Perioperative infusion of amino acids: effect of epidural blockade.

An integrated analysis of perioperative protein and glucose metabolism using stable isotope kinetics

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Abstract

The present project investigated the effect of epidural blockade with local anesthetic on the catabolic stress response during and after abdominal surgery in patients receiving intravenous amino acid infusion. The kinetics of protein and glucose metabolism were determined by an isotope dilution technique using stable isotope L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose.

The intraoperative provision of amino acids induced a positive protein balance independently of the anesthetic technique. In addition, amino acids supplementation did not influence gluconeogenesis, while whole body glucose uptake decreased independently of the type of anesthesia used.

The postoperative infusion of amino acids inhibited protein breakdown and stimulates protein synthesis, thus rendering protein balance positive regardless of analgesic. The effect of amino acids on postoperative glucose metabolism was characterized by a decrease glucose clearance indicating a state of insulin resistance and by a decrease in endogenous glucose production.

Résumé

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

L'infusion d'acides aminés pendant l'intervention a induit une balance protéique positive indépendamment de la technique anesthétique employée. La production endogène du glucose n'a pas été modifiée par l'infusion d'acides aminés, par contre, celle-ci a diminuée la prise périphérique du glucose indépendamment de la technique anesthétique employée.

L'infusion d'acides aminés après l'intervention a empêché la dégradation protéique et a stimulé la synthèse protéique, résultant en une balance protéique positive, indépendamment de la technique anesthétique employée. L'effet de l'infusion d'acides aminés sur le métabolisme glucidique postopératoire a été caractérisé par la diminution de la prise périphérique du glucose, indiquant le développement d'un état de résistance à l'insuline (en périphérie) et aussi par la diminution de la production endogène du glucose.

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Contribution of Authors

Two manuscripts entitled "*Intraoperative infusion of amino acids induces anabolism independently of the type of anesthesia*" and "*Postoperative infusion of amino acids induces a positive protein balance independently of the type of analgesia used*" are included as a part of this thesis. The following describes the contribution of the authors to the manuscript:

I was involved in developing the study design of both manuscripts. In collaboration with Dr. Carli and Dr. Asenjo I recruited the study patients and performed the experiments in the surgical patients (stable isotopes infusions). I collected the samples and prepared them for analysis. Dr. Wykes and I performed gas chromatography and Mass spectrometry analysis.

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

Glucose exerts its anticatabolic action through the suppression of gluconeogenesis and reduced need for gluconeogenic amino acids released from the muscle [1]. However, the increased blood glucose levels observed in patients undergoing colon surgery and during perioperative dextrose infusion [2-5] raised doubts on the value of infusing dextrose because hyperglycemia is associated with several disadvantageous clinical effects [6-10].

Studies in surgical patients have demonstrated that infusion of amino acids spares protein and induces an anabolic state because it directly stimulates whole body and muscle protein synthesis, and indirectly attenuates protein breakdown [11]. Neuraxial block of afferent and efferent stimuli (epidural analgesia) with local anesthetics, by decreasing the excretion of catabolic hormones and decreasing insulin resistance, has been shown to attenuate postoperative nitrogen excretion, to minimize the rise in whole body protein breakdown and to arrest the fall in muscle protein synthesis in patients receiving parenteral nutrition [12-15].

With the understanding of the anabolic properties of infusing amino acids and the greater capacity of epidural blockade in decreasing endogenous glucose production (EGP), the main goal of this study is to test the hypothesis that perioperative supplementation of amino acids induces a greater positive protein balance in patients receiving epidural blockade compared to patients receiving general anesthesia alone during surgery and patient controlled analgesia (PCA) as postoperative pain management technique. To quantify the dynamic changes in protein and glucose metabolism whole body protein breakdown, amino acid oxidation, protein synthesis, and EGP and glucose clearance were

determined by an isotope dilution technique using stable isotope L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose.

2. Literature Review

2.1 Catabolic response to surgery.

Major surgery induces a catabolic state resulting in a net loss of body protein [16, 17] . This is primarily the result of increased excretion of urea and other nitrogenous products in the urine, although individuals with large open wounds (such as flame burns) will also exude serum and lose proteins through the injured tissues. The pattern of protein catabolism is related to the extent of the injury in a dose-responsive manner. That is, the greater the injury, the larger the nitrogen loss. The response pattern follows a time course, with nitrogen excretion increasing in the first few days after injury, peaking for several days or weeks, and then gradually returning to equilibrium as the inflammation resolves and/or the wound heals [18]. The major loss of body protein arises from skeletal muscle [19, 20] although there is some evidence that the gastrointestinal tract may initially respond by releasing amino acids in the first 48 h [21, 22]. The hormonal and inflammatory environment is a major regulator of this catabolic response. Initially, insulin levels are low and then they gradually rise, although insulin resistance is present. Resistance to growth hormone has also been observed and insulin-like growth factor-1 usually remains at subnormal concentrations throughout a catabolic course [23]. The elaboration of the counterregulatory hormones cortisol, glucagon and catecholamines is increased, and these factors play a central role in the response. In fact, the response pattern can be mimicked by infusing these substances into animals or humans [24]. More specifically, cortisol has a pronounced effect in upregulating glutamine (GLN) synthesis in skeletal muscle, [25] and glucagon appears essential in enhancing hepatic uptake of this amino acid and facilitating ureagenesis [26]. In addition to the hormonal response,

inflammatory factors, such as the proinflammatory cytokines, leukotrienes and other factors, contribute to the catabolic response, either directly or indirectly stimulating elaboration of catabolic hormones, causing anorexia through central nervous system mechanisms and increasing body temperature [27]. The increased protein breakdown serves to mobilize amino acids for synthesis of new protein tissue in wounds, for proliferation of macrophages, and other cellular components involved in the healing process, and for synthesis of acute phase proteins and glucose in the liver. Up to 1500 g of lean tissue can be lost after major abdominal surgery [28, 29]. As proteins represent structural and functional components, erosion of lean body mass can initiate immunosuppression, delayed wound healing, decreased muscle strength and fatigue which may result in prolonged convalescence and increased morbidity [30-32].

2.2. Epidural analgesia modulates the catabolic response to surgery

The epidural space contains nerve roots, fat, spinal arteries and lymphatic, as well as a valveless venous system [33, 34] that communicates directly with both the intracranial sinuses via the basovertebral veins and the general circulation via the azygos vein. Dorsal and ventral spinal nerve roots covered by dura mater pass across the epidural space, and drugs within this space can act on any nerve that traverses it - whether it is motor, sensory or autonomic. Epidural analgesics may prevent the release of neurotransmitters from afferent pain fibers, block receptors to neurotransmitters released by primary afferent pain fibers, or interrupt the transmission of pain-related information in the dorsal horn of the spinal cord [35]. Drugs introduced into the epidural space also have the potential to pass both into the brain and the general circulation depending on their pharmacokinetics. Experimentally, epidural local anesthetics suppress perioperative adrenaline and noradrenaline production [36-38]. Recently, epidural analgesia with an intensive and standardized regimen of early feeding and mobilization has been shown to reduce hospital stay in patients undergoing gastrointestinal surgery [13, 39]. This has led to the suggestion [40] that ‘epidurals’ have the most favorable effects on outcome when used for gastrointestinal surgery. Increased oxygen consumption from the stress response is abolished by epidural analgesia [38] and postoperative plasma levels of adrenocorticotrophic hormone, cortisol, aldosterone and glucose, and urinary cortisol levels are lower after surgery with epidural blockade than with other methods of analgesia [41, 42]. Epidural opioids reduce the stress response to a lesser extent than local anesthetics, suggesting that pain-related pathways are only partly responsible [42]. Epidural analgesia at the C3-C4 level achieves a more profound reduction in the

endocrine response to surgery than blockade at lower levels, suggesting that phrenic, as well as thoracic and lumbar spinal, sensory fibers carry nociceptive impulses after gastrointestinal surgery [43]. Due to superior pain control during mobilization, epidural analgesia with local anesthetics facilitates a more rapid return in the patient's mobility after surgery when compared with intravenous opioids. Since prolonged bed rest has been associated with significant muscle atrophy and a decrease in whole-body protein synthesis, early postoperative ambulation and mobilization might well contribute to the anticatabolic effect of epidural analgesia [44]. Subsequently, the reports of three large, randomized, clinical trials in major abdominal and vascular surgery [45, 46] and major procedures in high-risk patients [47, 48] have been published, all with negative outcome effects of epidural analgesia, except for the previously demonstrated, improved pulmonary outcome [49, 50]. At first glance these negative findings might be extremely disappointing and inconsistent with the potential of continuous epidural analgesia to improve outcome due to its various well-established stress-reducing effects [51, 52]. However, there may be several explanations for the negative outcome data from epidural analgesia, which emphasizes the need for reconsideration and re-design of such outcome studies [53]. Firstly, existing outcome studies have predominantly been opioid based, which will not provide stress-reducing effects as observed when local anesthetics are included [51, 52]. Secondly, these studies often do not allow sufficient interpretation to be made, as there is no specific information on the type of epidural analgesia with regard to level of insertion in relation to the surgical incision, dose of the local anaesthetic, exact amount of opioid and success and duration of the epidural regimen [54]. Thirdly, and probably most importantly, epidural outcome studies almost invariably do not provide

specific information about the overall perioperative care regimen that is provided, with regard to use of tubes, drains, restriction of feeding, mobilization, nursing care regimen, pre-admission information on hospitalization, etc., all of which, in recent years, have been demonstrated to be of the utmost importance for the reduction of postoperative morbidity and need for hospitalization [55-57]. Thus, the advantageous physiological effects of a continuous postoperative epidural regimen on outcome may be overridden by the negative effects of a semi-starved, partly immobilized patient with unnecessary prolonged used of nasogastric tubes, drains, catheters and traditional use of time for hospitalization. In addition, epidural outcome studies rarely provide information on fluid management, which is another component that has to be considered in multimodal rehabilitation in fast-track surgery, as fluid excess may have negative outcome effects on cardiopulmonary function, gastrointestinal recovery and overall morbidity[58, 59].

2.2.1 Epidural and protein metabolism

Symmetrical sensory loss from T4 to S4 dermatomes has been shown to attenuate the postoperative loss of body nitrogen, to blunt the rise in protein breakdown and amino acid oxidation, and to arrest the fall in muscle protein synthesis following abdominal surgery [37, 60-62]. The obvious shortcoming of nitrogen balance measurement is that the contribution from dynamic changes in protein synthesis and degradation cannot be separated. These studies were conducted in patients receiving parenteral nutrition throughout the perioperative period. Therefore, the effects of analgesia could not be separated from those of nutrition. A three-hour infusion of 4mg/kg/min glucose did not

affect protein breakdown and synthesis, but improved oxidative glucose utilization, and decreased amino acid oxidation [4]. In a subsequent study [5] provision of exogenous glucose and amino acids improved protein balance, indicating that the anabolic effect of epidural analgesia requires energy and substrate supply. Perioperative epidural blockade with local anesthetics (and opioids), in contrast to patient controlled analgesia (PCA) with intravenous morphine, facilitated the uptake and oxidative utilization of glucose two days after abdominal surgery. This was accompanied by a decrease in protein oxidation indicating a favorable shift from a protein dominated to a more glucose orientated oxidative substrate utilization.

2.2.2 Epidural and glucose metabolism

It has long been recognized that epidural blockade with local anesthetics inhibits or prevents the hyperglycemic response to surgical procedures performed on the lower part of the body [61, 63]. The mechanisms involved in this modifying effect of neural blockade on perioperative glucose homeostasis and tolerance have not yet 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. Perioperative intravenous glucose tolerance tests carried out in patients undergoing inguinal herniotomy or transabdominal hysterectomy with neural blockade were normal, while glucose tolerance was impaired during inhalational anesthesia [62, 64]. 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 epidural blockade could only partly be explained by a lower glucose release from the splanchnic region [65]. In contrast a suppressory effect of epidural blockade on endogenous glucose production was observed by Shaw, who studied parenterally fed patients between one and five days after various surgical interventions [66]. This inhibitory influence of epidural blockade on glucose production was accompanied by a decrease in urea synthesis, an indirect parameter of protein catabolism. According to the results of a recent study, perioperative epidural blockade had no influence upon glucose production and plasma glucose concentration in fasted patients two days after colorectal surgery [67]. However, epidural blockade in contrast to PCA with intravenous morphine stimulated uptake and oxidative utilization of glucose indicating an increased insulin sensitivity [67]. This improved utilization of glucose was accompanied by a decrease in endogenous protein oxidation lending further support to the concept of a significant interdependence of glucose and protein metabolism after surgery.

2.3. Nutrition

2.3.1 Rationale of nutritional support

The major goal of nutritional management of surgical patient 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 [68]. 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 [68]. Although most clinicians would agree that nutritional support is useful in the nutritional management of selected surgical patients, only few studies addressed the mechanisms, whereby parenteral feeding modulates the metabolic and endocrine responses to surgical trauma.

Exogenous glucose suppresses gluconeogenesis, thereby reducing the need for muscle protein breakdown to supply gluconeogenic amino acids [1]. If the rate of gluconeogenesis from amino acids is decreased, that amount of nitrogen is available for reincorporation into protein rather than for excretion as urea. The inhibitory effect of exogenous glucose upon gluconeogenesis has been shown to depend on the dose of glucose and the physiologic state of the subject. Glucose infused at 4 mg kg –1 min –1 almost completely suppresses endogenous glucose production in healthy volunteers, while glucose applied at the same dose in patients after trauma and during sepsis decreases glucose production by 50% only [69, 70]. Glucose administration at a higher rate fails to further inhibit glucose production in surgical patients, but results in an increase in glucose oxidation and a more pronounced decrease of urea synthesis, an

indirect parameter of protein catabolism [69]. It has, therefore, been postulated that once enough glucose is given to achieve the maximal inhibition of gluconeogenesis the justification of administering higher doses resides in the ability of the body to oxidize the infused glucose. Hence, provision of exogenous amino acids and, thereby counteracting the loss of amino acids oxidized for energy production has been regarded a second mechanism responsible for the protein preservation in critically ill patients. Studies on volunteers and surgical patients [11, 71, 72] have demonstrated that amino acids infusion spares protein and induces an anabolic state because it directly stimulates whole body and muscle protein synthesis, and indirectly attenuates protein breakdown. However, the effects on glucose metabolism are conflicting. Studies on volunteers and diabetic patients suggest that infusion of amino acids stimulates gluconeogenesis [73, 74]. In contrast, in surgical and in trauma patients it has been found that, by infusing an equivalent amino acids mixture at the same rate, EGP decreases [75, 76]. Skeletal muscle glucose uptake is also reduced in some [77, 78] but in not all studies [73, 79].

2.3.2 Limitations of nutritional support: alternative approaches

Although provision of adequate quantities of glucose, either alone or together with amino acids, attenuates protein losses in critically ill patients via an increase in whole body synthesis, the elevated rate of protein catabolism continues unaltered [70]. This limited effectiveness of nutritional support during catabolic illness has been attributed to the nonsuppressibility of gluconeogenesis by glucose administration and the impaired capacity of stressed patients to oxidize glucose, which is given in excess of the amount required for most efficient inhibition of gluconeogenesis. Only half of the glucose infused

at 4 mg kg –1 min – 1 was directly oxidized after surgical or accidental trauma, and this percentage further fell when glucose was administered at higher rates [70, 80].

The fact that even vigorous nutritional support fails to entirely curtail protein catabolism after trauma and during sepsis 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 ameliorate protein catabolism in surgical and injured patients [81-83]. In order to overcome insulin resistance, a prominent feature of the endocrine response to surgery, insulin, however, has to be applied in high doses [81]. At the same time administration of excessive amounts of glucose is required to maintain normoglycemia. This issue rises potential metabolic concerns because excessive carbohydrate and caloric intake might cause fatty infiltration of the liver [84] and significantly stimulates carbon dioxide production [6].

2.4 Assessment of metabolism

2.4.1 Infusion of stables isotopes

Although generally adequate for clinical management purposes, observations based on plasma levels of metabolites alone allow no insight into the biochemical events that produce the observed value. Since, for instance, an increased blood glucose concentration might be the consequence of accelerated release, diminished glucose uptake or both, inferences made from static observations can be misleading.

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 intake and the excretion of nitrogen. With this method the contribution from alterations in protein synthesis and protein degradation cannot be separated. Negative nitrogen balance can, therefore, occur if the protein breakdown and amino acid oxidation increase and synthesis remain the same or if breakdown and oxidation rates remain unchanged and the rate of protein synthesis decrease.

In contrast the measurement of protein turnover, amino acid oxidation and protein synthesis gives a dynamic picture of the movement of protein in the body. Calculation of substrate kinetics means the determination of the rate of appearance (Ra) of a substrate and, at least in the steady state, the rate of disappearance (Rd) of the substrate. Other factors, such as the half life, mean residence time, and clearance rate can also be derived. Turnover rates of amino acids and glucose can be followed in vivo by using stable isotope tracers, i.e. substrates, which have been labeled by stable, non radioactive isotopes (^{2}H, ^{13}C) and the subsequent measurement of isotopic enrichments in plasma.

If a tracer is given as a constant infusion, and the kinetics of the tracee are best described by a single-pool model, then when an isotopic equilibrium is achieved the rate of appearance (Ra) of the unlabeled substrate (tracee) can be calculated by equation 1:

$$Ra = F/E_p \qquad (1)$$

Where F is the tracer infusion rate and E_p is the isotopic enrichment at plateau.

2.4.2 Protein kinetics

The discovery of the dynamic nature of protein in the body can be attributed to the pioneering work of Schoenheimer and associates [85]. These investigators used stable isotopes of amino acids to show that proteins were continually being broken down (catabolized) and resynthesized. In fact, only about 20% of protein synthesis comes from daily amino acid intake. Thus about 80% of protein synthesis involves a recycling of amino acids from protein breakdown. A high rate of protein turnover *per se* can have no effect on net protein balance if catabolism is elevated to the same extents as synthesis. In light of these consideration the measurement of both protein synthesis and breakdown are necessary to assess adequately the regulation of protein kinetics. A brief overview of the possible techniques is provided.

2.4.2.1 Constant infusion of ^{15}N-glycine (end product method)

The end product method was first used by Picou and Taylor-Roberts [86]. The rationale behind this method considers a metabolic pool of nitrogen (N) into which amino acids enter from the diet or from breakdown of body protein. Amino acids can leave the

metabolic pool to be incorporated into protein (synthesis) or to be degraded and the N excreted in the urine, mainly as urea. The authors assumed:

1. a constant size of the metabolic pool throughout infusion
2. no recycling of ^{15}N isotope
3. no isotopic discrimination
4. no difference in the way in which amino acids from the diet or from endogenous protein breakdown are handled
5. ^{15}N-glycine is a valid tracer for total amino N

If there is no change in the size of the metabolic pool over the course of the experiment, then the rate at which amino acids leave the pool must be equal to the rate at which they arrive. Therefore:

$I + C = S + E_t = Q$

Where I is the N intake, C is catabolism, S is synthesis, Et is the sum of the rate of excretion of urinary urea N (E_u) and the rate of excretion of nonurea N, and Q is the total turnover rate of flux amino N.

When ^{15}N-glycine is infused until an isotopic equilibrium is achieved, the fraction of the isotope excreted as urea (f) should be the same as the percentage of total amino N entering the pool that is excreted as urea. Thus

$$Q = Eu/f = F/(t/t)$$

Where t/t is the tracer/tracee ratio of either urinary urea or ammonia N.

Methods relying on kinetics of an essential amino acid

Many different techniques fall within this general category. They are representative of whole-body techniques in general in that they are useful approaches for estimating

protein turnover in humans with stable isotopes in certain circumstances, while in the situations of significantly altered physiological state (exercise, cold exposure) the model become unreliable. The principal advantage with these methods is tat, with appropriate priming of the relevant pools, it is possible to collect reliable data within 90 minutes of the start of infusion.

2.4.2.2 Constant infusion of [1-^{13}C]-leucine

the general rationale of this method considers the entire intracellular space as a single compartment. In the fasting state, the sole source of the essential amino acid leucine for protein synthesis and oxidation is that derived from the breakdown of endogenous proteins [87]. The flux of L-[1-^{13}C] leucine represents the total movement of leucine into and from the plasma pool. Oxidation of leucine results in its conversion to ^{13}C-carbon dioxide. Therefore, leucine flux minus oxidation provides, indirectly, a measure of the rate of protein synthesis. The Ra of leucine intracellularly can be calculated from the quotient of the tracer infusion rate and a pooled value representing the average intracellular enrichment throughout the body. Plasma alpha-ketoisocaproic (KIC) enrichment has been proposed to provide such a value [88].

Under steady state conditions, the rate of appearance (Ra) of unlabelled substrate in plasma can be derived from the plasma enrichment (atom percent excess = APE) calculated by $Ra = I \cdot (APE_{inf}/ APE_{pl} - 1)$, 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 labelled tracer. 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 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 post adsorptive state, flux is equal to breakdown. Plasma α-[1-^{13}C]KIC enrichment will be used to calculate both flux and oxidation of leucine.

2.4.2.3 Use of alternative amino acids

The general principle described for leucine can be used for any other essential amino acid, with the exception that only isoleucine and valine have intracellular ketoacids analogous to the leucine/KIC system. Thus, with other amino acids, the Ra will result in a considerable underestimate of the total rate of breakdown. Lysine can be used to this purpose labeled with either alpha-^{15}N or 1-^{13}C. Plasma Ra lysine (and thus uptake) is measured, and then it is assumed that the lysine uptake is either irreversibly catabolized or incorporated into protein. The alpha-N of lysine does not participate in transamination reactions to any great extent, and lysine cannot be synthesized once the 1-carbon is lost to CO_2. Therefore, the rate of breakdown of lysine can be estimated by determining either the rate of lysine decarboxylation with 1-^{13}C-lysine tracer or the rate of incorporation of the alpha-^{15}N into urea. Unfortunately, it is not possible to use an approach described for the estimation of intracellular lysine enrichment analogous to the determination of KIC enrichment during the infusion of 1-^{13}C-leucine due to the lack of a unique catabolic product of lysine that cannot be derived from any source. Therefore, lysine Ra is underestimated, as is lysine oxidation. Rates of whole-body protein synthesis (S) and catabolism (C) are calculated as follows when labeled lysine is used as tracer.

$$S = (Ra\ lysine - lysine\ breakdown) / 3.4\ mM\ lysine/gN \times 6.25$$

When the rate of lysine catabolism is determined from the incorporation of alpha-^{15}N into urea,

Lysine breakdown = (Ra_N urea x 2μmol N/μmol urea x E_{UN}) / E_{ly}

Where Ra_N urea is the rate of appearance of urea from nonrecycled N, E_{UN} is urinary urea N enrichment, and E_{ly} is plasma lysine enrichment. When 1-^{13}C-lysine is used, lysine breakdown is calculated as being equal to lysine oxidation. Thus, protein breakdown and synthesis are both underestimated with this method.

2.4.3 Glucose kinetics

2.4.3.1 Gluconeogenesis and glycogenolysis

Whole body glucose production is depending on the metabolic state, composed to a varying extent of glycogenolysis and gluconeogenesis. The exact contribution of glycogen to total glucose production is difficult to quantify, but after an overnight fast estimates range as high as 90% [89]. With more prolonged fasting (60 hours), hepatic glycogen becomes depleted and gluconeogenesis becomes the entire source of glucose production. Glucose Ra is most commonly determined with hydrogen isotopes (^2H or ^3H) as tracers but its use of does not allow to differentiate between the two metabolic pathways. Quantification of gluconeogenesis involves the infusion of a 13C-labeled precursor (alanine or lactate) and measurement of the precursor enrichment and the enrichment of glucose at isotopic equilibrium. The fraction of glucose Ra (F_{Ra}) coming from gluconeogenesis from that precursor is then calculated as follows.

$$F_{Ra} = E_{glucose} / E_{precursor}$$

Other precursors used to quantify gluconeogenesis are 1-^{13}C-pyruvate and 2-^{14}C-acetate.

2.4.3.2. Glucose uptake

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 [51]. 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. This methodology differs from the glucose clamp technique in the sense that the metabolic milieu is not altered by the small amount of glucose tracer infused during the study.

Intraoperative infusion of amino acids induces anabolism independently of the type of anesthesia.

3.1 Abstract

Previous intraoperative studies conducted either in a fasting state or during glucose infusion reported no difference in the effect of the type of anesthesia on protein metabolism. The purpose of this study was to test the hypothesis that nutritional supplementation with amino acids induced a greater protein balance in patients receiving epidural combined to a light general anesthesia compared to patients receiving general anesthesia alone. Sixteen patients were randomly assigned to receive either general anesthesia with desflurane (control group) or general anesthesia combined with epidural analgesia (EDA group). A primed constant infusion of stable isotope tracers L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose was started before surgery (3 hours of fasted state) and continued for other 3 hours during surgery together with an infusion of amino acids (fed state). Arterial blood and expired air samples were collected into the fasted and fed states when the tracers were assumed to have reached a steady state, and protein and glucose kinetics were studied. Endogenous rate of appearance (Ra) of leucine decreased to a similar extent in both groups ($P < 0.05$), and protein synthesis increased, with no difference between the two groups ($P < 0.05$). As a result, net protein balance (protein synthesis – protein breakdown) became positive to the same extent in both groups ($P < 0.05$). Leucine oxidation did not change in both groups ($P > 0.05$). After amino acids infusion, in both groups EGP remained unchanged ($P = 0.08$). Glucose clearance decreased in both groups. ($P < 0.05$). Blood glucose, plasma cortisol, serum insulin and glucagon concentrations increased to the same extent in both groups ($P < 0.05$). Intraoperative provision of amino acids induced a positive protein balance. Epidural anesthesia did not provide any additional benefit beyond the anabolism obtained with

amino acids. In both groups EGP was not significantly decreased while glucose clearance decreased independently of the type of anesthesia used.

3.2 Introduction

Gluconeogenic amino acids released during muscle proteolysis become the major source of precursors for *de novo* glucose synthesis [1]. It has been proposed that perioperative inhibition of gluconeogenesis by anesthetic or pharmacologic interventions causes a decrease in protein breakdown, leading to a better preservation of whole body protein economy [1, 4]. In a previous study, [2] intraoperative endogenous glucose production (EGP), an indicator of gluconeogenesis, was significant less than preoperative values. This decrease was more pronounced in patients receiving epidural combined with a light general anesthesia than in patients with general anesthesia alone. However, in both groups, protein breakdown decreased to the same extent, and net protein balance, measured as protein synthesis – protein breakdown, was negative, implying that the type of anesthesia did not affect protein metabolism.

Glucose exerts its anticatabolic action through the suppression of gluconeogenesis and reduced need for gluconeogenic amino acids released from the muscle [1]. Thus, in a subsequent investigation, [3] dextrose was infused in the same groups of patients receiving the same anesthesia protocol as above with the intent to decrease EGP to a greater extent and to achieve a positive protein balance. It was also hypothesized that the benefits on protein metabolism would have been more pronounced in the epidural group as a result of its greater ability to reduce EGP. However, even if epidural anesthesia caused a major decrease in EGP, net protein balance remained negative [3]. Furthermore, the fall in protein breakdown was smaller (18%) than when dextrose was not infused (23%), and blood glucose levels increased up to 10 mmol/l in both epidural and general anesthesia [3]. The lack of anticatabolic action of exogenous glucose, associated with

hyperglycemia raised doubts on the value of infusing dextrose during surgery. Hyperglycemia which is associated with several disadvantageous clinical effects [6-10] and efforts should be made to avoid it..

Efforts should be made to attenuate perioperative protein breakdown and establish a positive protein balance because, if catabolism is intense and untreated, postoperative convalescence can be protracted with increased morbidity as a result of perioperative erosion of lean body mass, immunosuppression, delayed wound healing, decreased muscle strength and fatigue [28]. Studies in surgical patients have demonstrated that infusion of amino acids spares protein and induces an anabolic state because it directly stimulates whole body and muscle protein synthesis, and indirectly attenuates protein breakdown [11].

With the understanding of the anabolic properties of infusing amino acids and the greater capacity of epidural blockade in decreasing EGP, the main goal of this study is to test the hypothesis that supplementation of amino acids during surgery induces a greater positive protein balance in patients receiving an intra-operative epidural blockade compared to patients receiving general anesthesia alone. At the same time, to put in evidence possible relationship between protein and glucose metabolism during amino acids infusion, whole body glucose kinetics was studied. To quantify the dynamic changes in protein and glucose metabolism whole body protein breakdown, amino acid oxidation, protein synthesis, and EGP and glucose clearance were determined by an isotope dilution technique using stable isotope L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose.

3.3 Methods

Patients

The study was approved by the ethics committee of the Montreal General Hospital and informed consent was obtained from 16 patients undergoing elective colorectal surgery. No patient was suffering from cardiac, hepatic, renal or metabolic disorders or receiving any medication known to affect glucose metabolism. None of the participants had developed more than 10% weight loss over the preceding 3 months or had a hemoglobin < 100 g/L. Patients were randomly assigned to receive either general anesthesia with desflurane (control group) or the same general anesthesia combined with epidural analgesia (EDA group).

Anesthesia and amino acids infusion

General anesthesia was induced in all patients with 5mg/kg of thiopentone, and 5 μg/kg of fentanyl in the control group or 1.5 μg/kg of fentanyl in the EDA group. Tracheal intubation was facilitated by 0.6 mg/kg rocuronium, and the lungs were ventilated to normocapnia (35-40 mmHg) with 30% oxygen enriched with air. In the EDA group, an epidural catheter was inserted between T9-11 before induction of general anesthesia. Neuraxial blockade was established with 0.5% bupivacaine to achieve a bilateral sensory block from T4-S5 and maintained with intermittent boluses of bupivacaine 0.25%. General anesthesia in the control group was maintained with desflurane at end-tidal concentrations as required to keep heart rate within 20% of preoperative values. In the EDA group desflurane was administered at end-tidal 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 given to achieve complete surgical muscle relaxation throughout the surgery. Intravenous fluid was given as NaCl 0.9% solution at a rate of 8 ml/kg/h. All patients were covered with a warming blanket during surgery to maintain normothermia. Hemodynamic monitoring was performed using a three-lead electrocardiogram monitor and radial artery catheterisation for continuous blood pressure measurement.

Isotope kinetics study was started 3 hours before surgery (fasted state), and followed by the fed state that commenced with the surgical incision and during which 10% amino acids without electrolytes (Travasol™: Baxter, Montreal, Canada) were infused at a rate of 0.02ml/kg/min, equivalent to 2.9 g/kg/day rover a 3 hours period.

Experimental protocol

The kinetics of whole body leucine metabolism, i.e., rate of appearance (R_a) of leucine, and leucine oxidation were measured by an isotope dilution technique using the stable isotope tracers L-[1-^{13}C]leucine, and $NaH^{13}CO_3$, while the kinetics of whole body glucose metabolism, i.e., rate of appearance (R_a) of glucose was measured by an isotope dilution technique using the stable isotope tracer [6,6-$^2H_2$]glucose (Cambridge Isotope Laboratories, Cambridge, MA). All isotope solutions were prepared under sterile conditions in the hospital pharmacy. An aliquot of tracer was dissolved in a known volume of sterile water to achieve a solution of 100mg/ml. The solution was passed through a 0.22-µm filter into injection bottles. The bottles were sealed off, heat sterilized at 121°C for 15 min, and kept at 4°C until administration. Each set of solutions was confirmed to be free of pyrogens.

All patients were studied on the day of surgery between 7:00 and 8:00 AM after fasting for ~32 h. Only clear fluids were allowed until midnight the day preceding the operation because of bowel preparation as required for colorectal surgery. No premedication was given. A superficial vein in the dorsum of the hand was cannulated, to provide access for the infusion of the isotope. Under local anesthesia the contralateral artery was cannulated with a 22-gauge catheter for sampling of arterial blood. Blood and expired air samples were collected before the isotope infusion to determine baseline isotope enrichments. Primed doses of 1 μmol/kg $NaH^{13}CO_3$, 4 μmol/kg L-[1-^{13}C]leucine, and 22 μmol/kg [6,6-$^{2}H_2$]glucose, were administered and followed immediately by a continuous infusion of 0.06 μmol/kg/min L-[1-^{13}C]leucine and 0.22 μmol/kg/min [6,6-$^{2}H_2$]glucose. Four arterial blood and expired-air samples were collected at 150, 160, 170, and 180 min into the isotope infusion (fasted state), when the tracers were assumed to have reached an isotopic steady state. Thereafter, anesthesia was induced and surgery begun. At the same time the infusion of amino acids was started and the rate of infusion of L-[1-^{13}C]leucine was doubled to 0.12 μmol/kg/min. Four arterial blood and expired-air samples were taken at 330, 340, 350, and 360 min into the isotope infusion (fed state). Plasma samples for the analysis of blood glucose and plasma hormones (insulin, glucagon, cortisol) were drawn at 150 and 330 minutes of isotopes infusion. A graphic illustration of the study protocol is presented in Fig 1.

Analytical methods

Each blood sample was transferred immediately to a heparinized tube and centrifuged at 4°C. The plasma obtained was stored at −70°C until analysis. Expired-air samples were collected through a mouthpiece in a 2-liter latex bag and transferred immediately to 20-ml

vacutainers to await $^{13}CO_2$ isotope enrichment analysis. During artificial ventilation, expired gases were collected by means of a one-way valve into a 5-liter bag. Production of CO_2 was measured by indirect calorimetry (Datex Deltatrac, Helsinki, Finland) over a 20-min period of steady state during both fasted and fed states. Plasma α-KIC enrichment was determined by electron impact selected-ion monitoring gas chromatography-mass spectrometry, as previously described [4]. Expired $^{13}CO_2$ enrichment was analyzed by means of isotope ratio mass spectrometry and used to calculate leucine oxidation. In each analysis run, duplicate injections were always performed, and their means were taken to represent enrichment. Plasma glucose was derivatized to its pentaacetate compound and analyzed by electron impact ionization gas chromatography-mass spectrometry, as previously described [4]. Isotopic enrichment of [6,6-$^2H_2$]glucose was calculated as molecules per cent excess on duplicate injections of 4 samples at isotopic steady state and one baseline sample. Plasma concentration of glucose was measured by a glucose oxidase method using a glucose analyzer 2 (Beckman Instruments, Fullerton, CA). Circulating concentrations of insulin and glucagon were measured by sensitive and specific double-antibody radioimmunoassays (Amersham International, Bucks, UK). Cortisol plasma concentration was measured using the Ciba Corning ACS 180 automated immunoassay (Ciba Corning Diagnostic, East Walpole, MA).

Calculations

Whole body leucine and glucose kinetics were calculated by conventional isotope dilution practice using a two-pool stochastic model during steady-state conditions, obtained at each phase of the studies, and before and during surgery. At isotopic steady state, the R_a of unlabeled substrate can be derived from the plasma enrichment [molecules

percent excess (MPE)] calculated by $R_a = (MPE_{inf}/MPE_{pl} - 1) \cdot F$, where F is the infusion rate of the labelled tracer (µmol/ kg/min), MPE_{inf} is the tracer enrichment in the infusate, and MPE_{pl} is the tracer enrichment in plasma at steady state. The MPE value used in this calculation represents the mean of the MPE values determined during each isotopic plateau. The accuracy of the isotopic enrichments at isotopic plateau was tested by evaluating the scatter of the MPE values around their mean, expressed as a coefficient of variation (CV). A CV < 5% was used as a confirmation of a valid plateau. . 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 dietary intake or the rate of infusion of L-[1-^{13}C]leucine (µmol/kg/h) or both. Inspection of that formula indicates that, when studies are conducted in the post absorptive state, flux is equal to breakdown. Enrichment of plasma α-ketoisocaproate (α-KIC) during infusion of L-[1-^{13}C]leucine has been used to determine whole body leucine kinetics. During amino acids infusion, net leucine flux was calculated by subtracting the leucine infusion rate from the total Ra of leucine. This steady-state reciprocal pool model is considered to represent the intracellular precursor pool enrichment more precisely than leucine itself [87]. In the calculation of oxidation, a factor of 0.76 was applied during the fasted state and account for the fraction of ^{13}C-carbon dioxide released from leucine but retained within slow turnover rate pools of the body. A factor of 0.92 was used during feeding [4].

In the fasted state or when glucose intake is zero, Ra glucose equals the rate of endogenous glucose production. The glucose clearance rate, an indicator of the tissue

ability to take up glucose, was calculated as the Ra glucose divided by the corresponding glucose plasma concentration.

Sample size and statistical analysis

Based on expected difference in protein balance of 10 μmol/kg/min between the two groups (SD= 5 μmol/kg/min; power 80% and P= 0,05) a total number of 16 patients was calculated to be sufficient [5]. Data are presented as means ± SD. 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.

3.4 Results

Patient characteristics (Table 1)

There were no differences between the two groups regarding age, weight, sex and the American Society of Anesthesiologists (ASA) classification. Type of surgery, duration of surgery, estimated blood loss and the amount of crystalloid fluids administered were comparable in both groups.

Hemodynamics, and other variables (Table 2)

Blood pressure and oxygen saturation taken before surgery and 120 minutes after the start of surgery were similar in both groups. Heart rate at 120 minutes after the start of surgery were lower in the EDA than in the control group. The hematocrit decreased in both groups. Body temperature (°C) did not change during surgery and amino acids infusion in both groups. Carbon dioxide production decreased to the same extent in both groups during anesthesia and surgery. The end-tidal desflurane concentrations were lower in the EDA group than in the control group at 120 minutes after skin incision.

Leucine kinetics (Tables 3a and 3b)

An isotopic plateau of α-[1-^{13}C]KIC, and expired $^{13}CO_2$ was achieved in all patients allowing steady state calculations (CV < 5%, Fig. 2). The baseline values of rate of appearance (Ra) of leucine, leucine oxidation and protein synthesis were different between groups, (Table 3a) but the difference between the fasted and fed states was similar in the two groups (Table 3b). Endogenous rate of appearance (R_a) of leucine decreased to a similar extent in both groups ($P < 0.05$), while protein synthesis increased, with no difference between the two groups ($P < 0.05$), thus net protein balance became

positive to the same extent in both groups ($P < 0.05$). Leucine oxidation did not change in both groups ($P > 0.05$) (Table 3a and 3b).

Glucose kinetics (Table 4)

An isotopic plateau of [6,6-$^2H_2$]glucose was achieved in all patients (CV < 5%), (Fig 3) allowing steady state calculations. Blood glucose increased from 5.0 mmol/l to 8.0 mmol/l during the fed state in both groups ($P < 0.05$). There were not differences in the EGP during the fasted state between the epidural and the general anesthesia groups. Following the infusion of amino acids EGP decreased by an average of 6.6% and this was not significant in both groups. Glucose clearance decreased in both groups by 50% after the infusion of amino acids.

Hormones (Table 5)

Basal values of serum insulin and glucagon, and plasma cortisol were similar in both groups, and increased after amino acids infusion to the same extent.

3.5 Discussion

The results of this study indicate that patients receiving amino acids infusion during surgery where in a positive protein balance, with no difference between epidural and general anesthesia.

The same lack of influence on body protein economy by the type of anesthesia (general alone vs general plus epidural) has been found in previous intraoperative studies, conducted either in a fasting state [2] or during a glucose infusion [3], and the net protein balance was negative in both states, lending support to the contention that during surgery the type of substrate infused is the only factor influencing protein metabolism.

Studies conducted in the postoperative period have shown that the type of anesthesia influences protein metabolism. In fact, epidural blockade, compared to intravenous opioids, improved nitrogen balance, [15] blunted the fall in muscle fractional synthetic rate, [60] attenuated the rise in whole body protein breakdown and oxidation, [13] and enhanced protein synthesis [5]. These positive effects where observed only in association with parenteral nutrition or with an infusion of a mixture of amino acids and glucose. If only glucose was provided, the net protein balance became negative and epidural analgesia was unable to modify protein synthesis and breakdown when compared to intravenous opioids with patient controlled analgesia (PCA) [4].

One would conclude that, intraoperative epidural blockade does not affect protein metabolism regardless to the type of nutritional support, while after surgery it exerts an anabolic effect only during nitrogenous nutritional supplementation.

In an attempt to explain the present findings, one would hypothesize that the lack of an enhanced anabolic effect by intraoperative epidural anesthesia might be the result of an

attenuation of whole-body protein breakdown due to general anesthesia itself. A decrease in protein breakdown and synthesis has been shown to occur in patients undergoing colorectal surgery during inhalational desflurane and intravenous propofol anesthesia [90]. Similarly, a decrease in all aspects of protein metabolism was reported during enflurane anesthesia for total abdominal hysterectomy [91]. In view of these findings, the inhibitory effect of anesthesia, observed in the present study, might have masked the positive effect on protein synthesis that one would otherwise have expected with epidural anesthesia.

Previous results suggested that whole body and muscle protein synthesis is stimulated almost linearly within the normal physiological range of plasma amino acids, and that two-to threefold increase in plasma concentrations of amino acids is required to saturate the system [92]. In view of this, the rate of infusion of amino acids was selected to be 2.9g/kg/day, with the intention to achieve a plasma amino acid concentration two-to threefold above basal value [93]. Three hours of amino acid infusion is sufficient for maximal incorporation of amino acids into whole body and tissue compartments. Beyond this time, in fact, muscle protein synthesis falls back to values near the basal state despite amino acids infusion continuing at the same rate [94].

It is interesting to observe that, although the real quantity of amino acids administered was 0.36g/kg/d and less than the daily recommended intake of 1.5 g/kg/d, [95] a consistent anabolic effect was shown, and therefore in agreement with previous findings demonstrating that amino acids are more efficiently utilized for maintaining lean body mass when given in divided doses rather than with continuous infusion [94].

With regard to the present findings on glucose metabolism, the baseline values of EGP were not modified after the infusion of amino acids, and this was independently of the type of anesthesia used, while glucose clearance decreased to the same extent with both techniques.

The fact that epidural anesthesia had the same effect of general anesthesia alone on EGP is in contrast with previous intraoperative studies using a similar protocol and conducted during either saline infusion or feeding with dextrose [2, 3]. In those studies, epidural anesthesia decreased EGP more than general anesthesia alone and it was probably due to the suppression of glucagon secretion exerted only by epidural anesthesia. In contrast, in the present study, serum glucagon increased to the same extent in both groups and this is related to the infusion of amino acids that stimulated glucagon secretion [73, 74].

The small, non significant decrease in EGP (8% on average) after the infusion of amino acids is in agreement with a previous findings in surgical patients undergoing major abdominal surgery where a perioperative infusion of amino acids mixture at 2g/kg/day [75] decreased endogenous glucose production by 8%, but failed to reach a statistic significance.

In volunteers, on the contrary, amino acids increased EGP. Tappy and colleagues using a stable isotopes technique showed an increase in EGP by 84%, following an infusion of amino acids at a rate of 4.8 g/kg/day [73]. Such increase had been explained as a result of a direct effect of amino acids acting as substrate for the gluconeogenic pathway and indirectly by raised plasma glucagon concentration which stimulates gluconeogenesis [73]. This contrasting effect of amino acids might be explained considering that anesthesia by itself decreases EGP [2, 90, 96, 97] and therefore, by inhibiting

gluconeogenesis, it might have increased the available amino acids for synthetic pathways.

In spite of the lack of change in EGP in both groups, a significant increase in blood glucose to values around 8 mmol/l was observed, implying that glucose uptake was decreased as reflected by a decrease in glucose clearance. The decrease in glucose uptake can be due to either anesthesia or amino acid infusion or both. Other studies have reported a decreased glucose utilization in brains of anesthetized animals [98, 99] and this could affect whole body glucose turnover, as the human central nervous system represents 50-80% of basal whole body glucose disposal after an overnight fast [100]. A decrease in muscular activity would be also an expected consequence of general anesthesia and this could contribute to a significant decrease in glucose utilization because muscle uptake accounts for about 20% of basal glucose consumption in a postabsorpitive state [101]. Thus, the reduction in glucose clearance could be simply related to the decrease in energy requirements of some tissues as reflected by the decrease in whole body oxygen consumption observed under anesthesia. Amino acids also could decrease peripheral glucose uptake. In volunteers, in fact, amino acids inhibited glucose transport/phosphorylation resulting in a decreased intracellular utilization of glucose [102].

In conclusion, intraoperative provision of amino acids induces a positive protein balance independently of the anesthetic technique. In addition, amino acids supplementation did not influence gluconeogenesis, while whole body glucose uptake decreased independently of the type of anesthesia used.

3.6 Appendix

Table 1. Patient Characteristics

	Control	EDA
Number	8	8
Age (yr)	57 ± 15	63 ± 16
Height (cm)	172 ± 7	169 ± 6
Weight (Kg)	71 ± 14	73 ± 16
Sex (Male/Female)	7/1	6/2
ASA (I/II/III)	2/6/0	3/5/0
Type of surgery		
Hemicolectomy/colectomy	4	5
Sigmoid resection	2	1
Anterior resection	2	2
Duration of surgery (min)	198 ± 40	201 ± 38
Estimated blood loss (ml)	163 ± 74	158 ± 95
Crystalloids (ml)	1928 ± 332	1882 ± 420

Values are mean ± SD; Control = general anesthesia alone; EDA = epidural blockade combined with general anesthesia; ASA = American Society of Anesthesiologists classification

Table 2. Hemodynamic, and other clinical variables.

	Control		EDA	
	Before surgery	During surgery	Before surgery	During surgery
Heart rate (beats/min)	77 ± 12	82 ± 14	73 ± 15	68 ± 5 †
Mean arterial pressure (mmHg)	90 ± 12	88 ± 8	93 ± 16	81 ± 5
Hematocrit (%)	37.4 ± 2.4	30.86 ± 1.9 *	36.3 ± 2.6	31.3 ± 1.7 *
SpO2 (%)	98 ± 2	100 ± 1	98 ± 1	99 ± 1
Temperature (° C)	36.2 ± 0.7	36.6 ± 0.5	36.1 ± 0.5	36.7 ± 0.6
CO_2 production (ml/min)	197.9 ± 46.3	169.9 ± 47.1 *	205.8 ± 40.5	159.3 ± 38.4 *
Desflurane End Tidal concentrations (%)		5.1 ± 1.2		3.3 ± 0.5 †

Values are means ± SD. Control = general anesthesia alone; EDA = epidural blockade combined with general anesthesia; SpO2 = arterial oxygen saturation. * = $P < 0.05$ versus before surgery, † = $P < 0.05$ versus control.

Table 3a. Leucine Kinetics

	Control		EDA	
	Fasted	Fed	Fasted	Fed
R_a Leucine (μmol/kg/h)	97.0 ± 11.0	107.3 ± 6.2	125.0 ± 10.9	146.0 ± 11.5
Endogenous R_a Leucine (μmol/kg/h)	97.0 ± 11.0	53.5 ± 6.2 *	125.0 ± 10.9	93.0 ± 12.2 *
Leucin oxidation (μmol/kg/h)	15.1 ± 3.6	15.0 ± 6.4	18.6 ± 5.9	21.7 ± 5.4
Protein Synthesis (μmol/kg/h)	82.0 ± 8.5	92.3 ± 4.0 *	106.4 ± 13.8	124.3 ± 6.9 *
Protein balance (μmol/kg/h)	- 15.1 ± 3.6	38.76 ± 6.4 *	- 18.6 ± 5.9	31.3 ± 6.1 *

Values are means ± SD. R_a = rate of appearance; Control = General anesthesia alone; EDA = Epidural anesthesia combined with general anesthesia. * = $P < 0.05$ versus fasted.

Table 3b. Differences in leucine kinetics between fast and fed status

	Difference Fed-Fasted		
	Control	EDA	P values
Endogenous R_a Leucine (μmol/kg/h)	- 43.51 ± 11.84	- 32.01 ± 16.49	0.13
Oxidation (μmol/kg/h)	- 0.09 ± 4.49	3.14 ± 5.39	0.21
Synthesis (μmol/kg/h)	10.35 ± 10.42	17.90 ± 15.35	0.27
Protein balance (μmol/kg/h)	53.86 ± 4.49	49.91 ± 5.77	0.15

Values are means ± SD, Ra = rate of appearance; Control = General anesthesia alone; EDA = Epidural anesthesia combined with general anesthesia

Table 4. Glucose kinetics

	Control		EDA	
	Fasted	Fed	Fasted	Fed
Glucose (mmol/l)	4.9 ± 0.5	8.2 ± 0.7 *	5.1 ± 0. 8	8.5 ± 1.1 *
EGP (μmol/kg/min)	12.05 ± 2.57	11.25 ± 1.36	12.68 ± 1.44	11.49 ± 1.87
Glucose clearance (ml/kg/min)	2.28 ± 0.81	1.30 ± 0.19 *	2.07 ± 0.71	1.41 ± 0.21 *

Values are means ± SD. EGP = endogenous glucose production; Control = General anesthesia alone; EDA = Epidural anesthesia combined with General anesthesia.
* = $P < 0.05$ versus fasted, † = $P < 0.05$ versus control.

Table 5. Plasma concentrations of hormones

	Control		EDA	
	Fasted	Fed	Fasted	Fed
Insulin (pmol/l)	37.6 ± 16.6	92.2 ± 48.3 *	45.5 ± 22.3	101.1 ± 62.5 *
Cortisol (nmol/l)	398 ±188.6	796 ± 213.5 *	392. 9 ±121.8	695.1 ± 127.9 *
Glucagon (pmol/l)	28.3 ± 8	46.5 ± 8 *	27.1 ± 9	40.8 ± 14 *

Values are mean ± SD.

Control = General anesthesia alone; EDA = Epidural anesthesia combined with General anesthesia.

* = $P < 0.05$ versus fasted, † = $P < 0.05$ versus control.

Figure 1: Time course of the infusion of isotopes and collection of plasma and expired air samples (Ø) indirect calorimetry (open rectangles), and collection of plasma for the determination of metabolic substrates and hormones (x) in the fasted state and during the infusion of amino acids.

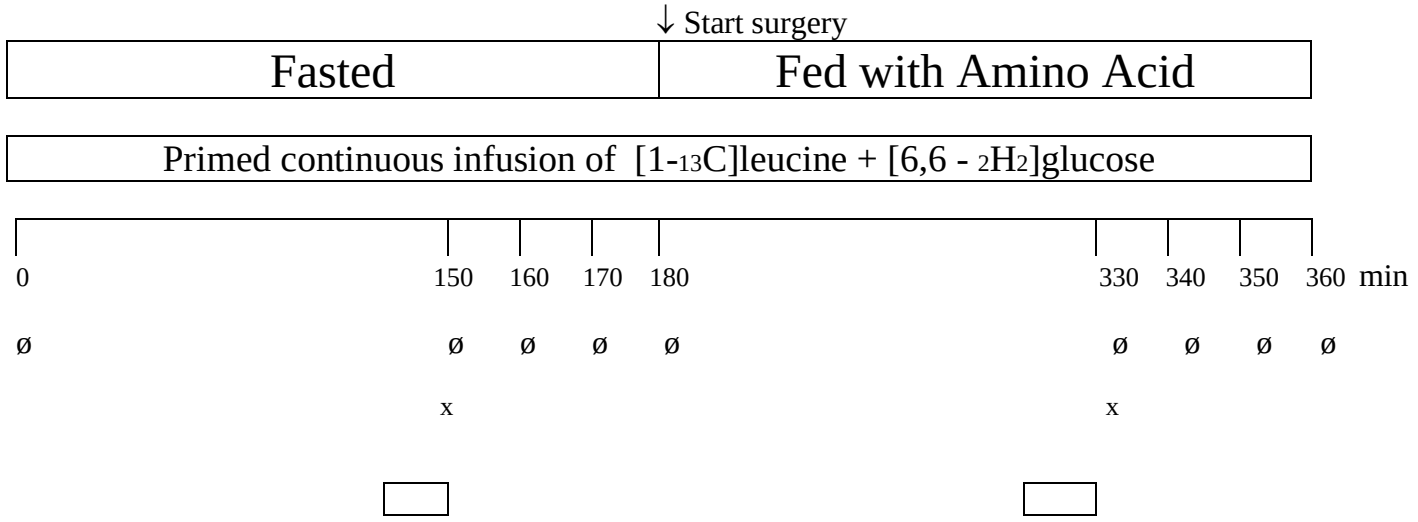


Fig.2. Plateau enrichments expressed in atom percent excess (APE) of [1-^{13}C]α-ketoisocaproic (KIC) and of ^{13}C-carbon dioxide. (□) = epidural; (▲) = control

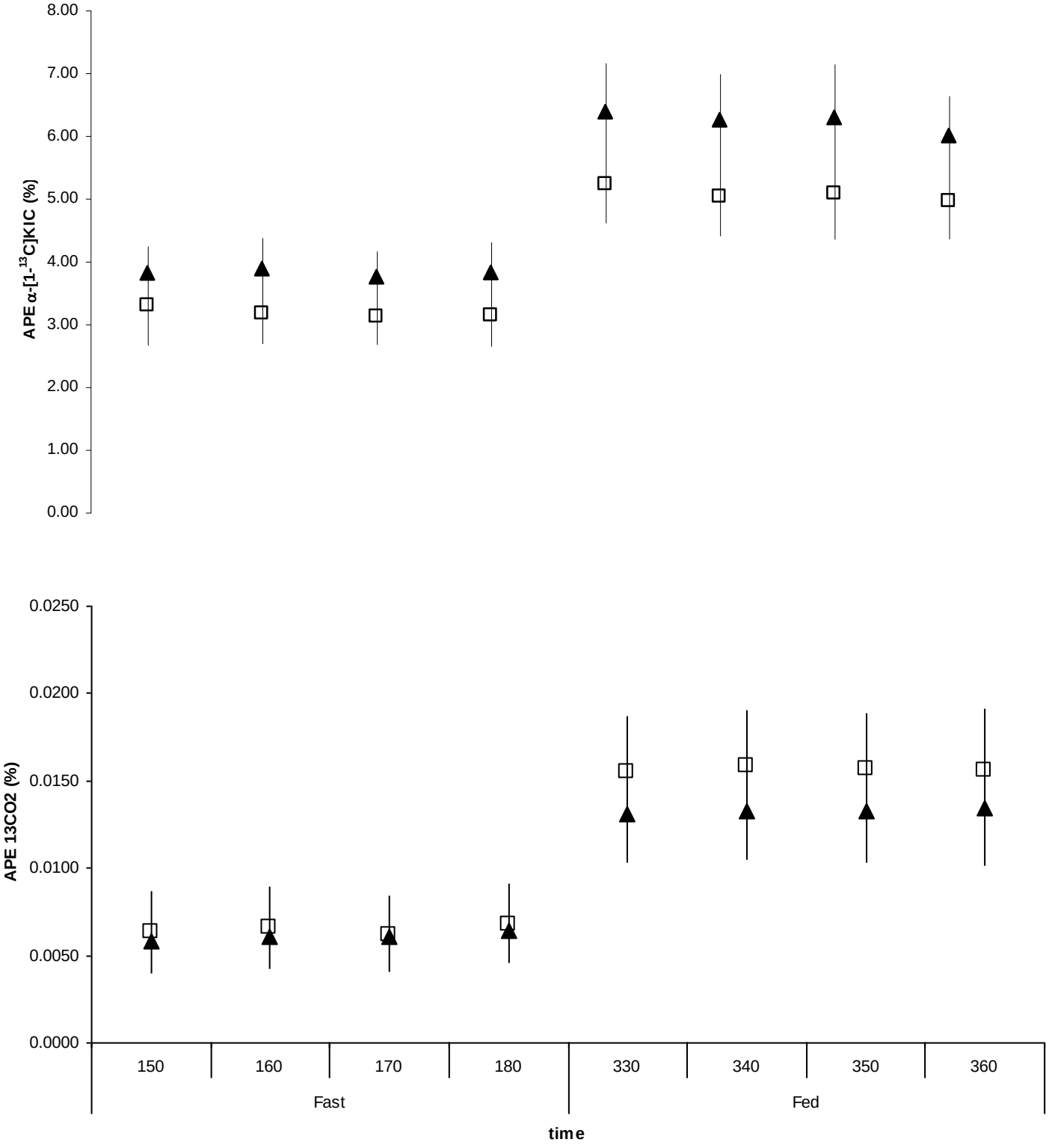
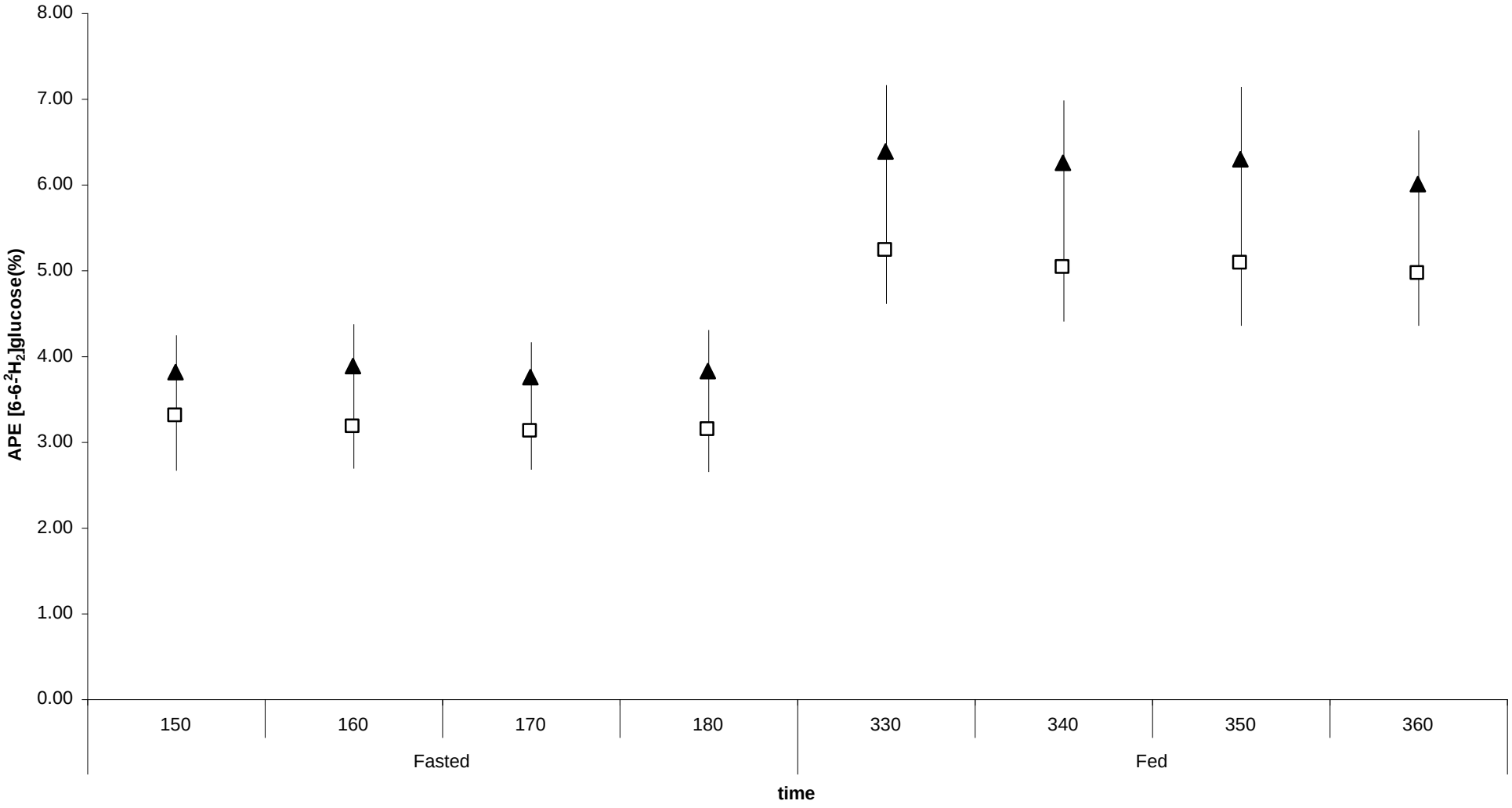


Figure 3. Plateau enrichments expressed in atom percent excess (APE) of [6,6-$^2H_2$]glucose. (□) = epidural anaesthesia; (▲) = General anesthesia



3.7 Summary of study I and introduction to study II

The first part of this project investigated the effect of epidural blockade on the catabolic response during an intraoperative infusion of amino acids. It could be demonstrated that neuraxial blockade with epidural local anesthetics did not affected protein metabolism and the anabolic effect observed in the two groups of patients were the result of the amino acid infusion. However, the catabolic response to stress is still active during the postoperative period and the anabolic properties of amino acids should be tested also in this phase and in addition, it should be studied if they could be more pronounced in patients receiving epidural analgesia. The second part of this study addressed exactly this issue.

Postoperative infusion of amino acids induces a positive protein balance independently of the type of analgesia used.

4.1 Abstract

Background: Net loss of body protein is a prominent feature of the catabolic response to surgical tissue trauma. Epidural analgesia with hypocaloric dextrose has been demonstrated to attenuate leucine oxidation but was unable to make protein balance positive. The present study was set to determine whether an infusion of amino acids on the second day after colon surgery would revert the catabolic state and promote protein synthesis, while maintaining glucose homeostasis in patients receiving epidural analgesia as compared to patient controlled analgesia with morphine (PCA).

Methods: Sixteen patients undergoing colorectal surgery were randomly assigned to epidural blockade (Group EDA) or PCA (Group PCA) as analgesic techniques and underwent a 6-h stable isotope infusion study (3h fasted, 3h fed) on the second postoperative day. Whole body glucose kinetics and protein turnover were measured using[6,6-$^{2}H_2$]glucose and l-[1-^{13}C]leucine as tracer.

Results: The infusion of amino acids caused a decrease in endogenous glucose rate of appearance in both groups ($P < 0.05$), with greater changes in the PCA group ($P < 0.05$). Administration of amino acids suppressed the appearance of leucine from protein breakdown in both groups ($P < 0.05$) although the decrease was greater in the PCA group ($P < 0.05$). Leucine oxidation increased in both groups ($P < 0.05$) with greater change in the epidural group ($P < 0.05$). Protein synthesis increased to the same extent in both groups ($P < 0.05$). Protein balance became positive after the infusion of amino acids, and the effect was greater in the PCA group ($P < 0.05$).

Conclusions: Infusion of amino acids decreased the endogenous glucose production and induced a positive protein balance independent of the type of analgesia provided, although such effects were greater in the PCA group.

4.2 Introduction

Strategies to preserve lean body mass after major surgery have targeted protein breakdown and amino acid oxidation as principal mechanisms inducing a catabolic state [56, 103]. Manipulation of the endocrine response *per se*, either by inhibiting catabolic hormones such as catecholamines, cortisol and glucagon or stimulating insulin and insulin-growth factors, has resulted in significant suppression of the catabolic response [104, 105]. Neuraxial block of afferent and efferent stimuli (epidural analgesia) with local anesthetics, by decreasing the excretion of catabolic hormones and decreasing insulin resistance, has been shown to attenuate postoperative nitrogen excretion, to minimize the rise in whole body protein breakdown and to arrest the fall in muscle protein synthesis in patients receiving parenteral nutrition [12-15].

Subsequent studies aimed at controlling the feeding regimen and assessing the effect of postoperative epidural analgesia on aspects of protein and glucose metabolism identified the necessity of providing sufficient nutritional substrate in order to manipulate effectively the catabolic state [4, 5, 106]. The studies were conducted on the second postoperative day following an overnight fasting and using a fasted and fed 6-h period to mimic the metabolic response associated with feeding. Stable isotopic methodology was chosen to determine the dynamic effect of feeding and to quantify the changes in glucose and protein metabolism.

In patients receiving epidural analgesia, but not PCA, administration of hypocaloric dextrose suppressed the postoperative increase in amino acid oxidation but had no impact on whole body protein breakdown and synthesis [4]. In a subsequent study using the same methodology in which intravenous amino acids were supplied with dextrose,

protein balance became positive and endogenous protein breakdown and glucose production decreased independent of the postoperative analgesia used (epidural vs parenteral opioids) [5]. Protein synthesis increased in both groups but was higher in the epidural group.

This series of studies, conducted under controlled feeding conditions, confirmed the modulatory role of epidural blockade on protein economy and glucose production and utilization.

In spite of these positive results, administration of hypocaloric dextrose was associated with an increase in circulating blood glucose (average 10 mmol/l). Acute hyperglycemia has been shown to be responsible for increased morbidity and mortality in both surgical and medical patients [6, 8-10], and therefore it can be argue whether glucose should be used in the postoperative period.

Amino acids infusion in volunteers causes a fall in whole body protein breakdown, and an increase in protein synthesis resulting in a positive protein balance despite an increase in oxidation [71, 72]. In addition, endogenous glucose production has been reported to either increase or remain unchanged, indicating that amino acids can also be acting as substrate for gluconeogenic pathways [73, 74]. Studies conducted in patients following major surgery and trauma showed that amino acid infusion stimulated protein synthesis, with a small decrease in endogenous glucose production[11, 75]. This could imply that amino acids supplied might have been made available for synthetic rather than oxidative or gluconeogenic pathways.

The present study was designed to determine whether an infusion of amino acids on the second day after colon surgery in patients receiving either epidural or parenteral opioid analgesia would reverse the catabolic state and maintain glucose homeostasis.

4.3 Methods

Patients

Sixteen patients scheduled to undergo elective colon resection for benign and malignant lesions were studied between October 2004 and March 2005. Exclusion criteria were: over 20% loss of body weight in the last 6 months, evidence of metastatic disease, severe cardiac and respiratory diseases, diabetes and albumin below 35 g/L and anemia (hemoglobin less that 100g/L). The Ethics Committee of the MUHC approved the study (REC#03-039), and informed consent was obtained from all patients. The patients were assigned to two groups, A (patient controlled analgesia), and B (epidural analgesia), using computer-generated randomization schedule.

Anesthesia and Surgical Care

No premedication was administered. General anesthesia in both groups consisted of propofol, nitrous oxide in 40% oxygen, desflurane, fentanyl and rocuronium. In group B, before induction of anesthesia, an epidural catheter was inserted at a thoracic vertebral levels between T6 and T10 and bupivacaine 0.5% administered to achieve a bilateral sensory block between T4 and S5. At the end of surgery, analgesia in group A was maintained with patient controlled analgesia (PCA) with intravenous morphine, adjusted to obtain a visual analog scale (VAS) less than 4 at rest (scale 0=no pain to 10=worst pain imaginable). Group B received a continuous epidural infusion with a mixture of 0.1% bupivacaine and 3 μg/ml fentanyl administered at a rate between 8 and 15 ml/h with supplemental top-ups of bupivacaine 0.125% to maintain a sensory block from T7 to L3 and VAS less than 4 at rest.

During surgery patients were kept normothermic using a warming blanket spread over the body, and well hydrated with 0.9% isotonic sodium chloride solution infused at a rate of 6ml/kg/h, followed by Ringer lactate at a rate of 100 ml/h during the first 48 h.

Experimental protocol

All patients were studied on the second postoperative day beginning at 08.00 AM. The protocol included 2 periods: a fasted state of 3 hours followed by a 3-hour fed state during which patients received a solution of 10% amino acids without electrolytes (Travasol™; Baxter, Montreal, Canada) infused for 3 hours at a rate of 0.02ml/kg/min equivalent to 2.9 g/kg/day.

The kinetics of whole-body leucine and glucose were measured using an isotope dilution technique and the stable isotope tracers l-[1-^{13}C]leucine, and [6,6-$^{2}H_2$]glucose (Cambridge Isotope Laboratories, Cambridge, Mass). A superficial vein in the dorsum of the hand was cannulated and the catheter kept patent with heparinized saline to withdraw blood samples. A second catheter was placed in a vein of the contralateral arm to provide access for the infusion of the tracers. After collecting blood and expired-air samples to determine baseline enrichments, 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 and followed by a continuous infusion of L-[1-^{13}C]leucine (0.06 μmol/kg/min) and [6,6-$^{2}H_2$]glucose (0.22 μmol/kg/min) for a total period of 6 h (3h of fasted state, and 3h of fed state). During the latter period the dose of –L-[1-^{13}C]leucine was increased to 0.12 μmol/kg/min. Toward the end of the fasted and fed states, four blood and expired-air samples were collected at 10-min intervals to determine isotope enrichments. Blood samples for the analysis of plasma concentrations of glucose and hormones (cortisol,

glucagon and insulin) were collected once during each state, at 150 and 330 min into the isotopic infusion. Each blood sample was immediately transferred to a heparinized tube and centrifuged at $4^{o}C$ (3000 rpm for 15 min) and then stored at $-70^{o}C$ until analysis. Expired-air samples were collected in a 2-L latex bag and then transferred immediately to 10-ml tubes (BD Vacutainer; Becton Dickinson, Franklin Lakes, NJ) for CO_2 isotope enrichment analysis.

Whole-body oxygen consumption (VO_2) and carbon dioxide production VCO_2 were measured using indirect calorimetry (Vmax 29N; SensorMedics, Yorba Linda, Calif) in the last hour of the fasted ad fed states. Measurements were performed for 20 min on each occasion, and average values of VO_2 and VCO_2 and calculated respiratory quotient (RQ) were calculated, with a coefficient of variation less than 10%. A graphic illustration of the study protocol is presented in Fig 1.

Isotopic enrichments

Plasma α-KIC was analyzed to represent intracellular leucine enrichment and it was determined by electron impact selected-ion monitoring gas chromatography-mass spectrometry, as previously described [4] . Expired $^{13}CO_2$ enrichment was analyzed by means of isotope ratio mass spectrometry (Analytical Precision AP2003, Manchester, United Kingdom). Plasma glucose was derivatized to its pentaacetate compound and the [6,6-$^{2}H_2$] glucose enrichment determined by gas chromatography-mass spectrometry using electron impact ionization (ref). In each analysis run, duplicate injections were performed, and their means of enrichment at 4 time points was taken to represent enrichment at isotopic steady state.

Plasma metabolites and hormones

Plasma concentration of glucose was measured by a glucose oxidase method using a glucose analyzer 2 (Beckman Instruments, Fullerton, CA). Circulating concentrations of insulin and glucagon were measured by sensitive and specific double-antibody radioimmunoassays (Amersham International, Bucks, UK). Cortisol plasma concentration was measured using the Ciba Corning ACS 180 automated immunoassay (Ciba Corning Diagnostic, East Walpole, MA).

Calculations

Whole body leucine kinetic was calculated by conventional isotope dilution practice using a two-pool stochastic model during steady-state conditions, obtained at each study phase (fasted or fed). When an isotopic steady state exists, the rate of appearance (R_a) of a substrate in plasma can be derived from the plasma enrichment [atom percent excess (APE)] calculated by $R_a = (APE_{inf}/APE_{pl} - 1) \cdot F$, where F is the infusion rate of the labeled tracer (μmol/ kg/min), APE_{inf} is the isotopic enrichment in the infusate, and APE_{pl} is the tracer enrichment in plasma at steady state. The APE value used in this calculation represents the mean of the APE values determined during each isotopic plateau. The accuracy of the isotopic enrichments at isotopic plateau was tested by evaluating the scatter of the APE values above their mean, expressed as a coefficient of variation (CV). A CV < 5% was used as a confirmation of a valid plateau. 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 dietary intake or the rate of infusion of

L-[1-^{13}C]leucine (µmol/kg/h) or both. Inspection of that formula indicates that, when studies are conducted in the postabsorptive state, flux is equal to breakdown. Enrichment of plasma α-ketoisocaproate (α-KIC) during infusion of L-[1-^{13}C]leucine has been used to determine whole body leucine kinetics. During amino acid infusion, net leucine flux was calculated by subtracting the leucine infusion rate from the total Ra of leucine. This steady-state reciprocal pool model is considered to represent the intracellular precursor pool enrichment more precisely than leucine itself [87]. In the calculation of oxidation, a factor of 0.76 was applied during the fasted state and account for the fraction of ^{13}C-carbon dioxide released from leucine but retained within slow turnover rate pools of the body. A factor of 0.92 was used for the fed state [87].

In the fasted state, the R_a glucose was equal to the endogenous production of glucose. In the physiological steady state, whole body glucose uptake equals the rate of endogenous glucose production. The glucose clearance, an index of the ability of the tissues to take up glucose was calculated as R_a glucose divided by the corresponding plasma glucose concentration.

Sample size and statistical analysis

Based on expected difference in protein synthesis of 10 µmol/kg/min between the two groups (SD= 5 µmol/kg/min; power 80% and P= 0,05) a total number of 16 patients was calculated to be sufficient [5]. All data are presented as mean ± SD. Analysis of dependent variables were performed using two-factorial analysis of variance with repeated measures. Significant effects induced by parenteral nutrition were assumed when P values for time dependency were less than 0.05. Influences by analgesic regimen

were accepted as significant when the analgesic or the interaction term of the analysis of variance were less than 0.05.

4.4 Results

Demographic characteristics and clinical data were similar in both groups (Table 1). A plateau in the enrichments of plasma [1-^{13}C][alpha]-ketoisocaproate (Fig 2), expired ^{13}C-carbon dioxide (Fig 3) and [6,6-$^{2}H_2$]glucose (Fig 4) was achieved in the fasted and fed states (coefficient of variation <5%), allowing the application of steady state equation to calculate glucose and protein kinetics. *Glucose and leucine kinetics (Table 2)*

In the fasted state, endogenous R_a glucose was higher in the PCA group compared with the epidural group. The infusion of amino acids caused a decrease in endogenous R_a glucose in both groups, with greater changes in the PCA group. Glucose clearance decreased in both groups by over 50% and there was no difference between the two groups studied.

Rate of appearance of leucine (equivalent to protein breakdown in the fasted state) leucine oxidation, protein synthesis and protein balance during the fasted state were similar in both groups. Administration of amino acids suppressed leucine appearance from protein breakdown in both groups although the decrease was greater in the PCA group. Leucine oxidation increased in both groups with greater change in the epidural group. Protein synthesis increased to a similar extent in both groups. Protein balance was negative in the fasted state and became positive during the infusion of amino acids. This net anabolic effect was greater in the PCA group.

Glucose and hormones (Table 3)

Circulating concentration of glucose during the fasted state was similar in both groups and the small increase observed after the amino acid infusion was not significant.

During the fed state plasma concentration of insulin and glucagon increased by the same magnitude, while no changes were observed in plasma cortisol and insulin/glucagon ratio (I/G ratio).

Gaseous exchange (Table 4)

VO_2, VCO_2 and RQ were not affected by the infusion of amino acids in either groups (Table 4). VCO_2 was higher in the epidural group during both the fasted and fed states.

Amino Acid Profile (Table 5)

A concentration of the branched-chain amino acids (BCAAs) acids L-leucine, L-isoleucine and L-valine has been measured before and after the infusion of the mixture of amino acids.

4.5 Discussion

The results of the present study indicate that the infusion of amino acids during the second postoperative day induced a positive protein balance, regardless of the type of analgesia provided, although balance was greater in the PCA group. At the same time, amino acid infusion decreased endogenous glucose production in both groups without affecting plasma glucose concentrations.

The data provided by the isotopic analysis allows us to dissect the different components of whole-body protein metabolism, and understand the principal metabolic mechanism governing protein economy. From the present findings, a 3-h infusion of amino acids, equivalent to 0.36 g/kg/day, during the second postoperative day, suppressed protein breakdown by over 25%. Using leucine as a presentative amino acid, approximately, 30-40% of the amino acids made available from proteolysis were oxidized, while 12 to 16 % were redirected towards protein synthesis.

In a previous investigation using a similar protocol and infusing the same amount of amino acids supplemented by dextrose, at a rate of 4mg/kg/min, it was found that protein balance was positive in both groups, but patients who received epidural analgesia had a slightly greater increase (27 μmol/kg/h) compared to patients treated with PCA (25 μmol/kg/h). [5] It was proposed that the greater insulin sensitivity in patients with epidural, as reflected by a greater increase in glucose clearance in these patients, could be responsible for such difference. Such difference was not evident in the present study, as shown by the same variation in glucose clearance in both groups. In addition, the modifications of the hormonal levels were the same whether patients had epidural or PCA. Therefore, it is not possible to explain the observed anabolic differences between the two analgesic techniques on the basis of the underlying hormonal mechanisms.

It has been shown consistently that epidural analgesia compared to intravenous opioid analgesia attenuates the postoperative nitrogen loss in patients undergoing upper abdominal surgery [12, 14, 15]. However, nitrogen balance cannot differentiate the contribution from changes in protein synthesis and in protein degradation. Therefore, it cannot provide any information about how changes in protein balance are achieved. In other studies conducted using the stable isotopes technique, epidural analgesia was found to attenuate protein breakdown or decrease leucine oxidation [4, 13]. These beneficial effects should be considered in relation to the type of nutritional support. In fact, in a previous study, conducted after an overnight fast, and using the same measurement methodology, epidural analgesia attenuated postoperative protein breakdown without affecting protein synthesis, resulting in patients being in negative protein balance[2]. When dextrose was infused, epidural compared to PCA induced a decrease in leucine oxidation during the second postoperative day, but protein balance remained negative in both groups with no differences between the analgesic techniques, indicating that hypocaloric amounts of dextrose could not reverse negative postoperative protein balance [4].

To quantify the difference in magnitude in anabolism between nutritional support and type of analgesia, the infusion of amino acids in the current study caused an average increase in protein balance of 36,7 μmol/kg/hr while the increase in protein balance was 7,3 μmol/kg/hr as a result of the analgesia technique. Similarly, in the study where glucose and amino acids were infused together, the protein balance increased by 26 μmol/kg/hr while epidural accounted for only an increase of 2.1 μmol/kg/hr [5]. Therefore, in both studies the effects of amino acids on protein balance were 5-10 times more powerful than the type of analgesia used.

In the present study the rate of infusion of amino acids was 2.9g/kg/day, such that the plasma amino acid concentration was maintained two-to threefold above basal value [93].Three hours of

amino acid infusion was found to be sufficient for maximal incorporation of amino acids into whole body and tissue compartments [94]. The quantity of amino acids administered was 0.36g/kg/d that is less than the daily recommended intake of 1.5 g/kg/d, [95] nevertheless a consistent anabolic effect was shown. This is in agreement with previous findings demonstrating that amino acids are more efficiently utilized for maintaining lean body mass when given in divided doses rather than with continuous infusion [94].

Suppression of gluconeogenesis reduces the need for muscle protein breakdown to supply gluconeogenic amino acids. If the rate of gluconeogenesis from amino acids is decreased, that amount of nitrogen is available for reincorporation into protein rather than for excretion as urea. In the present study EGP was slightly decreased (15-30%) while in the previously described study, [5] where the nutritional support was amino acids plus glucose, EGP was almost totally suppressed (80-90%). Paradoxically, amino acid oxidation was similar in both studies, but protein balance was greater in the present study. Therefore, even if the inhibition of EGP should spare amino acids, these do not become automatically available for the synthetic pathways.

Glucose is produced endogenously by both glycogenolysis and gluconeogenesis. Under normal overnight postabsorpitive conditions, glycogenolysis constitutes approximately 50% of whole body glucose production, with the remainder being derived from gluconeogenesis [107]. Gluconeogenesis progressively increases with the duration of fasting, contributing to > 90% of glucose production after 42 h of fasting [108]. Considering the long perioperative fasting the endogenous glucose production was primarily of gluconeogenic origin in our study.

The observed reduction in EGP during an amino acid infusion is in agreement with previous studies in the postoperative period and on the third day after trauma where an infusion of an amino acids mixture at a similar rate decreased endogenous glucose production by 8%-12% [75,

76]. However, in contrast with these observations in patients, infusion of amino acids in volunteers increased endogenous glucose production. Tappy and colleagues showed an increase in EGP and gluconeogenesis by 84% and 235% respectively, following an infusion of amino acids at a rate of 4.8 g/kg/day [73]. In contrast Krebs measured EGP by stable isotopes and glycogenolysis by ^{13}C nuclear magnetic resonance spectrometer [74] in volunteers after an overnight fasting and found that gluconeogenesis increased by 100% after the amino acids infusion at a rate of 4.8g/kg/day. Such increase had been explained as a result of a direct effect of amino acids acting as substrate for the gluconeogenic pathway and indirectly by raised plasma glucagon concentration which stimulates gluconeogenesis.

Despite the decrease in EGP in both groups, a decrease in glucose clearance was observed implying that glucose uptake was decreased. The amino acids effect of decreasing glucose consumption has been found also in studies on volunteers where it has been demonstrated that they inhibit glucose transport/phosphorylation resulting in a decrease in intracellular utilization of glucose [102].

In conclusion, a short-term infusion of amino acids after colo-rectal surgery inhibits protein breakdown and stimulates protein synthesis, thus rendering protein balance positive regardless of analgesic. The effect of amino acids on postoperative glucose metabolism was characterized by a decrease glucose clearance indicating a state of insulin resistance and by a decrease in endogenous glucose production.

4.6 Appendix

Table 1. Patients characteristics

	PCA	Epidural analgesia
Number	8	8
Age (yr)	59 ± 16	65 ± 14
Height (cm)	165 ± 8	166 ± 7
Weight (Kg)	72 ± 14	68 ± 16
Sex (Male/Female)	4/4	5/3
ASA (I/II/III)	3/5/0	2/5/1
Type of surgery		
Hemicolectomy/colectomy	4	5
Sigmoid resection	3	2
Anterior resection	1	1

Values are mean ± SD, PCA = Patient Controlled Analgesia, ASA = American Society of Anesthesiologists classification

Table 2 Protein and glucose kinetics

	PCA		Epidural analgesia		P values		
	Fasted	Fed	Fasted	Fed	Nutrition*	Analgesia†	Interaction‡
Endogenous Ra Glucose (µmol/kg/min)	15.19 ± 2.29	10.71 ± 1.25	12.5 ± 2.1	10.3 ± 1.1	< 0.0001	**0.019**	0.078
Glucose clearance (ml/kg/min)	2.88 ± 0.77	1.14 ± 0.24	2.3 ± 0.3	1.1 ± 0.2	< 0.0001	0.083	0.067
Ra Leucine (µmol/kg/h)	124.9 ± 12.4	143.9 ± 11.9	125.9 ± 10.4	161.3 ± 14.7	< 0.0001	0.038	0.058
Endogenous Ra Leucine (µmol/kg/h)	124.9 ± 12.4	90.4 ± 11.9	125.9 ± 10.4	108.5 ± 15.5	< 0.0001	**0.042**	0.066
Leucin oxidation (µmol/kg/h)	17.9 ± 4.6	30.7 ± 6	17.1 ± 4.3	37.3 ± 3.4	< 0.0001	0.078	**0.028**
Protein Synthesis (µmol/kg/h)	107 ± 11.7	113.2 ± 11.5	108.2 ± 8	124 ± 15.2	0.014	0.165	0.26
Protein Balance (µmol/kg/h)	-17.9 ± 4.6	22.8 ± 5.9	-17.7 ± 3.6	15.5 ± 3.4	< 0.0001	**0.033**	**0.025**

Values are means ± SD, Ra = rate of appearance. PCA = Patient Controlled Analgesia.

* Probability that values are influenced by parenteral alimentation. † Probability that values are influenced by the type of analgesia whether nutrition was administred or not. ‡ Probability that the effect of nutrition is greater in one distinct analgesic group

Table 3. Plasma concentrations of glucose and hormones

	PCA		Epidural analgesia		P values		
	Fasted	Fed	Fasted	Fed	Nutrition*	Analgesia†	Interaction‡
Glucose (mM)	5.85 ± 1.49	6.44 ±1.20	5.79 ± 1.03	6.21 ± 0.44	0.26	0.843	0.732
Insulin (pM)	67.00 ± 50.09	155.00 ± 93.94	64.38 ± 30.40	123.63 ± 56.47	**0.002**	0.345	0.40
Glucagon (pM)	39.7 ± 20	90.3 ± 41	34.8 ± 20	72.8 ± 28	**0.0001**	0.280	0.548
Cortisol (nM)	342.53 ± 250.93	355.52 ± 157.63	299.37 ± 113.85	322.98 ± 151.67	0.843	0.559	1
I/G ratio	1.7 ± 1.05	1.7 ± 1.05	1.7 ± 2.1	1.7 ± 1.4	0.743	0.403	0.559

Values are presented as mean ± SD. PCA = Patient Controlled Analgesia

* Probability that values are influenced by parenteral alimentation. † Probability that values are influenced by the type of analgesia whether nutrition was administred or not. ‡ Probability that the effect of analgesia is greater in one of the two nutritional states

Table 4 : Gaseous Exchange in the Fasted and Fed state

	PCA		Epidural analgesia		P values		
	Fasted	Fed	Fasted	Fed	Nutrition*	Analgesia†	Interaction‡
REE/day (Kcal/day)	1532 ± 461	1797 ± 575	1883 ± 527	2067 ± 885	0.2	0.08	0.8
VO_2 (L/min)	0,222 ± 0,074	0,258 ± 0,087	0,272 ± 0,085	0,292 ± 0,060	0.3	0.1	0.7
VCO_2 (L/min)	0,170 ± 0,042	0,193 ± 0,058	0,223 ± 0,048	0,245 ± 0,045	0.2	**0.005**	1
RQ	0,78 ± 0,08	0,75 ± 0,05	0,83 ± 0,08	0,84 ± 0,1	0.8	**0.03**	0.6

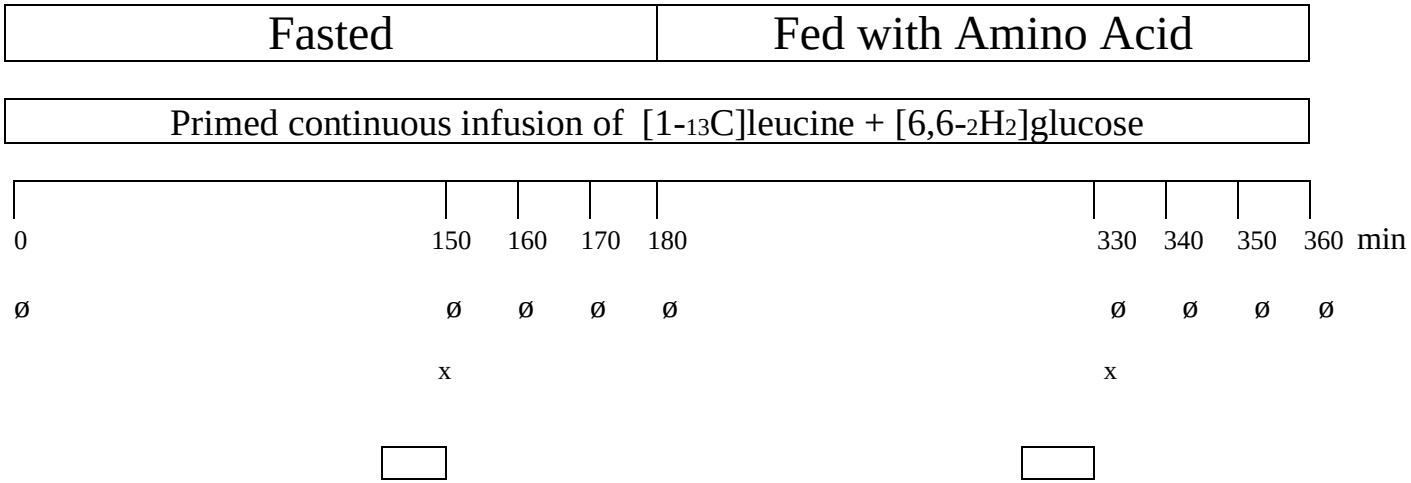
Values are presented as mean ± SD. PCA = Patient Controlled Analgesia

* Probability that values are influenced by parenteral alimentation. † Probability that values are influenced by the type of analgesia whether nutrition was administred or not. ‡ Probability that the effect of analgesia is greater in one of the two nutritional states

Table 5: Branched-chain amino acids (BCAAs) levels before and after the infusion of the mixture of amino acids. Values are mean ± SD.

	Before infusion	After infusion
L-Valine (μmol/L)	154 ± 37	316 ± 64
L-Isoleucine (μmol/L)	38 ± 9	169 ± 27
L-Leucine (μmol/L)	101 ± 25	243 ± 41

Figure 1: Time course of the infusion of isotopes and collection of plasma and expired air samples (Ø) indirect calorimetry (open rectangles), and collection of plasma for the determination of metabolic substrates and hormones (x) in the fasted state and during the infusion of amino acids.



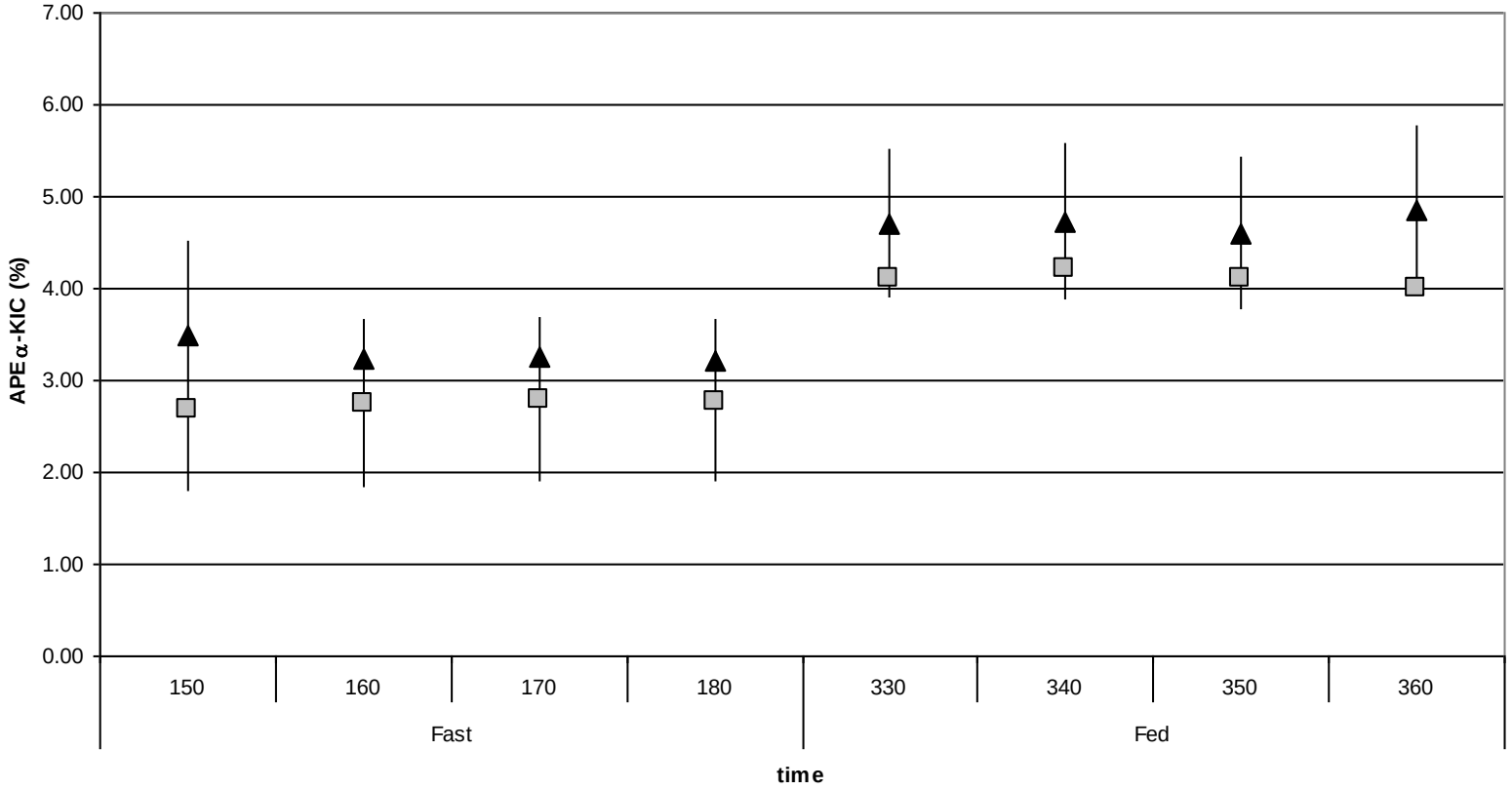


Fig.2. Plateau enrichments, expressed in atom percent excess (APE) of [1-^{13}C]α-ketoisocaproic (KIC). (□) = epidural; (▲) = PCA

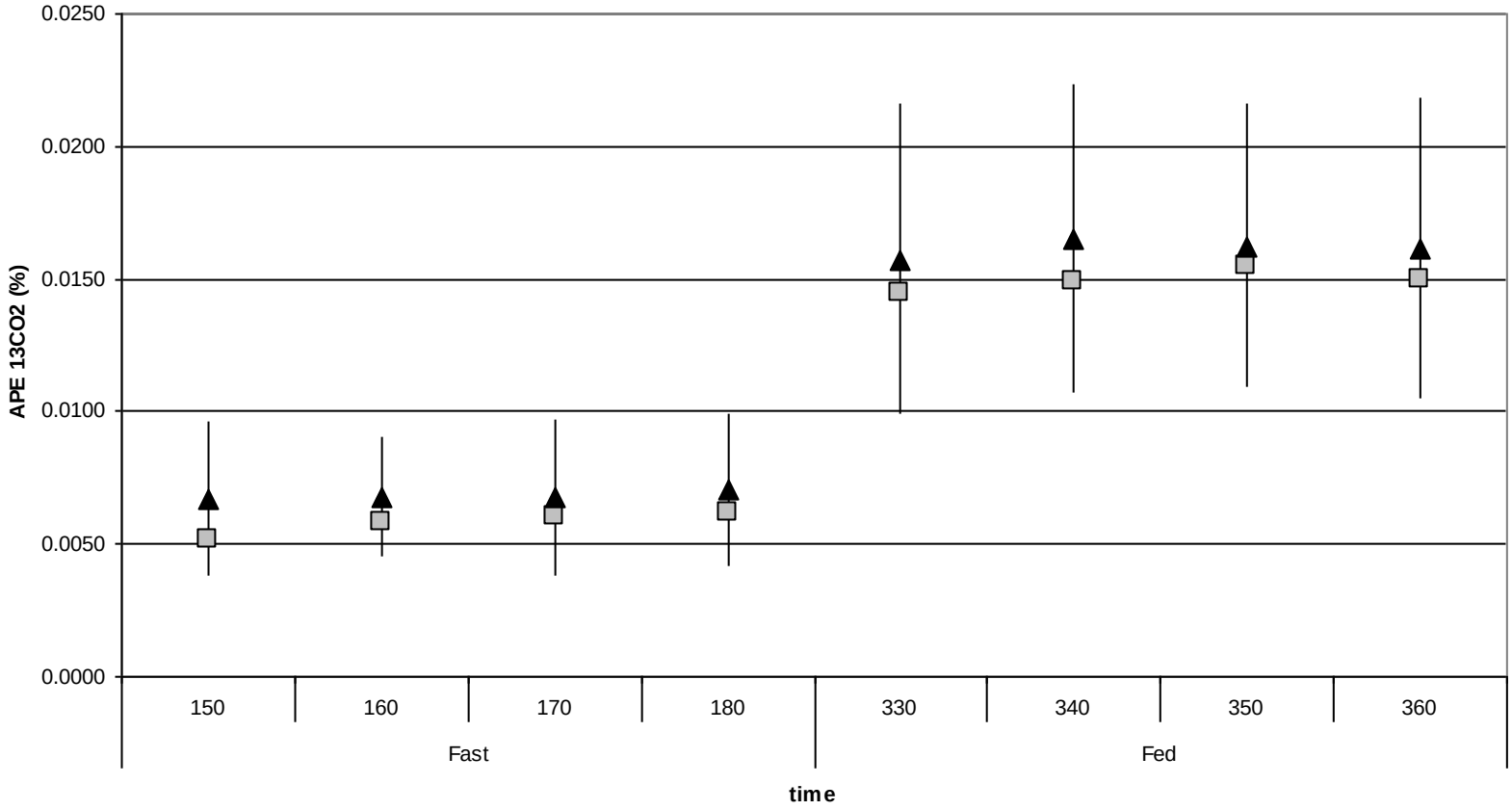


Fig 3. Plateau enrichments, expressed in atom percent excess (APE) of ^{13}C-carbon dioxide. (□) = epidural; (▲) = PCA

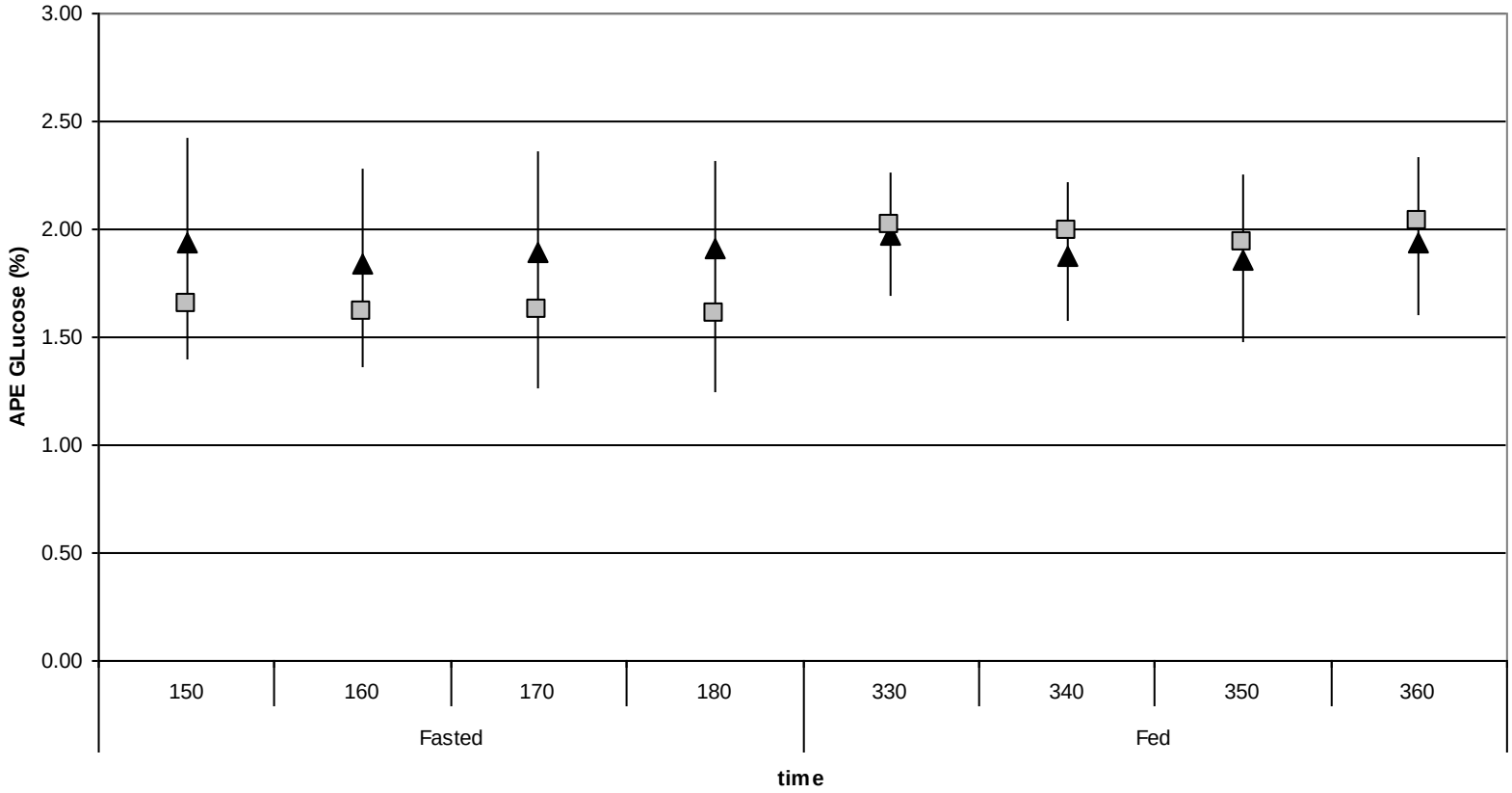


Fig. 4. Plateau enrichments expressed in atom percent excess (APE) of [6,6-$^2H_2$]glucose. (□) = epidural; (▲) = PCA

5. General Discussion

The main finding of this study is that perioperative supplementation of amino acids is the most efficient way to induce a positive protein balance in both the periods evaluated in this study; during and after surgery. The type of anesthesia/analgesia used in the perioperative period had minor repercussions on protein metabolism. The idea that a complete block of nociception with epidural blockade could result in a less catabolic stress response and in a more positive protein balance is not true. In the intraoperative period the addition of an epidural block, at thoracic level, to general anesthesia did not make protein balance more positive compared to general anesthesia alone. On the contrary, although not significant, protein balance is more positive in the general anesthesia alone group. In the postoperative period also, the epidural block did not make protein balance more positive compared to PCA. On the contrary patients treated with PCA had a slightly higher protein balance compared to patients treated with epidural analgesia. This little benefit observed in PCA group although statistically significant in the second manuscript is responsible for a 15% increase in protein balance while the effect of amino acids infusion alone is responsible of an average 40% increase in protein metabolism in both groups. This implies that anesthetic or analgesic techniques have a little power to modulate protein turnover and that nutrition, especially nitrogen supply, remains the most effective way to induce a positive protein balance in the 2 studied periods; during and after surgery.

This failure of epidural in improving protein homeostasis is unexpected. The typical postoperative catabolism is caused by an increase of the stress hormones. Among these cortisol, epinephrine and glucagon are the most known. Pilot studies done in the 80’ have

clearly shown a minor increase in these catabolic hormones when epidural blockade was active compared to patients managed without epidural blockade of nociceptive stimulus. In the present studies we have measured only cortisol and glucagone and despite their levels were lower in patients with epidural blockade this did not translate into a protein homeostasis benefit for them. It is possible to hypothesize that modern surgery is much less invasive now compared to when those pilot studies have been done and hence it is much less stressful for human body. Cortisol and glucagon levels in fact had a modic increase in both groups compared to base line values and consequently the stress response could have been not enough intense to put in evidence a benefit from analgesia technique.

The effects of amino acids on glucose metabolism were contrasting. The intraoperative infusion of amino acids did not affect EGP while the postoperative infusion decreased EGP. This implies that the presence or the absence of anesthesia and surgery modifies the effects of amino acid infusion on glucose metabolism and further studies should investigate the reasons for such a difference. Glucose clearance decreased in both groups independently of the timing of administration of amino acids indicating that the provision of amino acids can induce a state of insulin resistance. Further study should evaluate if the development of a state of perioperative insulin resistance affects clinical outcome.

6. Final conclusion and summary

The main goal of this study was to test the hypothesis that perioperative supplementation of amino acids induced a greater positive protein balance in patients receiving epidural blockade compared to patients receiving general anesthesia alone during surgery and patient controlled analgesia (PCA) as postoperative pain management technique. It has been found that infusion of amino acids inhibited protein breakdown and stimulates protein synthesis, thus rendering protein balance positive in both groups. This implies that it is not possible to modify protein turnover with anesthetic or analgesic techniques and that nitrogen supply remains the most effective way to induce a positive protein balance in the perioperative period. Further research is warranted to find the right doses and timing of perioperative amino acids infusion. Nutrition supplementation could start much before the surgery according to new pre-habilitation theories.

7. References

1. Wolfe, R.R., *Carbohydrate metabolism and requirements*, in *Clinical nutrition: parenteral nutrition*, J. Rombeau and M. Caldwell, Editors. 1993, WB Saunders Company: Philadelphia. p. 113-131.
2. Lattermann, R., et al., *Epidural blockade modifies perioperative glucose production without affecting protein catabolism.* Anesthesiology, 2002. **97**(2): p. 374-81.
3. Lattermann, R., et al., *Perioperative glucose infusion and the catabolic response to surgery: the effect of epidural block.* Anesthesia & Analgesia, 2003. **96**(2): p. 555-62.
4. Schricker, T., L. Wykes, and F. Carli, *Epidural blockade improves substrate utilization after surgery.* American Journal of Physiology - Endocrinology & Metabolism, 2000. **279**(3): p. E646-53.
5. Schricker, T., et al., *The anabolic effect of epidural blockade requires energy and substrate supply.* Anesthesiology, 2002. **97**(4): p. 943-51.
6. Askanazi, J., et al., *Nutrition for the patient with respiratory failure: glucose vs. fat.* Anesthesiology, 1981. **54**(5): p. 373-7.
7. Kwoun, M.O., et al., *Immunologic effects of acute hyperglycemia in nondiabetic rats.* Jpen: Journal of Parenteral & Enteral Nutrition, 1997. **21**(2): p. 91-5.
8. Nordenstrom, J., et al., *Increasing glucose intake during total parenteral nutrition increases norepinephrine excretion in trauma and sepsis.* Clinical Physiology, 1981. **1**(5): p. 525-34.
9. Rassias, A.J., et al., *Insulin infusion improves neutrophil function in diabetic cardiac surgery patients.* Anesthesia & Analgesia, 1999. **88**(5): p. 1011-6.
10. van den Berghe, G., et al., *Intensive insulin therapy in the critically ill patients.[see comment].* New England Journal of Medicine, 2001. **345**(19): p. 1359-67.
11. O'Keefe, S.J., et al., *The influence of intravenous nutrition on protein dynamics following surgery.* Metabolism: Clinical & Experimental, 1981. **30**(12): p. 1150-8.

12. Brandt, M.R., et al., *Epidural analgesia improves postoperative nitrogen balance.* British Medical Journal, 1978. **1**(6120): p. 1106-8.
13. Carli, F., et al., *Protein metabolism after abdominal surgery: effect of 24-h extradural block with local anaesthetic.* British Journal of Anaesthesia, 1991. **67**(6): p. 729-34.
14. Tsuji, H., et al., *Effects of epidural administration of local anaesthetics or morphine on postoperative nitrogen loss and catabolic hormones.* British Journal of Surgery, 1987. **74**(5): p. 421-5.
15. Vedrinne, C., et al., *Nitrogen-sparing effect of epidural administration of local anesthetics in colon surgery.* Anesthesia & Analgesia, 1989. **69**(3): p. 354-9.
16. Schricker, T., et al., *Postoperative protein sparing with epidural analgesia and hypocaloric dextrose.* Annals of Surgery, 2004. **240**(5): p. 916-21.
17. Simmons, P.S., et al., *Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range.* Journal of Clinical Investigation, 1984. **73**(2): p. 412-20.
18. Wilmore, D.W., *The effect of glutamine supplementation in patients following elective surgery and accidental injury.* Journal of Nutrition, 2001. **131**(9 Suppl): p. 2543S-9S; discussion 2550S-1S.
19. Aulick, L.H. and D.W. Wilmore, *Increased peripheral amino acid release following burn injury.* Surgery, 1979. **85**(5): p. 560-5.
20. Wilmore, D.W., et al., *Effect of injury and infection on visceral metabolism and circulation.* Annals of Surgery, 1980. **192**(4): p. 491-504.
21. Bessey, P.Q. and K.A. Lowe, *Early hormonal changes affect the catabolic response to trauma.* Annals of Surgery, 1993. **218**(4): p. 476-89; discussion 489-91.
22. Molina, P.E. and N.N. Abumrad, *Gut-derived proteolysis during insulin-induced hypoglycemia: the pain that breaks down the gut.* Jpen: Journal of Parenteral & Enteral Nutrition, 1994. **18**(6): p. 549-56.
23. Ross, R.J. and S.L. Chew, *Acquired growth hormone resistance.* European Journal of Endocrinology, 1995. **132**(6): p. 655-60.

24. Bessey, P.Q., et al., *Combined hormonal infusion simulates the metabolic response to injury.* Annals of Surgery, 1984. **200**(3): p. 264-81.
25. Max, S.R., et al., *Dexamethasone regulates glutamine synthetase expression in rat skeletal muscles.* American Journal of Physiology, 1988. **255**(3 Pt 1): p. E397-402.
26. Krishna, M.G., et al., *Glucagon response to exercise is critical for accelerated hepatic glutamine metabolism and nitrogen disposal.* American Journal of Physiology - Endocrinology & Metabolism, 2000. **279**(3): p. E638-45.
27. Watters, J.M., et al., *Both inflammatory and endocrine mediators stimulate host responses to sepsis.* Archives of Surgery, 1986. **121**(2): p. 179-90.
28. Wilmore, D.W., *Postoperative protein sparing.* World Journal of Surgery, 1999. **23**(6): p. 545-52.
29. Kinney, J.M. and D.H. Elwyn, *Protein metabolism and injury.* Annual Review of Nutrition, 1983. **3**: p. 433-66.
30. Smeets, H.J., et al., *Differential effects of counterregulatory stress hormones on serum albumin concentrations and protein catabolism in healthy volunteers.* Nutrition, 1995. **11**(5): p. 423-7.
31. Romijn, J.A., *Substrate metabolism in the metabolic response to injury.* Proceedings of the Nutrition Society, 2000. **59**(3): p. 447-9.
32. McClave, S.A., H.L. Snider, and D.A. Spain, *Preoperative issues in clinical nutrition.* Chest, 1999. **115**(5 Suppl): p. 64S-70S.
33. Bradshaw, B.G., S.S. Liu, and R.C. Thirlby, *Standardized perioperative care protocols and reduced length of stay after colon surgery.[see comment]*. Journal of the American College of Surgeons, 1998. **186**(5): p. 501-6.
34. Kehlet, H. and T. Mogensen, *Hospital stay of 2 days after open sigmoidectomy with a multimodal rehabilitation programme.[see comment]*. British Journal of Surgery, 1999. **86**(2): p. 227-30.
35. Buggy, D.J. and G. Smith, *Epidural anaesthesia and analgesia: better outcome after major surgery? Growing evidence suggests so.[see comment]*. BMJ, 1999. **319**(7209): p. 530-1.

36. Stanton-Hicks, M., *Subarachnoid and extradural analgesic techniques.* Acute Pain, ed. Butterworths. 1985, London: Smith, G. Covino, BG. 228-256.
37. Miller, R., *Anesthesia*. 2nd edition ed, ed. C. Livingstone. 1986, New York. 1061-1111.
38. Shafer, A.L. and A.J. Donnelly, *Management of postoperative pain by continuous epidural infusion of analgesics.[erratum appears in Clin Pharm 1991 Nov;10(11):824]*. Clinical Pharmacy, 1991. **10**(10): p. 745-64.
39. Kehlet, H., *Surgical stress: the role of pain and analgesia.* British Journal of Anaesthesia, 1989. **63**(2): p. 189-95.
40. Hosoda, R., M. Hattori, and Y. Shimada, *Favorable effects of epidural analgesia on hemodynamics, oxygenation and metabolic variables in the immediate post-anesthetic period.* Acta Anaesthesiologica Scandinavica, 1993. **37**(5): p. 469-74.
41. Kouraklis, G., et al., *Epidural analgesia attenuates the systemic stress response to upper abdominal surgery: a randomized trial.* International Surgery, 2000. **85**(4): p. 353-7.
42. Christensen, P., et al., *Influence of extradural morphine on the adrenocortical and hyperglycaemic response to surgery.* British Journal of Anaesthesia, 1982. **54**(1): p. 23-7.
43. Segawa, H., et al., *The role of the phrenic nerves in stress response in upper abdominal surgery.* Anesthesia & Analgesia, 1996. **82**(6): p. 1215-24.
44. Ferrando, A.A., et al., *Prolonged bed rest decreases skeletal muscle and whole body protein synthesis.* American Journal of Physiology, 1996. **270**(4 Pt 1): p. E627-33.
45. Park, W.Y., J.S. Thompson, and K.K. Lee, *Effect of epidural anesthesia and analgesia on perioperative outcome: a randomized, controlled Veterans Affairs cooperative study.* Annals of Surgery, 2001. **234**(4): p. 560-9; discussion 569-71.
46. Norris, E.J., et al., *Double-masked randomized trial comparing alternate combinations of intraoperative anesthesia and postoperative analgesia in abdominal aortic surgery.[see comment].* Anesthesiology, 2001. **95**(5): p. 1054-67.

47. Rigg, J.R., et al., *Epidural anaesthesia and analgesia and outcome of major surgery: a randomised trial.[see comment]*. Lancet, 2002. **359**(9314): p. 1276-82.

48. Peyton, P.J., et al., *Perioperative epidural analgesia and outcome after major abdominal surgery in high-risk patients.[see comment]*. Anesthesia & Analgesia, 2003. **96**(2): p. 548-.

49. Ballantyne, J.C., et al., *The comparative effects of postoperative analgesic therapies on pulmonary outcome: cumulative meta-analyses of randomized, controlled trials.[see comment]*. Anesthesia & Analgesia, 1998. **86**(3): p. 598-612.

50. Kehlet, H. and K. Holte, *Effect of postoperative analgesia on surgical outcome.* British Journal of Anaesthesia, 2001. **87**(1): p. 62-72.

51. Kehlet, H., *Modification of responses to surgery by neural blockade: clinical implications*, in *Neural blockade in clinical anesthesia and management of pain*, M. Cousins and P. Bridenbaugh, Editors. 1998, Lippincott-Raven: Philadelphia. p. 129-175.

52. Holte, K. and H. Kehlet, *Epidural anaesthesia and analgesia - effects on surgical stress responses and implications for postoperative nutrition*. Clinical Nutrition, 2002. **21**(3): p. 199-206.

53. Holte, K. and H. Kehlet, *Postoperative epidural analgesia and outcome - a research agenda*, in *Evidence-based resource in anaesthesia and analgesia*, M. Tramer, Editor. 2003, BMJ books. p. 175-183.

54. de Leon-Casasola, O.A., *When it comes to outcome, we need to define what a perioperative epidural technique is.[see comment][comment]*. Anesthesia & Analgesia, 2003. **96**(2): p. 315-8.

55. Kehlet, H., *Multimodal approach to control postoperative pathophysiology and rehabilitation.* British Journal of Anaesthesia, 1997. **78**(5): p. 606-17.

56. Kehlet, H. and J.B. Dahl, *Anaesthesia, surgery, and challenges in postoperative recovery.* Lancet, 2003. **362**(9399): p. 1921-8.

57. Kehlet, H. and D.W. Wilmore, *Multimodal strategies to improve surgical outcome.* American Journal of Surgery, 2002. **183**(6): p. 630-41.

58. Holte, K., N.E. Sharrock, and H. Kehlet, *Pathophysiology and clinical implications of perioperative fluid excess.[see comment]*. British Journal of Anaesthesia, 2002. **89**(4): p. 622-32.

59. Brandstrup, B., et al., *Effects of intravenous fluid restriction on postoperative complications: comparison of two perioperative fluid regimens: a randomized assessor-blinded multicenter trial.[see comment]*. Annals of Surgery, 2003. **238**(5): p. 641-8.

60. Carli, F. and D. Halliday, *Continuous epidural blockade arrests the postoperative decrease in muscle protein fractional synthetic rate in surgical patients.* Anesthesiology, 1997. **86**(5): p. 1033-40.

61. Kehlet, H., et al., *Effect of epidural analgesia on metabolic profiles during and after surgery.* British Journal of Surgery, 1979. **66**(8): p. 543-6.

62. Houghton, A., et al., *Glucose tolerance during anaesthesia and surgery. Comparison of general and extradural anaesthesia.* British Journal of Anaesthesia, 1978. **50**(5): p. 495-9.

63. Engquist, A., et al., *The blocking effect of epidural analgesia on the adrenocortical and hyperglycemic responses to surgery.* Acta Anaesthesiologica Scandinavica, 1977. **21**(4): p. 330-5.

64. Jensen, C.H., et al., *Effect of epidural analgesia on glucose tolerance during surgery.* Acta Anaesthesiologica Scandinavica, 1980. **24**(6): p. 472-4.

65. Lund, J., et al., *Effect of extradural analgesia on glucose metabolism and gluconeogenesis. Studies in association with upper abdominal surgery.* British Journal of Anaesthesia, 1986. **58**(8): p. 851-7.

66. Shaw, J.H., et al., *The effect of extradural blockage upon glucose and urea kinetics in surgical patients.* Surgery, Gynecology & Obstetrics, 1987. **165**(3): p. 260-6.

67. Carli, F., et al., *Effect of epidural blockade on glucose metabolism after colorectal surgery: a stable isotope kinetic study in the fasted and fed state.* Anesthesiology, 1999. **91**: p. A916.

68. Kinney, J.M., *Metabolic responses to injuries*, in *Nutritional support of the seriously ill patients*, R. Winters and H. Green, Editors. 1983, Academic Presss: New York. p. 5-12.

69. Shaw, J.H., S. Klein, and R.R. Wolfe, *Assessment of alanine, urea, and glucose interrelationships in normal subjects and in patients with sepsis with stable isotopic tracers.* Surgery, 1985. **97**(5): p. 557-68.

70. Shaw, J.H. and R.R. Wolfe, *An integrated analysis of glucose, fat, and protein metabolism in severely traumatized patients. Studies in the basal state and the response to total parenteral nutrition.* Annals of Surgery, 1989. **209**(1): p. 63-72.

71. Volpi, E., et al., *Exogenous amino acids stimulate net muscle protein synthesis in the elderly.* Journal of Clinical Investigation, 1998. **101**(9): p. 2000-7.

72. Volpi, E., et al., *The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly.* Journal of Clinical Endocrinology & Metabolism, 2000. **85**(12): p. 4481-90.

73. Tappy, L., et al., *Effects of infused amino acids on glucose production and utilization in healthy human subjects.* American Journal of Physiology, 1992. **262**(6 Pt 1): p. E826-33.

74. Krebs, M., et al., *Direct and indirect effects of amino acids on hepatic glucose metabolism in humans.[see comment]*. Diabetologia, 2003. **46**(7): p. 917-25.

75. Humberstone, D.A., J. Koea, and J.H. Shaw, *Relative importance of amino acid infusion as a means of sparing protein in surgical patients.* Jpen: Journal of Parenteral & Enteral Nutrition, 1989. **13**(3): p. 223-7.

76. Long, C.L., et al., *Effect of amino acid infusion on glucose production in trauma patients.* Journal of Trauma-Injury Infection & Critical Care, 1996. **40**(3): p. 335-41.

77. Flakoll, P.J., et al., *Short-term regulation of insulin-mediated glucose utilization in four-day fasted human volunteers: role of amino acid availability.* Diabetologia, 1992. **35**(4): p. 357-66.

78. Pisters, P.W., et al., *The effects of euglycemic hyperinsulinemia and amino acid infusion on regional and whole body glucose disposal in man.* Metabolism: Clinical & Experimental, 1991. **40**(1): p. 59-65.

79. Boden, G. and L. Tappy, *Effects of amino acids on glucose disposal.* Diabetes, 1990. **39**(9): p. 1079-84.

80. Wolfe, R.R., et al., *Investigation of factors determining the optimal glucose infusion rate in total parenteral nutrition.* Metabolism: Clinical & Experimental, 1980. **29**(9): p. 892-900.

81. Sakurai, Y., et al., *Stimulation of muscle protein synthesis by long-term insulin infusion in severely burned patients.* Annals of Surgery, 1995. **222**(3): p. 283-94; 294-7.

82. Woolfson, A.M., R.V. Heatley, and S.P. Allison, *Insulin to inhibit protein catabolism after injury.* New England Journal of Medicine, 1979. **300**(1): p. 14-7.

83. Brandi, L.S., et al., *Insulin resistance after surgery: normalization by insulin treatment.* Clinical Science, 1990. **79**(5): p. 443-50.

84. Hall, R.I., et al., *Pathogenesis of hepatic steatosis in the parenterally fed rat.* Journal of Clinical Investigation, 1984. **74**(5): p. 1658-68.

85. Schoenheimer, R., S. Rutner, and D. Rittenberg, *Studies in protein metabolism; metabolic activity of body proteins investigated with 1-leucine containing 2 isotopes.* J. Biol. Chem., 1939. **130**: p. 730-732.

86. Picou, D. and T. Taylor-Roberts, *The measurement of total protein synthesis and catabolism and nitrogen turnover in infants in different nutritional states and receiving different amounts of dietary protein.* Clin. Sci., 1969. **36**: p. 283-296.

87. Matthews, D., et al., *Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-C13]-leucine.* Am. J. Physiol., 1980. **238**: p. E473-479.

88. Wolfe, R.R., et al., *Isotopic analysis of leucine and urea metabolism in exercise humans.* J. Applied Physiology, 1982. **52**: p. 458-466.

89. Cahill, G.F., *Starvation in man.* N. Engl. J. Med., 1970. **282**: p. 668-675.

90. Schricker, T., et al., *Integrated analysis of protein and glucose metabolism during surgery: effects of anesthesia.* Journal of Applied Physiology, 2001. **91**(6): p. 2523-30.

91. Carli, F., et al., *Effect of general anaesthesia on whole body protein turnover in patients undergoing elective surgery.* Br J Anaesth, 1990. **65**(3): p. 373-9.

92. Wolfe, R.R., *Regulation of muscle protein by amino acids.* J Nutr, 2002. **132**(10): p. 3219S-24S.

93. Castellino, P., et al., *Effects of insulin and amino acids on glucose and leucine metabolism in CAPD patients.* Journal of the American Society of Nephrology, 1999. **10**(5): p. 1050-8.

94. Bohe, J., et al., *Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids.* Journal of Physiology, 2001. **532**(Pt 2): p. 575-9.

95. Hoffer, L.J., *Protein and energy provision in critical illness.* American Journal of Clinical Nutrition, 2003. **78**(5): p. 906-11.

96. Schricker, T., et al., *Effect of desflurane/remifentanil anaesthesia on glucose metabolism during surgery: a comparison with desflurane/epidural anaesthesia.* Acta Anaesthesiologica Scandinavica, 2004. **48**(2): p. 169-73.

97. Schricker, T., K. Klubien, and F. Carli, *The independent effect of propofol anesthesia on whole body protein metabolism in humans.* Anesthesiology, 1999. **90**(6): p. 1636-42.

98. Savaki, H.E., et al., *Local cerebral glucose consumption in the rat. I. Effects of halothane anesthesia.* Journal of Comparative Neurology, 1983. **213**(1): p. 36-45.

99. Shulman, R.G., D.L. Rothman, and F. Hyder, *Stimulated changes in localized cerebral energy consumption under anesthesia.* Proceedings of the National Academy of Sciences of the United States of America, 1999. **96**(6): p. 3245-50.

100. Baron, A.D., et al., *Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans.* American Journal of Physiology, 1988. **255**(6 Pt 1): p. E769-74.

101. Sbai, D., et al., *Effect of halothane anesthesia on glucose utilization and production in adolescents.* Anesthesiology, 1995. **82**(5): p. 1154-9.

102. Krebs, M., et al., *Mechanism of amino acid-induced skeletal muscle insulin resistance in humans.* Diabetes, 2002. **51**(3): p. 599-605.

103. Wilmore, D.W., *From Cuthbertson to fast-track surgery: 70 years of progress in reducing stress in surgical patients.[see comment]*. Annals of Surgery, 2002. **236**(5): p. 643-8.

104. Lattermann, R., et al., *Intraoperative epidural blockade prevents the increase in protein breakdown after abdominal surgery.* Acta Anaesthesiologica Scandinavica, 2001. **45**(9): p. 1140-6.

105. Kehlet, H., *Manipulation of the metabolic response in clinical practice.* World Journal of Surgery, 2000. **24**(6): p. 690-5.

106. Schricker, T., et al., *Effect of epidural blockade on protein, glucose, and lipid metabolism in the fasted state and during dextrose infusion in volunteers.* Anesthesiology, 2000. **92**(1): p. 62-9.

107. Landau, B.R., et al., *Contributions of gluconeogenesis to glucose production in the fasted state.* Journal of Clinical Investigation, 1996. **98**(2): p. 378-85.

108. Chandramouli, V., et al., *Quantifying gluconeogenesis during fasting.* American Journal of Physiology, 1997. **273**(6 Pt 1): p. E1209-15.