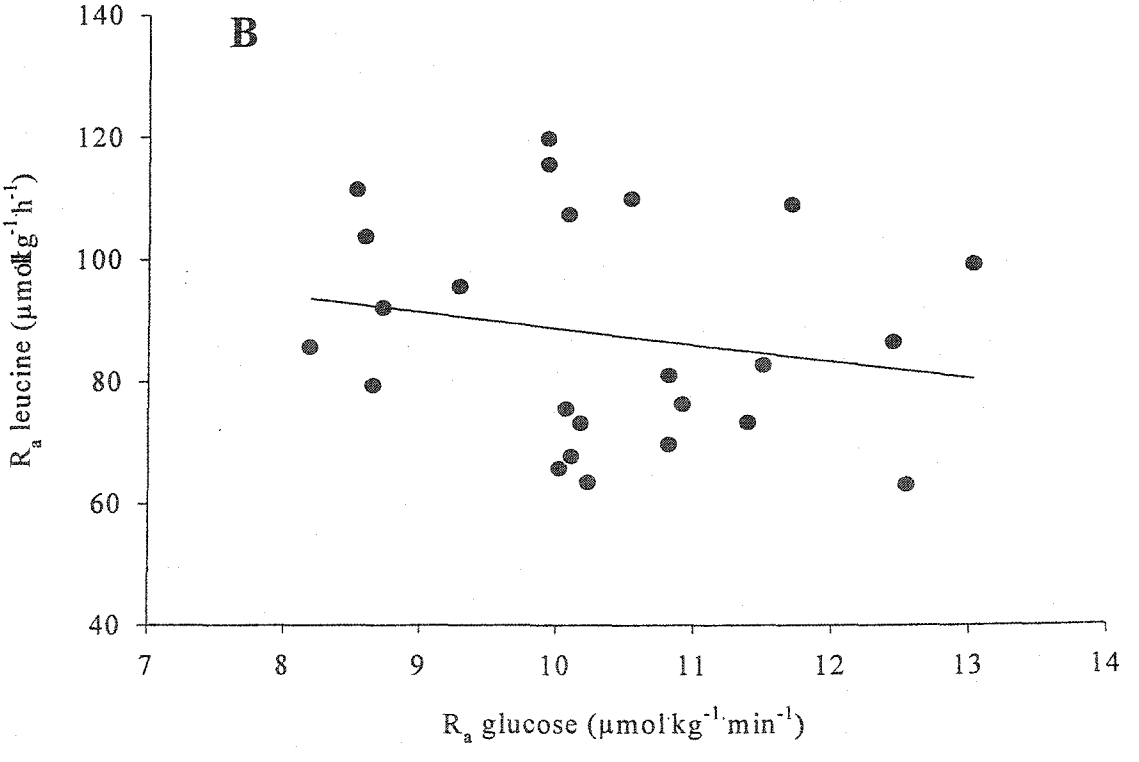


Figure 5.3. Correlation between R_a leucine and R_a glucose in the EDA group (A, r = 0.51
$P < 0.05$) and in the control group (B, r = -0.198, $P > 0.05$).

5.7 Summary of study I and introduction of study II

The first part of this project investigated the effect of epidural blockade on the catabolic responses during and immediately after surgery in fasting patients. It could be demonstrated that neuraxial blockade with epidural local anesthetic attenuated the increase in plasma glucose concentration, mediated by a lower glucose production and not by a higher glucose utilization. However, epidural blockade did not exert any specific influence on perioperative protein metabolism.

These findings are in line with the results of previous studies, demonstrating that perioperative epidural blockade does not exert any anticatabolic effects after the operation when calorie intake is low (Hjortso et al, 1985) or absent (Schricker et al, 2000a). However, epidural blockade has frequently been shown to modulate postoperative protein economy by moderate amelioration of nitrogen losses in patients receiving nutritional support (Kehlet, 1998), leading to the conclusion that the protein-sparing influence of epidural blockade requires adequate energy supply (Schricker et al, 2000a).

These findings, however, were obtained on the days following the operation. Because the combined effect of epidural blockade and glucose administration on the catabolic responses during the acute phase of the stress response had not been investigated, the second part of this study addressed this issue in patients undergoing abdominal surgery.

PERIOPERATIVE GLUCOSE INFUSION AND THE CATABOLIC RESPONSE TO SURGERY: THE EFFECT OF EPIDURAL BLOCKADE

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submitted to

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6.1 Abstract

Background: The study tested the hypothesis that the suppression of endogenous glucose production by intravenous glucose is more pronounced in patients receiving epidural blockade. It was further determined if the modification of glucose production has an impact on perioperative protein catabolism.

Methods: Sixteen patients undergoing colorectal surgery received either general anesthesia and epidural blockade with bupivacaine (EDA, n=8) or general anesthesia alone (control, n=8). All patients received intravenous glucose at 2 $mg \cdot kg^{-1} \cdot min^{-1}$ during and after surgery. Glucose and protein kinetics were determined during and two hours after the operation by stable isotope tracers [6,6-$^2H_2$]glucose and L-[1-^{13}C]leucine. Plasma concentrations of glucose, lactate, free fatty acids (FFA), insulin, cortisol and glucagon were also determined.

Results: Epidural blockade attenuated the increase in plasma glucose concentration compared to the control group ($P<0.05$). The rate of appearance of glucose (R_a glucose) and endogenous glucose production (EGP) were lower in the EDA group than in control subjects during (R_a glucose: EDA 13.2±1.0 vs. control 15.3±1.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P<0.05$; EGP: EDA 1.2±1.2 vs. control 3.8±1.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P<0.05$) and after the operation ($P>0.05$). Protein breakdown and amino acid oxidation decreased in both groups ($P<0.05$). Whole body protein synthesis and plasma concentrations of lactate and FFA remained unchanged. Insulin levels increased with both anesthetic techniques ($P<0.05$). Intraoperative plasma concentrations of cortisol and glucagon were lower in the EDA group ($P<0.05$).

Conclusion: The suppression of endogenous glucose production by exogenous glucose is more pronounced in presence of epidural blockade. However, epidural blockade does not modulate perioperative protein metabolism during glucose administration.

6.2 Introduction

Glucose exerts its anticatabolic action through the suppression of gluconeogenesis and the reduced need for gluconeogenic amino acids released from the muscle (Wolfe, 1993). While the protein preserving properties of glucose administration after surgery are well established, its use during the acute phase of surgical tissue trauma has produced conflicting results (Lund et al, 1986b; Obata et al, 1993; Sieber et al, 1989). On one side, glucose infused at 3 $mg \cdot kg^{-1} \cdot min^{-1}$ during gastrectomy has been shown to decrease plasma concentrations of branched-chain amino acids (BCAA), indicating an inhibition of whole body protein breakdown (Obata et al, 1993). On the other side, intravenous glucose at the same dose did not affect plasma BCAA concentrations during cholecystectomy (Lund et al, 1986b) and did not improve nitrogen balance in patients undergoing craniotomy (Sieber et al, 1989). Although it was proposed that this discrepancy may be due to the different types of surgery studied (Obata et al, 1993), it also has to be noted that plasma amino acid concentrations and nitrogen excretion values represent only indirect parameters of protein breakdown and protein oxidation.

Besides its influence on protein metabolism, intraoperative glucose infusion typically leads to a significant increase in plasma glucose concentration. This raises metabolic concern because acute hyperglycemia is associated with several disadvantageous clinical effects (Allison, 1980; Askanazi et al, 1981; Nordenström et al, 1981; Rassias et al, 1999; van den Berghe et al, 2001).

Numerous studies have demonstrated that neuraxial blockade with local anesthetics inhibits the intraoperative increase in plasma glucose concentrations, particularly during operations below the umbilicus (Kehlet 1998; Schricker et al, 1998). It was recently demonstrated that epidural blockade blunts the hyperglycemic response to colorectal

surgery in fasting patients by preventing the increase in endogenous glucose production (Lattermann et al, 2002). Despite the suppressory effect on glucose production, however, epidural blockade failed to modify protein catabolism in the absence of nutritional support (Lattermann et al, 2002).

Several studies investigated the influence of intraoperative glucose administration on protein metabolism, but very few were controlled for the type of analgesia. In particular, two studies addressed the effect of epidural blockade on the catabolic response in patients receiving intravenous glucose. Patients in one group received a combination of epidural blockade and general anesthesia, while patients in the control group received general anesthesia alone. The findings of the first study showed that the combination of epidural analgesia and general anesthesia was accompanied by a lower splanchnic glucose release when compared to the control group (Lund et al, 1986a). In the other study, epidural blockade was associated with higher amino acid plasma concentrations, but did not modify splanchnic amino acid uptake, indicating no effect on the rate of *de novo* glucose synthesis from gluconeogenic amino acids (Lund et al, 1987). However, the measurement of splanchnic amino acid metabolism does not provide insight into the kinetics of whole body protein metabolism and therefore the influence of epidural blockade and glucose administration on protein breakdown and amino acid oxidation remains to be determined.

The present study was designed to test the hypothesis that the suppression of endogenous glucose production by intravenous provision of glucose (2 $mg \cdot kg^{-1} \cdot min^{-1}$) would be more pronounced in patients receiving combined epidural blockade and general anesthesia than in patients with general anesthesia alone. It was also proposed that a greater decrease in glucose production in the epidural group would lead to a more accentuated inhibition of protein breakdown and amino acid oxidation. To provide an integrated analysis of

glucose and protein metabolism during and immediately after surgery, glucose production, glucose clearance, as well as whole body protein breakdown, amino acid oxidation and protein synthesis were assessed by the stable isotope tracers [6,6-$^{2}H_{2}$]glucose and L-[1-^{13}C]leucine.

6.3 Methods

Patients

The study was approved by the Ethics Committee of the Montreal General Hospital. Sixteen patients undergoing elective colorectal surgery were admitted to the study and written informed consent was obtained from all subjects. No patient was suffering from cardiac, hepatic, renal or metabolic disorders or receiving any medication known to affect metabolism. None of the participants had developed more than 5% weight loss over the preceding three months or had a plasma albumin concentration <35 g/l. We further excluded patients with anemia (hemoglobin <10 g/dl) and patients who had received chemotherapy or radiotherapy during six months before surgery.

Anesthesia

Patients were randomly assigned to receive either a combination of epidural blockade with bupivacaine and general anesthesia (EDA group) or general anesthesia alone (control group). In the EDA group an epidural catheter was inserted between T 9 and T 11 before the operation. Neuraxial blockade was established with 0.5% bupivacaine to achieve a bilateral sensory block from T 4 to S 5 and maintained with intermittent boluses of bupivacaine 0.25%. General anesthesia in all patients was induced with 5 mg/kg thiopentone and 1.5 μg/kg fentanyl in the EDA group or 5 μg/kg fentanyl in the control group, respectively. Endotracheal intubation was facilitated with 0.6 mg/kg rocuronium and patients` lungs were ventilated with 30% oxygen in air to maintain normocapnia. It has to be noted that no nitrous oxide was used because it has the same molecular weight as $^{13}CO_2$ and thus would interfere with the isotope ratio measurement of expired $^{13}CO_2$. General anesthesia in the control group was maintained using desflurane at endtidal

concentrations as required to keep heart rate within 20% of preoperative values. In the EDA group desflurane was administered at endtidal concentrations of approximately 3 Vol% in order to achieve tolerance of the endotracheal tube and to prevent awareness. The degree of muscle relaxation was monitored using train-of-four ratio and supplemental doses of rocuronium were applied as needed for complete surgical muscle relaxation. Fluid was given as NaCl 0.9% solution at a rate of 10 $ml \cdot kg^{-1} \cdot h^{-1}$ intraoperatively and 6 $ml \cdot kg^{-1} \cdot h^{-1}$ thereafter. All patients were covered with a warming blanket during surgery to maintain normothermia. While patients in the control group received morphine i.v. for postoperative pain relief, the epidural blockade was maintained in the EDA group with bupivacaine 0.25% as required to maintain sensory blockade from T8 to L3. Hemodynamic monitoring was performed using a three lead electrocardiogram monitor and radial artery catheterization for continuous blood pressure measurement.

Glucose infusion

All subjects received a solution of crystallized beet dextrose (10% dextrose anhydrous, Avebe, Foxhol, Holland) infused at 2 $mg \cdot kg^{-1} \cdot min^{-1}$ starting with the surgical incision. This dose of glucose has been chosen, as previous studies showed that intraoperative plasma glucose concentrations in metabolically healthy surgical patients infused with glucose at 3 $mg \cdot kg^{-1} \cdot min^{-1}$ regularly exceeded 10 mmol/l, the physiological threshold for renal glucose excretion (Lund et al, 1986b; Obata et al, 1993; Sieber et al, 1989). The solution was prepared by the local pharmacy under sterile conditions and was tested for sterility, stability, and absence of pyrogens before intravenous infusion. Beet dextrose was chosen because of its low ^{13}C content and therefore the lack of perturbation of $^{13}CO_2$ enrichment in expired air (Carli et al, 1997).

Study protocol

Plasma kinetics of leucine and glucose were determined before, during and two hours after surgery by stable isotope tracer technique using primed continuous infusions of [6,6-$^{2}H_2$]glucose and L-[1-^{13}C]leucine (Cambridge Isotope Laboratories, Cambridge, MA). Sterile solutions of the isotopes were prepared as previously described and were kept at 4 °C until administration (Lattermann et al, 2002).

All patients were studied on the day of surgery beginning between 7:00 and 08:00 after fasting for approximately 36 hours. Due to preparation of the bowel as required for the colorectal surgery, patients received only clear fluids until midnight the day before the operation. A catheter was placed in a superficial vein in the dorsum of the hand and kept patent with a slow saline 0.9% infusion. A superficial vein of the contralateral arm was cannulated to provide access for the infusion of [6,6 $^{2}H_2$]glucose and L-[1-^{13}C]leucine. After warming the hand in a heated air box to obtain arterialization of venous blood, blood and expired air samples were taken to determine baseline enrichments. Thereafter, priming doses of $NaH^{13}CO_3$ 1 μmol/kg, L-[1-^{13}C]leucine 4 μmol/kg and [6,6-$^{2}H_2$]glucose 22 μmol/kg were administered followed immediately by continuous infusions of [6,6-$^{2}H_2$]glucose 0.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and L-[1-^{13}C]leucine 0.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively (Schricker et al, 2000a). Isotope infusion was uninterrupted throughout the entire study period. Expired breath and arterialized blood samples for the determination of isotopic enrichments as well as for the measurement of metabolic substrates (glucose, lactate, free fatty acids (FFA)) and hormones (insulin, glucagon, cortisol) were collected according to figure 1.

Expired air samples were collected through a mouthpiece in a 3-l bag and transferred immediately to 10-ml vacutainers to await $^{13}CO_2$ isotope enrichment analysis. During controlled ventilation, expired gases were collected by a means of a one-way valve into a 5-l bag. Each blood sample was immediately transferred to a heparinized tube and centrifuged at 4 °C (3,000 g, 15 min). The plasma obtained was stored at -70 °C until analysis.

Gaseous Exchange

Whole body oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured by indirect calorimetry before and two hours after surgery using the open system indirect calorimetry device Deltatrac Metabolic Monitor (Datex Instrumentarium, Helsinki, Finland). VCO_2 was also measured during surgery (70 min after skin incision). The values of VO_2, VCO_2 and respiratory quotient (RQ) represent an average of the data obtained over a period of 20 min on each occasion, with a coefficient of variation <10%.

Analytical Methods

Plasma glucose was derivatized to its pentaacetate compound, and the [6,6-$^2H_2$]glucose enrichment was determined by gas chromatography-mass spectrometry using electron-impact ionization (Schricker et al, 1996). Plasma α-[1-^{13}C]ketoisocaproate (α-[1-^{13}C]KIC) enrichment was analyzed by electron-impact selected-ion monitoring gas chromatography-mass spectrometry (Mamer et al, 1988), except that t-butyldimethylsylyl rather than trimethylsylyl derivatives were used. Expired $^{13}CO_2$ enrichment for the calculation of leucine oxidation was determined by isotope ratio mass spectrometry (Analytical Precision AP2, 003, Manchester, UK).

Plasma glucose concentrations were measured with a glucose analyzer 2 (Beckman Instruments, Fullerton, CA) based on a glucose oxidase method. The plasma lactate assay was based on lactate oxidase using the synchron CX 7 system (Beckman Instruments, Fullerton, CA). Circulating concentrations of FFA were quantified by means of an enzymatic assay (Boehringer Mannheim, Laval, Quebec, Canada). Cortisol, insulin and glucagon plasma concentrations were measured by means of double antibody radioimmunoassays (Amersham International, Amersham, Bucks, UK).

Calculations

Under steady state conditions, the rate of appearance (R_a) of unlabeled substrate in plasma can be derived from the plasma enrichment (atom percent excess = APE) calculated by $R_a = I \cdot (APE_{inf} / APE_{pl} - 1)$, where APE_{inf} is the tracer enrichment in the infusate, APE_{pl} is the tracer enrichment in plasma at steady state and I is the infusion rate of the labeled tracer. The APE values used in this calculation represent the mean of the APE values determined during each isotopic plateau. Steady state conditions were assumed when the coefficient of variation (CV) of the APE values at isotopic plateau was <5%.

In a steady state, leucine flux is defined by the formula: Q = S + O = B + I, where S represents the rate of leucine uptake for protein synthesis, O is the rate of oxidation of leucine, B is the rate of leucine derived from endogenous protein breakdown and I is the rate at which leucine is entering the free pool from dietary intake. Inspection of this equation indicates that, when studies are conducted in the postabsorptive state, flux is equal to breakdown (Matthews et al, 1980). Plasma α-[1-^{13}C]KIC enrichment was used for calculating both flux and oxidation of leucine. The steady-state reciprocal pool model

represents the intracellular precursor pool enrichment more precisely than leucine itself (Schwenk et al, 1985). In the calculation of leucine oxidation, correction factors of 0.76 for the fasting state and 0.81 for the fed state were used to account for the fraction of $^{13}CO_2$ released from ^{13}C-labeled leucine oxidation but retained within slow turnover rate pools of the body (Matthews et al, 1980; Schricker et al, 2000).

In the fasted state, R_a glucose equals the rate of endogenous glucose production. During glucose infusion, endogenous glucose production was calculated by subtracting the glucose infusion rate from the total R_a glucose. The glucose clearance rate, an indicator of the tissues ability to take up glucose, was calculated as the R_a glucose divided by the corresponding glucose plasma concentration.

For metabolic substrates, hormones and hemodynamic parameters, the average of the two intraoperative measurements at 60 and 100 min after skin incision was calculated and is presented in the tables.

Statistics

Differences between the groups were analyzed using the Mann-Whitney U test. Within-group comparison of variables was made by analysis of variance for repeated measures with *post-hoc* analysis by Student-Newman-Keuls-test. A probability of $P<0.05$ was considered to be significant. Data are presented as means ± SD.

6.4 Results

There were no differences between the two groups regarding age, height, weight, gender and the ASA classification (table 1). The duration of surgery, estimated blood loss and the amount of cristalloid fluid administered were comparable in both groups.

Heart rate and mean arterial blood pressure (MAP) decreased during surgery in the EDA group and MAP was significantly lower than in the control group ($P<0.05$, table 2). Intraoperative body temperature was comparable between the groups (EDA 36.4 ± 0.5°C, control 36.3 ± 0.4°C). The end-tidal desflurane concentration was lower in the EDA group than in the control group at 60 min (EDA 3.5 ± 0.5 Vol%, control 5.6 ± 1.1 Vol%, $P<0.05$) and 100 min after skin incision (EDA 3.7 ± 0.5 Vol%, control 5.7 ± 1.2 Vol%, $P<0.05$).

Plasma glucose concentration increased during and after surgery in both groups ($P<0.05$, table 3), with higher values in the control group than in the EDA group ($P<0.05$). In all patients, isotopic plateau of [6,6-$^2H_2$]glucose, α-[1-^{13}C]KIC and expired $^{13}CO_2$ was achieved (CV <5%) allowing steady state calculations (figure 2). The rate of appearance (R_a) of glucose increased with both anesthetic techniques ($P<0.05$). In the EDA group R_a glucose was lower during ($P<0.05$) and after surgery ($P>0.05$) when compared to the control group. Endogenous glucose production decreased in both groups ($P<0.05$), with lower values in the EDA group than in the control group during ($P<0.05$) and after surgery ($P>0.05$). Glucose plasma clearance decreased in both groups ($P<0.05$) with no difference between the two anesthetic techniques.

R_a leucine decreased during surgery in both groups ($P<0.05$). However, the postoperative decrease in R_a leucine was significant only in the EDA group ($P<0.05$). Leucine oxidation

decreased during surgery ($P<0.05$), followed by an increase thereafter. Whole body protein synthesis remained unchanged with both anesthetic techniques.

There were no significant changes in whole body oxygen consumption and carbon dioxide production in both groups (table 4). The respiratory quotient increased postoperatively in the EDA group to higher values than in the control group ($P<0.05$).

Plasma concentrations of lactate and free fatty acids remained unaltered (table 5). In the control group plasma insulin concentrations increased during and after surgery ($P<0.05$), while the increase in the EDA group was statistically significant only after the operation ($P<0.05$). Plasma cortisol concentrations increased throughout the study period in both groups ($P<0.05$), with lower values during surgery in the EDA group compared to the control group ($P<0.05$). While plasma glucagon concentrations remained unchanged in the control group, they decreased intraoperatively in the EDA group to lower values than in control subjects ($P<0.05$).

6.5 Discussion

The results of this study demonstrate that epidural blockade enhances the glucose induced suppression of endogenous glucose production without affecting protein catabolism. This modifying effect of neuraxial blockade on glucose homeostasis is in agreement with its previously observed inhibitory influence on plasma glucose concentration and glucose production during surgery performed under fasted conditions (Lattermann et al, 2002; Lattermann et al, 2001). Furthermore epidural blockade blunts the endocrine responses as reflected in lower plasma concentrations of cortisol and glucagon.

Despite the well established inhibitory effect of epidural local anesthetics on the hyperglycemic and endocrine response to surgery, glucose infusion in the present study caused an increase in plasma glucose levels above 10 mmol/l, with or without epidural blockade. This finding gains clinical importance because acute hyperglycemia has been associated with impaired phagocytic capacity of polymorphonuclear leukocytes (Kwoun et al, 1997; Rassias et al, 1999) dysfunction of the complement system (Hostetter 1990), increased CO_2 production (Askanazi et al, 1981), stimulated sympathoadrenergic activity (Nordenström et al, 1981), electrolyte imbalances (Allison 1980) and increased morbidity and mortality in patients after major surgery (van den Berghe et al, 2001).

The measurement of plasma glucose concentration per se does not provide insight into the underlying metabolic changes, i.e. glucose production and uptake, and therefore the kinetics of glucose metabolism were assessed in the present study by a stable isotope tracer technique. The suppressive effect of epidural blockade on glucose production is in agreement with recent findings showing a reduction of intraoperative splanchnic glucose release during combined glucose infusion and epidural blockade (Lund et al, 1986a). It

has to be noted, however, that splanchnic glucose release as calculated from splanchnic blood flow and the arteriohepatic venous difference of glucose, is not an accurate measure of glucose production, because it does not account for intestinal glucose uptake.

Despite the significant suppression of endogenous glucose production by exogenous glucose in the epidural group, hyperglycemia occurred independently of the anesthetic technique employed. This increase in plasma glucose concentration can therefore be largely ascribed to impaired glucose uptake, as reflected indirectly by reduced plasma glucose clearance. This finding is in agreement with two recent investigations, demonstrating a decrease in plasma glucose clearance during major abdominal surgery in fasting patients with or without epidural blockade (Lattermann et al, 2002; Lattermann et al, 2001). It has long been recognized that neuraxial blockade with epidural local anesthetic may completely abolish the endocrine stress response to lower abdominal (gynecological) surgery, as reflected in unchanged plasma concentrations of the counterregulatory hormones cortisol, epinephrine and norepinephrine (Kehlet 1998). In contrast, this effect is less pronounced during major and upper abdominal procedures, most likely a result of the insufficient afferent somatic and sympathetic blockade (Kehlet 1998). In accordance with this notion, the increase in plasma cortisol concentration observed in this study was attenuated but not completely blocked. Because cortisol represents an important mediator of the hyperglycemic response to surgery by counteracting the peripheral action of insulin on the glucose uptake system (Weissman 1990), it is suggested that the decrease in whole body glucose uptake in the present study can be ascribed to the incomplete inhibition of the endocrine stress response to major abdominal surgery by epidural analgesia.

Gluconeogenesis contributes more than 90% to total glucose production under perioperative conditions, a consequence of the fasting-induced depletion of glycogen stores and the stimulatory effect of counterregulatory hormones (Chandramouli et al, 1997; Gump et al, 1975). Because muscle protein is broken down to supply amino acids serving as precursors for *de novo* glucose synthesis, it has been hypothesized that any suppression of gluconeogenesis may reduce protein breakdown (Wolfe, 1993). This assumption is supported by recent studies demonstrating a positive correlation between glucose production and protein breakdown in surgical patients (Schricker et al, 2000; Schricker et al, 2001). Whole body protein breakdown in the present protocol decreased to a similar extent in both groups lending support to the assumption that protein catabolism is controlled by factors other than the demand for gluconeogenic precursors alone. Our finding of a depressed protein metabolism during surgery adds to our earlier results of a reduction in protein breakdown and oxidation by 20%, which occurred independently of the anesthetic technique employed, i.e. inhaled, intravenous or epidural (Carli et al, 1990; Lattermann et al, 2002; Schricker et al, 2001). The fact that amino acid oxidation during glucose administration decreased by more than 50% and that protein synthesis was preserved indicate a protein sparing effect of intraoperative hypocaloric glucose administration in both groups. It appears, however, that the anticatabolic effect of glucose was most pronounced during the intraoperative period, since amino acid oxidation increased two hours after the operation back to preoperative values.

It is of interest to note that, in contrast to the present findings, epidural blockade has been shown to facilitate the uptake and oxidative utilization of glucose infused at 4 $mg \cdot kg^{-1} \cdot min^{-1}$ two days after abdominal surgery (Schricker et al, 2000). This was accompanied by a decrease in amino acid oxidation, indicating a protein sparing effect of epidural

blockade in presence of nutritional support. A possible explanation for the different anticatabolic properties of epidural blockade might be the more profound stress response with significantly greater circulating levels of counterregulatory hormones during the intraoperative period compared with the postoperative phase.

In summary, the suppression of endogenous glucose production by hypocaloric glucose infusion was more pronounced in patients receiving epidural blockade than in patients with general anesthesia alone. However, epidural analgesia did not enhance the anticatabolic effect of intravenous glucose. Because significant hyperglycemia occurred independently of the type of anesthesia, it remains questionable if patients can benefit from intraoperative glucose administration.

6.6 Appendix

Table 6.1 Patient Characteristics

	Control	EDA
Number (n)	8	8
Age (yr)	60 ± 18	63 ± 12
Height (cm)	172 ± 8	172 ± 8
Weight (kg)	73 ± 10	73 ± 15
Gender (male / female)	4 / 4	4 / 4
ASA (II / III)	8 / 0	7 / 1
Type of surgery		
Hemicolectomy / Colectomy	2	3
Sigmoid Resection	5	4
Ileocolic Resection	1	1
Duration of Surgery (min)	143 ± 46	152 ± 58
Estimated blood loss (ml)	238 ± 74	272 ± 100
Cristalloids (ml)	3043 ± 479	3213 ± 394

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade.

ASA = American Society of Anesthesiologists' classification.

Table 6.2 Hemodynamics, SpO_2

	Before surgery	During surgery	After surgery
Heart rate (beats/min)			
Control	79 ± 11	69 ± 10	81 ± 13
EDA	73 ± 6	59 ± 11^{+}	71 ± 8
Mean arterial pressure (mmHg)			
Control	91 ± 11	83 ± 10	92 ± 8
EDA	93 ± 9	73 ± 3^{+*}	82 ± 11
SpO_2 (%)			
Control	97 ± 1	99 ± 1	98 ± 1
EDA	98 ± 1	99 ± 1	99 ± 1

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade.

SpO_2 = transcutaneous oxygen saturation.

$^{+}$ = $P < 0.05$ vs. before surgery. * = $P < 0.05$ vs. control.

Table 6.3 Glucose and Leucine Metabolism

	Before surgery	During surgery	After surgery
Glucose (mmol/l)			
Control	4.7 ± 0.7	9.7 ± 0.5^{+}	11.9 ± 1.4^{+}
EDA	5.2 ± 0.7	8.4 ± 1.3^{+*}	10.2 ± 1.3^{+*}
R_a Glucose ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
Control	10.6 ± 1.8	15.3 ± 1.8^{+}	16.9 ± 3.2^{+}
EDA	11.1 ± 1.3	13.2 ± 1.0^{+*}	15.1 ± 1.1^{+}
Endogenous glucose production ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
Control	10.6 ± 1.8	3.8 ± 1.7^{+}	5.4 ± 2.9^{+}
EDA	11.1 ± 1.3	1.2 ± 1.2^{+*}	3.0 ± 1.8^{+}
Glucose Clearance ($ml \cdot kg^{-1} \cdot min^{-1}$)			
Control	2.3 ± 0.6	1.6 ± 0.2^{+}	1.4 ± 0.2^{+}
EDA	2.2 ± 0.4	1.6 ± 0.2^{+}	1.5 ± 0.2^{+}
R_a Leucine ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)			
Control	104 ± 20	81 ± 17^{+}	87 ± 18
EDA	103 ± 6	90 ± 8^{+}	92 ± 5^{+}
Leucine oxidation ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)			
Control	12 ± 3	5 ± 2^{+}	12 ± 4
EDA	13 ± 3	6 ± 2^{+}	10 ± 5
Protein synthesis ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)			
Control	91 ± 18	76 ± 15	75 ± 16
EDA	91 ± 9	84 ± 11	83 ± 5

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade. R_a = rate of appearance.

$^{+}$ = $P < 0.05$ vs. before surgery. * = $P < 0.05$ vs. control.

Table 6.4 Gaseous Exchange

	Before surgery	During surgery	After surgery
VO_2 (ml/min)			
Control	194 ± 20	-	209 ± 42
EDA	203 ± 33	-	193 ± 39
VCO_2 (ml/min)			
Control	157 ± 16	156 ± 30	178 ± 36
EDA	164 ± 28	151 ± 28	177 ± 37
RQ			
Control	0.82 ± 0.04	-	0.85 ± 0.02
EDA	0.81 ± 0.04	-	$0.92 \pm 0.02^{+*}$

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade.

VO_2 = whole body oxygen consumption. VCO_2 = whole body carbon dioxide production.

RQ = respiratory quotient.

$^{+}$ = $P < 0.05$ vs. before surgery. * = $P < 0.05$ vs. control.

Table 6.5 Plasma Concentrations of Metabolites and Hormones

	Before surgery	During surgery	After surgery
Lactate (mmol/l)			
Control	1.0 ± 0.3	0.8 ± 0.4	1.0 ± 0.5
EDA	1.2 ± 0.3	0.9 ± 0.5	1.3 ± 0.8
Free fatty acids (μmol/l)			
Control	764 ± 251	649 ± 173	602 ± 222
EDA	692 ± 161	591 ± 236	509 ± 218
Insulin (pmol/l)			
Control	54 ± 10	91 ± 41^{+}	183 ± 70^{+}
EDA	68 ± 27	97 ± 55	128 ± 77^{+}
Cortisol (nmol/l)			
Control	288 ± 124	890 ± 160^{+}	940 ± 230^{+}
EDA	278 ± 57	616 ± 259^{+*}	911 ± 202^{+}
Glucagon (pmol/l)			
Control	15 ± 8	14 ± 6	15 ± 7
EDA	13 ± 3	9 ± 3^{+*}	11 ± 5

Values are mean ± SD. Control = general anesthesia. EDA = epidural blockade.

$^{+}$ = $P < 0.05$ vs. before surgery. * = $P < 0.05$ vs. control.

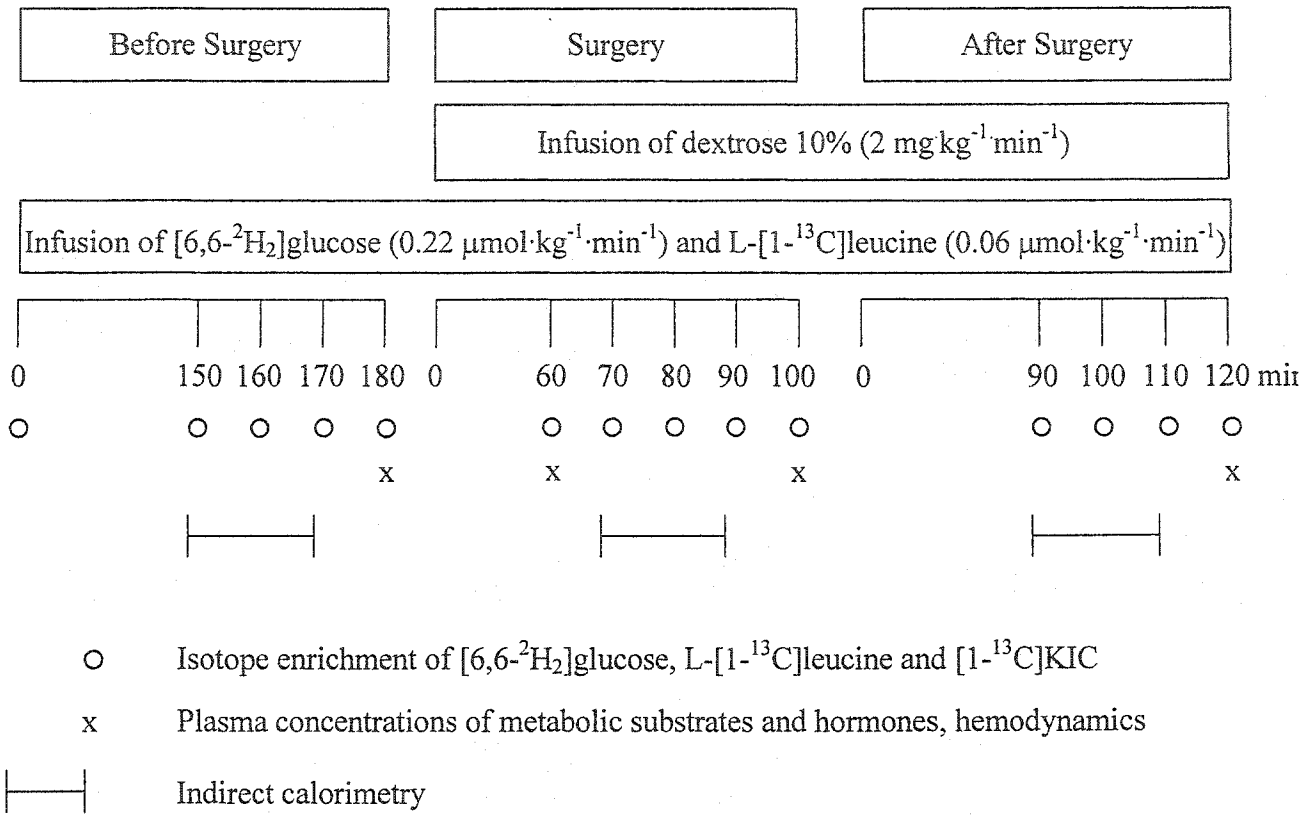

Figure 6.1 Study protocol

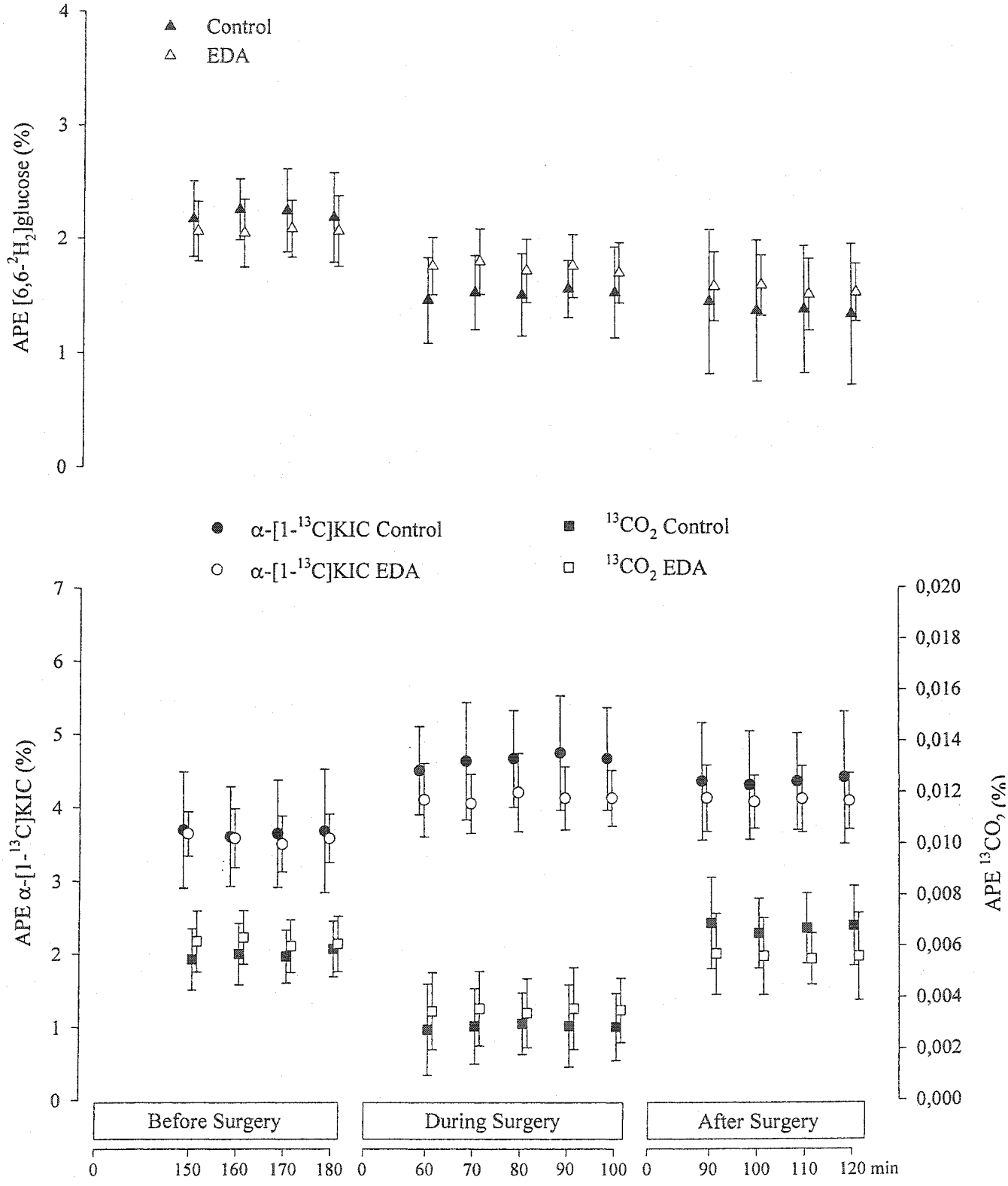

Figure 6.2. Isotopic enrichments (APE = atom percent excess) of [6,6-$^2H_2$]glucose, α-[1-^{13}C]KIC and $^{13}CO_2$.

7. Conclusion

7.1. Summary of Results

The present set of studies investigated the effect of epidural blockade on glucose and protein kinetics during and immediately after abdominal surgery both in the fasted state and during intravenous administration of glucose at 2 $mg \cdot kg^{-1} \cdot min^{-1}$.

Epidural blockade was associated with lower plasma glucose concentrations in fasting patients as well as during glucose infusion and this was mediated by lower endogenous glucose production rates. This suppressive effect of epidural blockade on glucose metabolism could be ascribed to the attenuation of the endocrine stress response, as reflected in lower glucagon and cortisol plasma concentrations during the operation. However, the kinetics of perioperative protein metabolism, i.e. protein breakdown, protein synthesis, amino acid oxidation were not modified by epidural blockade both in the fasted and fed state.

7.2. Significance and Future Research

There are numerous strategies of modifying the catabolic response after surgery, such as the type of anesthesia and analgesia (regional techniques, e.g. epidural blockade), the surgical approach (laparoscopic vs. open), provision of nutrition and specific nutrients such as glutamine, and hormonal interventions (insulin, growth hormone).

Epidural blockade with local anesthetics, initiated before and maintained after surgery, has frequently been demonstrated to attenuate postoperative protein catabolism (Brandt et al, 1978; Carli et al, 1997; Christensen et al, 1986; Vedrinne et al, 1989), and this anticatabolic effect of epidural blockade has been shown to require energy supply (Hjortso et al, 1985; Schricker et al, 2000).

In the present studies, epidural blockade failed to modify protein metabolism during the acute phase of the stress response, both in the fasted state and during intravenous administration of glucose. This stands in contrast to the well-established anticatabolic properties of epidural blockade in the days following surgery. The suppression of intraoperative protein metabolism occurred independently of the anesthetic technique, and it seems that different anticatabolic strategies need to be applied during the acute phase of the stress response than during the postoperative period.

Although single interventions applied in the postoperative period have been demonstrated to modulate protein catabolism, no evidence exists as to their beneficial impact on clinical outcome. However, there have been initial reports that a multimodal approach combining analgesic, anesthetic and surgical strategies with the provision of specific nutrients hold great promise for not only reducing the loss of body protein, but also for accelerating recovery and improving clinical outcome (Barratt et al, 2001; Brodner et al, 2001).

Despite the increasing knowledge about the separate anticatabolic action of these interventions applied in the days after the operation, little is known about their individual and combined effects during and immediately after surgery. The findings of the present thesis therefore suggest that future studies will have to further dissect the separate and combined metabolic effects of different anesthetic, surgical and nutritional regimens during the acute phase of the stress response.

Insulin is a well-recognized and extremely important anabolic regulator of metabolism, and it is currently believed that the reduction of insulin resistance represents a key factor for the normalization of protein economy in the surgical patient (Carli et al, 2001). In this context the rationale for long fasting periods before surgery has been questioned recently, as it could be shown that preoperative glucose infusion improves impaired insulin

sensitivity on the first day after surgery (Ljungvist et al, 1994). Furthermore, combined perioperative glucose and insulin administration has been demonstrated to normalize postoperative insulin sensitivity and substrate utilization, associated with decreased protein catabolism (Nygren et al, 1998).

However, the intraoperative effect of these interventions remains unknown and future studies will have to determine the optimal combination of anesthesia and analgesia, nutrition (glucose and other substrates, such as amino acids - glutamine - etc.) and hormones (insulin) that prove to be most successful in reducing protein catabolism during the acute phase of the stress response. The perioperative use of tracer methodologies in the fasted and fed state will further increase the understanding of the catabolic response during surgery and its possible modification by nutritional support.

Based on the growing evidence that reduced protein catabolism is associated with accelerated recovery and convalescence, reduced length of hospital stay, and improved clinical outcome after major abdominal operations (Barratt et al, 2001; Brodner et al, 2001; Carli et al, 2001), the preservation of whole body protein is of utmost clinical relevance for the surgical patient. The findings of the present studies indicate that separate anticatabolic strategies will have to be developed for each phase of the metabolic stress response in order to minimize total protein losses during the entire perioperative period.

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