Pressurized whey protein-based oral nutrition support promotes protein anabolism in surgical patients

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ABSTRACT

The objective of the present thesis was to determine whether an oral nutrition support regimen based on pressurized whey protein isolate and glucose improves the postoperative utilization of amino acids compared to glucose alone. Patients undergoing colorectal surgery were randomly assigned to receive an oral nutrition support regimen based on pressurized whey protein isolate and glucose or glucose alone. Leucine kinetics, serum hormone and substrate concentrations, resting energy expenditure and substrate utilization were measured before surgery and two days after surgery. The baseline characteristics of the two groups were similar before surgery. Postoperative leucine balance increased in the fed state in both groups but the change in leucine balance was significantly greater in the whey protein-based group. Only the whey protein-based group achieved positive leucine (protein) balance, which was attributed to greater suppression of protein breakdown. Protein synthesis was not affected by feeding or by nutrition regimen. Serum glucose and insulin increased in the fed state but patients in both groups remained normoglycemic. Fasting cortisol, total protein and albumin decreased after surgery in both groups. Postoperative VO_2, VCO_2 and RQ increased in both groups in the fed state; diet group did not affect the calorimetry parameters. Oral nutrition support based on pressurized whey protein isolate may help to avoid complications associated with postoperative body protein losses and hyperglycemia. Future research should focus on a direct comparison between oral and parenteral nutrition support and the functional clinical impact of minimizing perioperative fasting by implementing this oral nutrition regimen in the immediate perioperative period.

SOMMAIRE

L'objectif de la présente étude était à déterminer si un régime nutritif oral à base de protéines lactosérum traitée sous pression et glucose améliore l'utilisation post-opératif des acides aminées a comparé au glucose seul. Des patients subissant une intervention chirurgicale colorectale étaient assignés de façon aléatoire à recevoir un régime orale nutritif à base du glucose seul ou à base de protéines lactosérum traitées sous pression et glucose. Les paramètres suivants étaient quantifiées avant l'intervention et deux jours après l'intervention : cinétiques de leucine, la concentration des hormones et soustrait dans le sérum, la dépense d'énergie à la détente, et l'utilisation de la soustrait. Les deux groupes étaient homogènes avant l'intervention chirurgicale. La balance de leucine post-opérative a augmenté dans l'état nourrie pour les deux groupes, mais l'augmentation était plus grande dans le groupe nourri des protéines lactosérum. Seul le groupe nourri des protéines lactosérum a réussi une balance positive de leucine (des protéines), ce qui est attribuable à l'augmentation de la suppression de la destruction des protéines. La synthèse des protéines n'a pas été affectée par l'alimentation ou par le régime nutritif. Les niveaux de glucose et d'insuline dans le sérum ont augmente dans les deux groupes même si les deux groupes se trouvaient normoglycémiques. Les niveaux à jeun de cortisol, de protéines totales, et de l'albumen ont descendu après l'intervention chirurgicale dans les deux groupes. Les paramètres VO_2, VCO_2 et QR post-opératives ont augmenté dans les deux groupes à l'état nourri ; le régime nutritif n'a pas affecté les paramètres calorimétriques. Un régime nutritif oral à base de protéines lactosérum traitées sous pression peut aider à éviter les complications associées à la perte des protéines corporelles ainsi que l'hyperglycémie suivant une intervention chirurgicale. Les recherches futures devraient cibler une comparaison directe entre la nutrition orale et parentérale, afin de minimiser la période de jeun en implémentant ce régime nutritif oral dans la période périopératoire.

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LIST OF ABBREVIATIONS

α-KIC	α-ketoisocaproate
ACTH	Adrenocorticotropic hormone
ASPEN	American Society for Parenteral and Enteral Nutrition
BV	Biological value
C	Carbon
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis transmembrane conductance regulator
Cl^-	Chloride ion
CO_2	Carbon dioxide
CRP	C-reactive protein
EAR	Estimated average requirement
ERAS	Enhanced recovery after surgery
ESPEN	European Society for Parenteral and Enteral Nutrition
FSH	Follicle stimulating hormone
GC/MS	Gas Chromatography/ Mass Spectrometry
GH	Growth hormone
GLUT-4	Glucose transporter-4
GSH	Glutathione
HIV	Human immunodeficiency virus
HPA	Hypothalamic-pituitary-adrenal
I-κβα	Inhibitor-κβα
IL-1	Interleukin-1
IL-1β	Interleukin-1β
IL-2	Interleukin-2
IL-6	Interleukin-6
LH	Luteinizing hormone

MPE	Molecules percent excess
MUHC	McGill University health center
NF-κβ	Nuclear factor-κβ
NPU	Net protein utilization
NSAID	Non-steroidal anti-inflammatory
PCA	Patient-controlled analgesia
PCAAS	Protein digestibility corrected amino acid score
PER	Protein efficiency ratio
PFB-Br	Pentafluorobenzyl bromide
PPN	Peripheral parenteral nutrition
R_a	Rate of appearance
R_b	Isotope ratio at baseline
R_d	Rate of disappearance
REE	Resting energy expenditure
ROS	Reactive oxygen species
RQ	Respiratory quotient
R_s	Isotope ratio at steady state
TBA	Tetrabutyl ammonium hydrogen sulfate
TNF-α	Tumor necrosis factor-α
VCO_2	Carbon dioxide production
VO_2	Oxygen consumption

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I. LITERATURE REVIEW

1.1 The Stress Response to Surgery

Surgical injury triggers whole-body neurologic, endocrine, immune and metabolic responses (1, 2). Surgery activates and stimulates the sympathetic nervous system and provokes an endocrine response, changing the hormonal milieu and impacting metabolism. The stress response ultimately creates an environment that supports organ function, fights infection and promotes wound healing, improving survival after injury (3).

1.1.1. The neuroendocrine response to surgery

Surgical tissue injury causes pain signals to travel via afferent nerve fibers to the brain, stimulating the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (2, 3). Activation of the HPA axis causes the release of hypothalamic releasing factors such as corticotropin-releasing factor and vasoactive intestinal peptide. These hypothalamic releasing factors then stimulate the pituitary gland (1, 2).

The anterior pituitary secretes adrenocorticotropic hormone (ACTH), growth hormone (GH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) (2). ACTH provokes glucocorticoid secretion from the adrenal cortex (2). Under normal conditions, ACTH and cortisol operate via a feedback mechanism such that high concentrations of cortisol in the circulation suppress the release of ACTH. However, this feedback mechanism does not function as effectively following surgery, so circulating levels of ACTH and cortisol remain elevated (2). Cortisol has important effects on carbohydrate, protein and fat metabolism, which will be discussed later (2).

The anterior pituitary also secretes GH in response to hypothalamic growth hormone-releasing factor (1) and the magnitude of the response is proportional to the severity of tissue damage (2). GH causes changes in metabolism. More specifically, GH promotes protein synthesis while suppressing protein breakdown and triglyceride catabolism (2). Blood glucose concentration also increases in response to GH because GH inhibits cellular glucose uptake and promotes hepatic glycogenolysis (2).

The posterior pituitary is also stimulated by hypothalamic releasing factors to secrete vasopressin, which has anti-diuretic effects on the kidneys and is involved in maintaining blood pressure (2).

The catecholamines epinephrine and norepinephrine also play an important role in the response to surgery. The hypothalamus activates the sympathetic nervous system, which stimulates the release of epinephrine from the adrenal medulla (1, 2). Norepinephrine in the circulation is the result of spillover after its release from sympathetic nerve endings (2). Thus, increased levels of norepinephrine reflect greater sympathetic nervous system activity. Increased sympathetic nervous system activity causes a number of responses with effects on the cardiovascular system and other organs (2).

Insulin and glucagon concentrations are affected by surgery. Insulin is an anabolic hormone that plays an important role in controlling blood glucose concentrations as it promotes glucose uptake by insulin sensitive tissues and promotes the conversion of glucose into glycogen and triglycerides (2). Insulin concentration decreases upon induction of general anesthesia and throughout surgery (1, 2, 4, 5). Epinephrine, which peaks immediately after surgery, suppresses the insulin secretion postoperatively (1, 4). The normal cellular response to insulin is also inadequate following surgery, leading to an insulin resistant state (6). In contrast, glucagon promotes hepatic glycogenolysis and gluconeogenesis and concentrations of this hormone increase to a small extent following surgery (1, 2). However, this small increase in glucagon postoperatively is not thought to contribute to postoperative hyperglycemia (2).

1.1.2. Immune and inflammatory response

Cytokines are an important component of the immune response to surgery. Cytokines are a category of low molecular weight proteins that are released in response to tissue injury and are involved in modulating immune and inflammatory responses (2). Cytokines are produced at the site of the tissue injury primarily to improve wound healing (3). However, more severe tissue injury causes cytokines to move into the bloodstream and affect the entire body (3, 7). In

terms of the inflammatory response to surgery, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) act as "first responders" and are released from activated monocytes and macrophages in damaged tissues (2, 7, 8). IL-1β and TNF-α, in turn, stimulate the synthesis and release of interleukin-6 (IL-6), which is largely responsible for the acute phase response (2, 7).

The central feature of the acute phase response is altered hepatic synthesis of acute phase proteins (2, 9). Acute phase proteins are important during the postoperative inflammatory response as they mediate inflammatory and tissue repair processes (9). Positive acute phase proteins are a group of functionally diverse proteins so named because their synthesis in the liver increases during inflammation. For example, C-reactive protein (CRP) levels are elevated hours after surgery and remain elevated for around two weeks after (10, 11). Fibrinogen and α_2-macroglobulin synthesis also increase during acute and chronic inflammation (2, 11). Conversely, the plasma and serum concentrations of particular transport proteins, including albumin and transferrin, decrease (2, 9). This occurs primarily because amino acids are less available to synthesize these proteins and these proteins are broken down at a higher rate (9, 12). In a clinical setting, the acute phase response presents as fever, granulocytosis and changes in serum transport proteins as well as copper, zinc and iron (2).

1.1.3. The interaction between the immune system and the endocrine system

While neural signals from the site of injury initiate the stress response, the immune system is also capable of inducing an endocrine response. More specifically, interleukin-1 (IL-1), interleukin-2 (IL-2), IL-6, and TNF-α promote cortisol secretion (13-15). It is clear that cytokines alone can stimulate an endocrine response because epidural analgesia cannot fully suppress the rise in ACTH and cortisol after upper abdominal surgery (13). TNF-α and IL-6 have been shown to stimulate ACTH and cortisol secretion over and above the secretion due to nervous system stimulation (13). However, cortisol can inhibit further IL-6 production through a negative feedback loop (16).

1.1.4. The metabolic response to surgery

Postoperative carbohydrate metabolism is altered such that blood glucose levels are elevated. Surgery-induced cortisol and catecholamine secretion stimulates hepatic glucose production through both glycogenolysis and gluconeogenesis (2). Furthermore, epinephrine and cortisol suppress insulin concentration and induce peripheral insulin resistance resulting in decreased peripheral glucose clearance (2, 17). Surgery impairs insulin-mediated glucose transporter-4 (GLUT-4) translocation and suppresses non-oxidative glucose disposal (17). Ultimately, increased glucose production and decreased insulin action contribute to postoperative hyperglycemia. Hyperglycemia following surgery is associated with poor wound healing, infection as well as neurologic and pulmonary complications (2, 18).

Protein metabolism is significantly altered by surgery. After surgical injury, skeletal muscle- and visceral proteins to a lesser degree- are broken down and sent to visceral tissues to accommodate the body's demands and improve chances of survival after trauma (2, 3, 19). After surgery, protein breakdown provides amino acids necessary to synthesize acute phase, structural and plasma proteins (3). Increased cortisol levels provoke protein and amino acid breakdown. More specifically, cortisol causes skeletal muscle proteolysis. The amino acids generated from muscle protein breakdown are then transported to the liver where they are incorporated into acute phase proteins or alternatively undergo gluconeogenesis to form glucose (2). The cytokine TNF-α also causes skeletal muscle catabolism (1). When TNF-α binds to its receptor, it signals mitochondria to produce superoxide anions which undergo enzymatic metabolism and electron transfer to produce reactive oxygen species (ROS) (20). ROS activate the ubiquitin pathway leading to proteasomal degradation of inhibitor-κBα (I-κBα) (21). I-κBα is a protein that inhibits nuclear factor-κB (NF-κB) and once this protein is degraded, NF-κB can translocate to the nucleus where it induces the expression of genes involved in muscle proteolysis (20, 21). TNF-α also stimulates synthesis of another catabolic cytokine, IL-1 (20). Furthermore, during the acute phase response, absolute and fractional synthesis rates of acute phase

proteins are increased and skeletal muscle is catabolized to accommodate the synthesis of other proteins (22). If protein breakdown proceeds unabated, it leads to weight loss, loss of body protein and muscle wasting, the degree of which is inversely correlated with patient survival (2, 23). Skeletal muscle proteolysis is especially problematic for elderly surgical patients as age-related muscle loss means these patients have less spare body protein to sacrifice after surgery (3).

Fat metabolism in the postoperative patient is characterized by increased lipolysis as a result of changes in the hormonal milieu. Cortisol, epinephrine, glucagon and growth hormone stimulate lipolysis while insulin inhibits lipolysis (1, 2). Lipolysis liberates triglycerides that are broken down by lipoprotein lipase to glycerol and fatty acids. After surgical tissue injury, lipoprotein lipase activity increases in muscle and fatty acids are oxidized in muscle tissue (1). Fatty acids may also be oxidized in the liver, or otherwise re-esterified, or converted to ketone bodies (2). Glycerol liberated during lipolysis can be used in gluconeogenesis in the liver (2).

1.1.5. The effect of bowel preparation on metabolism after colorectal surgery

Bowel surgery presents a unique challenge to perioperative metabolic state because patients are required to cleanse the bowel in preparation for surgery. Bowel cleansing typically requires dietary restriction and fasting as well as use of laxatives or enemas to properly prepare the colon. Surgical preparation and associated preoperative fasting can cause dehydration and electrolyte abnormalities (24-27) and deplete hepatic glycogen stores. Preoperative fasting can ultimately induce an unfavorable metabolic state, which can enhance the catabolic response to surgery (28, 29). To make matters worse, patients typically continue to fast following surgery, which exacerbates the catabolic state and affects the patient's risk of morbidity following surgery (30).

1.1.6. The effect of anesthesia on the stress response to surgery

Anesthesia is one important modality used to modulate the surgery-stress response. Regional anesthesia, such as epidural analgesia with local anesthetics, is particularly effective in suppressing the endocrine response to surgical stress (31,

32). Epidural analgesia works by blocking afferent stimuli from damaged tissues. In doing so, it suppresses catabolic hormone secretion, helping to attenuate the metabolic effects of these hormones (32). However, epidural anesthesia cannot suppress the inflammatory response to tissue injury (31, 33). The level of catheter insertion and the duration of postoperative analgesia are important factors. A thoracic epidural blockade using local anesthetics provides optimal analgesia during and after abdominal surgery (31, 32, 34). Furthermore, a 48-hour epidural block decreases postoperative protein breakdown to a greater extent than a 24-hour epidural block (35).

Epidural blockade with local anesthetics initiated before surgery and maintained for 24-48 h after, can help ameliorate alterations in macronutrient metabolism. Early work established that epidural analgesia attenuates postoperative nitrogen losses (36-38). More recent studies have shown that epidural analgesia attenuates the typical postoperative decrease in protein synthesis (39) and suppresses postoperative protein breakdown (32). Furthermore, by suppressing catabolic hormone secretion, epidural analgesia can improve postoperative glucose metabolism. Glycogenolysis and gluconeogenesis are reduced, while insulin resistance decreases (40) and insulin sensitivity improves (41), ultimately reducing plasma glucose concentration (31-33). Intraoperative lipolysis also decreases during an epidural block (31).

Ideally, the epidural blockade is used in conjunction with nutrition support. Postoperative patients in the fasted state receiving epidural analgesia cannot achieve positive whole body protein balance (42-45). However, positive protein balance can be achieved when epidural analgesia is supplied together with intravenous amino acids (42, 43, 45) or intravenous amino acids and dextrose (44, 45).

A study compared the effects of PCA and epidural analgesia on functional exercise capacity in colorectal resection patients (46). Patients receiving epidural analgesia had lower postoperative pain and fatigue scores, allowing greater mobilization and greater intake of protein and calories (46). Greater intake of

protein and energy, along with improved metabolism, can explain why patients receiving epidural analgesia demonstrated less severe declines in functional exercise capacity. Furthermore, epidural analgesia with local anesthetics facilitates paralytic ileus recovery (2, 47, 48), which allows for earlier resumption of oral feeding (41). Epidural analgesia also prevents clot formation after lower body surgery (49) and is associated with better pulmonary function (49) and fewer cardiac complications (2, 50).

1.2 Perioperative Nutrition Support

1.2.1. The rationale of nutrition support

Nutrition management of surgical patients aims to provide energy, promote wound healing and resistance to infection while preventing loss of body proteins (51). Nutrition support is of the utmost importance because postoperative nutrient metabolism is significantly altered (1). Protein, carbohydrate and fat metabolism is modified such that the postoperative patient is hypermetabolic (1). Further, providing nutrition to modulate body protein losses is clinically relevant because survival of critically ill surgical patients is inversely correlated with the loss of lean body mass (23). Nutrition support regimens for this patient population are designed to supply a source of amino acids that can used to spare body protein and achieve protein balance (1, 51).

1.2.2. Parenteral nutrition

Supplying energy or nutrients intravenously in amounts that exceed an individual's theoretical requirements is known as hyperalimentation. Studies have shown that hyperalimentation following surgery can induce an anabolic response, resulting positive nitrogen balance (52, 53). However, hyperalimentation can also result in hyperglycemia. Hyperglycemia is associated with a number of postoperative complications including infection, neurologic and pulmonary complications (18). Also, hyperalimentation often requires a central venous line to deliver nutrients in higher concentrations than what can be delivered through a peripheral venous line and maintaining a central venous line is associated with a

number of postoperative complications. Thus, hyperalimentation is not the most desirable choice when designing a postoperative nutrition support regimen.

A group of researchers at McGill University have investigated hypocaloric peripheral parenteral nutrition (PPN) support of colorectal resection patients (29, 44, 45, 54). Hypocaloric glucose infused at a rate of about 600 kcal/d ((5.8 g/(kg.d)) minimized endogenous glucose production but did not spare body protein (54). Schricker and associates showed that patients receiving parenteral glucose ((5.8 g/(kg.d)) and amino acids ((2.9 g/(kg.d)) became hyperglycemic while patients receiving amino acids alone did not (45). Finally, Donatelli and colleagues supplied amino acids alone at a high rate ((2.9 g/kg.d)) to simulate a meal over three hours. This type of parenteral nutrition regimen induced an anabolic protein response without hyperglycemia (42).

Parenteral nutrition has demonstrated some preoperative benefit in severely malnourished patients or those at high risk of postoperative complications. However, these benefits were demonstrated in severely malnourished patients with upper gastrointestinal tract malignancies and so these results may not apply to colorectal resection patients who are well-nourished (55).

According to the 2009 European Society for Parenteral and Enteral Nutrition (ESPEN) guidelines regarding parenteral nutrition and surgery, the use of preoperative parenteral nutrition is only indicated in severely malnourished patients who cannot acquire adequate nutrition orally or enterally either because it is not feasible or not well tolerated (51). Parenteral nutrition was also indicated during the postoperative period in patients who cannot meet at least 60% of their energy needs enterally due to gastrointestinal obstruction or other intolerances (51). Similarly, the American Society for Parenteral and Enteral Nutrition (ASPEN) created guidelines regarding enteral nutrition support for adults (56). While acknowledging the benefits of enteral nutrition, the ASPEN guidelines state that there is not enough evidence to determine whether enteral nutrition is better than parenteral nutrition (56). However, the nutrition delivery algorithm indicates that enteral nutrition should be used when the individual has a functional

gastrointestinal tract (56). Furthermore, the Canadian clinical practice guidelines for nutrition support in mechanically ventilated, critically ill adult patients strongly recommend enteral nutrition above parenteral nutrition for the nutrition support of critically ill patients (57).

1.2.3. Enteral nutrition support

Current perioperative nutrition support guidelines are changing, favoring oral and enteral nutrition over parenteral nutrition (51, 57, 58). Hence the present study was designed to employ an oral nutrition regimen. According to the ASPEN guidelines, enteral nutrition support entails the involuntary provision of nutrients into the gastrointestinal tract via a tube (56). Similarly, oral nutrition uses the gastrointestinal tract and is preferred when the individual is capable of consuming sufficient nutrients to meet their needs (56). There are indications in the literature that oral and enteral nutrition offers specific advantages when compared to parenteral nutrition.

The route of nutrient administration affects glucose metabolism. A study by Magnusson and associates compared enteral and parenteral glucose in postoperative colorectal resection patients. Each nutrition regimen consisted of a 10% glucose solution administered at a rate of approximately 2500 ml/d (975 kcal/d). Blood glucose was significantly lower during the first three postoperative days in the enteral nutrition group. Furthermore, blood glucose decrement in response to insulin infusion dropped 52% from preoperative values in the parenteral group. Conversely, blood glucose decrement did not change from preoperative values in the enteral group indicating that insulin action was better preserved in the enteral group (59). Even though glucose-based parenteral nutrition support regimens can suppress endogenous glucose production (54), infusing glucose may be associated with hyperglycemia (44). Petrov and Zagainov reviewed randomized controlled trials comparing the effect of parenteral and enteral nutrition on blood glucose control in acute pancreatitis patients. Their review revealed that enteral nutrition reduced the relative risk of requiring insulin therapy and the relative risk of hyperglycemia compared to parenteral nutrition (60). On the basis of these findings, it is postulated that the

present oral nutrition regimen will not increase blood glucose concentrations beyond what would be expected in a parenteral nutrition regimen.

Given that one of the goals of postoperative nutrition support is to prevent body protein losses, a number of studies have assessed how enteral nutrition affects postoperative protein metabolism. Hochwald and colleagues studied changes in whole body protein kinetics in patients receiving enteral nutrition or intravenous fluids after upper gastrointestinal resection (61). Positive whole body protein balance was only achieved in the enterally fed group. This was due to significantly less protein breakdown in patients receiving enteral nutrition. Soop and associates assessed the effect of complete or hypocaloric early enteral feeding on nitrogen balance after colorectal surgery (62). Positive nitrogen balance was achieved in patients receiving complete enteral nutrition (62). Other work by the Enhanced Recovery After Surgery (ERAS) Study Group has demonstrated that consuming carbohydrate-based oral nutrition prior to surgery attenuates postoperative loss of muscle mass (assessed by mid-arm circumference) (63), muscular strength (6) and total nitrogen (64).

The relationship between the route of nutrition administration and postoperative protein metabolism is not entirely clear. Early work on this topic indicated that enteral nutrition was associated with less negative nitrogen balance after abdominal surgery and that protein and energy had a greater nitrogen-sparing effect when delivered enterally (65). Since then, a few studies have failed to find a difference between enteral and parenteral nutrition in terms of nitrogen balance (66, 67). However, it is important to note the limitations of nitrogen balance studies. More recently, Kudsk and associates assessed changes in constitutive and acute phase protein in patients with abdominal trauma receiving either parenteral or enteral nutrition (68). They found that patients who received enteral nutrition had higher levels of constitutive proteins and lower levels of acute phase proteins compared to patients receiving parenteral which they attributed to reduced incidence of sepsis in enteral nutrition patients (68).

A large proportion of the research comparing enteral and parenteral nutrition assesses whether the route of nutrient administration affects the risk of infection, postoperative complications and morbidity. Enteral nutrition support avoids many of the problems associated with the insertion and maintenance of intravenous catheters (55). Compared to parenteral nutrition, enteral nutrition support is associated with maintenance of the intestinal structure and function (55, 56, 69). Enteral nutrition also helps to maintain immune function (55, 70) and decrease septic complications (56, 70-72). Patients receiving enteral nutrition have fewer infectious (56, 70, 73, 74) and non-infectious complications (70, 74, 75). Patients receiving enteral nutrition also have shorter hospital stays following surgery (70, 74, 75) and greater chances of survival after surgical trauma (70). In one study, patients receiving parenteral nutrition reported greater postoperative distress and required more frequent doses of analgesic drugs (59). Enteral nutrition regimens also tend to be less costly than analogous parenteral regimens (55, 56).

1.3 Perioperative Nutrition and Multi-Modal, Enhanced-Recovery Protocols

1.3.1. Perioperative fast-track and enhanced-recovery protocols

Postoperative recovery protocols generally promote more efficient recovery and return of functional capacity allowing for a shorter hospital stay. Two different research groups, the Fast-Track Study Group and the ERAS Study Group, have developed similar and noteworthy protocols with the goal of enhancing and accelerating postoperative recovery. Both groups acknowledge that a multidisciplinary approach is needed to modify the metabolic aspects of the surgery-stress response before, during and after surgery.

The term fast-track surgery has been developed to describe a model of perioperative patient care with two goals: [1] reducing the time a patient spends in the hospital after surgery and [2] resuming normal activity after hospital discharge (41). The Fast-Track Surgery Study Group is composed of clinical investigators from a number of scientific disciplines (41). This groups goal is to review and

evaluate literature related to perioperative patient care and determine surgical and anesthetic practices that will facilitate the recovery process (41). The Group has determined a number of factors that are important in determining postoperative patient outcomes (41). The Fast-Track Surgery Study Group considers nutrition supplementation an important postoperative factor. Regarding nutrition supplementation, the Group notes that enteral nutrition support is preferred to parenteral nutrition support and that parenteral nutrition should only be delivered to individuals who cannot tolerate enteral nutrition (41). The group also notes that hyperalimentation should be avoided because it can cause hyperglycemia (41) which is associated with a number of unfavorable postoperative outcomes (18).

The ERAS clinical protocol has a number of components. It is important to first manage the patients anxiety level and educate the patient about what to expect during the perioperative period (76). Secondly, preoperative fasting and bowel preparation should be avoided (76). A standard anesthetic procedure involving epidural analgesia with local anesthetics and a low-dose opioid should be initiated preoperatively and the size of the surgical incision should be minimized (76). Epidural analgesia should continue for at least two days after surgery and non-steroidal anti-inflammatory drugs (NSAID) should be used to manage pain thereafter (76). The perioperative fluid management strategy involves not overloading the patient with intravenous fluids or saline while allowing resumption of oral fluid intake shortly after surgery (76). Postoperative nausea and vomiting should be combated using an antiemetic to facilitate resumption of oral intake. Postoperative nutrition management involves prompting patients to resume oral food consumption as soon as possible and oral nutrition supplements can be used to help meet nutrient requirements. Lastly, prolonged bed rest should be avoided and early mobilization should be encouraged (76).

1.3.2. Postoperative oral nutrition within an enhanced-recovery protocol

Traditionally, patients were prescribed ‘nil per os’ or nothing by mouth after gastrointestinal surgery. It is now clear that there are no advantages to this

approach (56, 77). Furthermore, early postoperative oral nutrition is now a valued part of multi-modal, enhanced recovery protocols (25, 26, 76, 78, 79). Patients receiving oral supplementation within an enhanced recover protocol consumed more calories and protein during the first five postoperative days (80). Within a multi-modal enhanced-recovery protocol, oral nutrition supplementation is associated with minimal insulin resistance (62) and nitrogen losses (62). Moreover, oral nutrition and an enhanced recovery program reduces risk of infection (76), time to the passage of flatus and stool (81) and ultimately reduces the length of hospital stay (25, 76, 81). Finally, this type of oral nutrition supplementation regimen is safe and well tolerated by patients (56, 81, 82). Although one study indicated that the duration of surgery impacts tolerance of oral feeding such that longer operative time was associated with less tolerance of early oral feeding (81).

Lewis and associates conducted a systematic review and meta-analysis of randomized controlled trials comparing enteral nutrition initiated within 24 hours of gastrointestinal surgery and nothing by mouth. This meta-analysis indicated that early enteral nutrition support was associated with decreased relative risk of infection, and decreased length of hospital stay but increased risk of vomiting (77).

1.4 Composition of the Experimental Oral Nutrition Support Regimens

Oral nutrition support is relatively simple and inexpensive way to provide nutrients. Further, the provision of oral nutrition support is associated with better metabolic and clinical outcomes. While previous studies have demonstrated the benefit of oral nutrition in abdominal surgery patients, relatively little research has been carried out to assess the optimal macronutrient composition, particularly in relation to amino acid and glucose substrates. The present study aimed to supply oral nutrition support regimens based on either whey protein and glucose or glucose alone. The oral nutrition support regimens were intended to induce an anabolic protein response while maintaining normoglycemia. An oral nutrition

support program of this type requires a high quality, low residue protein that is highly bioavailable and palatable. The present study aimed to extend previous findings regarding the beneficial effects of enteral nutrition on postoperative metabolism by: [1] showing the utility of oral nutrition support in preventing hyperglycemia associated with glucose-based parenteral nutrition support; [2] including pressurized whey protein to provide a more bioavailable and nutritionally complete source of amino acids compared to single amino acid mixtures.

1.4.1. Whey protein isolates

Whey is produced during the process whereby milk is used to make cheese. Of the proteins in cow's milk, approximately 20% are whey proteins (83). Whey proteins are considered complete proteins because they provide all of the indispensible amino acids (83, 84). Whey protein isolates generally contain higher concentrations of protein as well as less fat and lactose than whey protein concentrates (83, 85).

Three factors considered during protein quality assessment are amino acid composition, protein digestibility and bioavailability of amino acids (83, 86). Castellanos and associates reviewed the protein quality of a number of protein sources typically used as modular protein supplements. The methods used to assess protein quality included net protein utilization (NPU), biological value (BV), protein efficiency ratio (PER; weight gained divided by nitrogen consumed in animal models) and the protein digestibility corrected amino acid score (PCAAS), which is a measure of how well a particular protein will provide indispensable amino acids (84). In comparison with other complete protein sources like milk, casein, egg white and soy, whey achieved scores that were equal or greater on NPU, BV and PCASS (84). However, egg white and milk scored higher then whey in terms of PER. Comparisons of protein quality by other researchers indicate that egg may also score higher on NPU (85). Whey is also limiting in the amino acid tryptophan. The amino acid profile of whey protein is compared to Travasol ®, (parenteral amino acid solution), and to egg protein (the reference protein for adults) in Table 1.

Whey protein is rapidly digested (87-89). Boirie and associates fed healthy subjects a single serving of 30 g (approximately 0.45 g/kg) of whey protein, which produced a short but substantial increase in plasma amino acids. More specifically, the rate of appearance of exogenous leucine peaked approximately one hour after ingestion but returned to baseline within 240 min (88). Dietary leucine oxidation was significantly higher than baseline during this same period of time (88). Protein synthesis was significantly elevated above baseline between 40 min and 140 min. However, protein breakdown was not affected by whey protein consumption (88). In contrast with whey protein, casein is a slow digesting protein. In the same study by Boirie and colleagues, subjects consumed a single serving of casein containing the same amount of leucine as whey protein (88). Ingesting casein produced a longer but smaller increase in exogenous leucine rate of appearance and oxidation relative to whey protein. Casein inhibited protein breakdown to a greater extent than whey protein but had no effect on protein synthesis (88). Ultimately, whey protein and casein have different digestion rates, which affect protein balance (87-89). Individual nutrition goals should be used to select one type of protein over the other. The feeding period in the present study was limited to four hours, so the fast-digesting properties of whey protein were advantageous and desirable.

Dangin and associates demonstrated that it is possible to modify the fast-digesting properties of whey protein (87). One group of healthy subjects were asked to consume small portions of a 30 g serving of whey protein for 240 min (RPT-WP) while another group of subjects consumed a single 30 g serving of whey protein (WP). When whey protein was consumed in smaller portions over a longer period of time, it acted like a slow digesting protein (87). More specifically, protein breakdown decreased significantly from baseline while protein synthesis was not affected. Leucine oxidation was elevated to a smaller extent but over a longer period of time in the RPT-WP group compared to the WP group (87).

Whey proteins can help mediate inflammatory processes. Whey proteins are rich in cysteine and have been shown to increase the concentration of

lymphocyte glutathione (GSH), a major intracellular antioxidant (90). It is thought that particular peptides released during digestion of whey protein isolates are absorbed and used to generate cysteine, the rate-limiting amino acid for GSH synthesis (91). GSH neutralizes ROS in a reaction catalyzed by GSH peroxidase where the reduced form of GSH donates its sulfhydryl proton. ROS often increase after surgical tissue injury (20) and greater oxidative stress increases muscle proteolysis (21). Thus, increasing intracellular GSH through provision of whey protein isolates may decrease muscle protein catabolism by suppressing ROS generation.

Whey protein supplementation has demonstrated benefits in disease states that are associated with chronically low levels of GSH. Lothian and associates conducted a case study to see if an oral, whey protein-based supplement could provide benefit to an individual with obstructive lung disease (92). The supplement was designed to supply GSH precursors and after one month of supplementation, whole blood GSH concentration increased (92). Micke and colleagues studied the effect of a six-month period of whey protein supplementation on plasma GSH concentration in individuals with human immunodeficiency virus (HIV) (93). Whey protein supplementation led to a significant increase in plasma GSH (93). Grey and colleagues randomly assigned Cystic Fibrosis (CF) patients to receive either whey protein isolate or casein supplementation for three months (90). Lymphocyte GSH increased 46.6% from baseline in patients receiving whey protein-based supplementation (90).

1.4.2. Pressurized whey protein isolates

The whey protein isolates included in the present study have undergone high pressure processing. High hydrostatic pressure treatment is typically used in the preservation and modification of food (94). This type of processing is one of the most safe and successful ways to sterilize foods without the use of high temperature processing (94). This treatment is effective in inactivating enzymes and microorganisms while leaving other food constituents such as vitamins and food coloring mostly unchanged (94). During the process of high-pressure treatment, foodstuffs are subjected to short periods of hydrostatic pressures of up

to 550 MPa (94, 95). High pressure processing has been shown to denature proteins in a specific manner (94). While the primary structure of the protein is preserved, high-pressure processing causes irreversible changes in the secondary and tertiary structures of the protein (96). Studies suggest that the changes that occur in the secondary and tertiary structures of whey protein subjected to high pressure processing lead to improved bioactivity and protein digestibility (96). In comparison to native whey protein, pressurized whey protein is less resistant to pepsin hydrolysis because pressure treatment gives pepsin better access to the peptide bonds in the interior of β-lactoglobulin, a major protein component of whey protein (95). The partial unfolding of the protein molecule exposes the hydrophobic amino acids buried in the interior to proteolytic enzyme action. Because it is digested relatively easily, pressurized whey protein demonstrates a rapid release of low molecular weight peptides which are associated with increased intracellular levels of GSH (95). In a study involving healthy volunteers, a linear relationship was demonstrated between the dose of pressurized whey protein isolate and lymphocyte GSH levels. In this case, pressurized whey protein isolate supplementation of 45g/day increased lymphocyte GSH by 24% over the course of two weeks (96).

Pressurized whey protein isolates are an effective therapeutic agent in other patient populations. For example, pressurized whey protein isolate has been shown to induce GSH production in tracheal epithelial cells with mutations in the Cystic Fibrosis transmembrane conductance regulator (CFTR) gene (95). The CFTR is an anion channel that transports chloride (Cl^-) across epithelial cell membranes in many organs (97). Mutations in the CFTR gene can either diminish Cl^- transport or decrease the expression of the CFTR on the cell surface, causing CF (95, 97). Individuals with CF typically experience mucus build-up, as well as chronic lung infections and inflammation (98). Whey protein isolates are an important aspect of antioxidant therapy in CF patients because of their capacity to protect mutant CFTR cells from oxidative stress and associated inflammation. Lands and colleagues assessed whether one month of pressurized whey protein supplementation improved inflammatory and nutritional status in 9 children and

18 adults with CF (99). Children received 20 g/d while adults received 40 g/d. CRP decreased in a majority of patients but whole blood GSH was unchanged. Nutritional status, assessed by BMI, improved in both children and adults. Children showed improvements in lung function.

1.4.3. Glucose

Glucose was included in the oral nutrition support regimens used in the present study because glucose is a common ingredient in perioperative nutrition supplementation. Glucose also stimulates insulin secretion to a greater extent than protein alone, which may help inhibit cellular proteolysis (100).

1.5 Stable Isotope Tracers to Study Amino Acid Kinetics

While generally adequate for clinical management purposes, assessments based on metabolite plasma levels alone do not enable understanding of the biochemical events that produced the observed value. For example, in surgical patients protein balance is frequently measured by nitrogen balance, which is the difference between nitrogen intake and total nitrogen excretion. It is possible to reasonably estimate nitrogen intake, total daily renal nitrogen excretion and nitrogen content in the urine under clinical conditions. However, it is not possible to precisely quantify nitrogen losses through wound secretion, drains, feces and skin. Another limitation of nitrogen balance studies is the fact that changes in nutrition independently affects protein synthesis and protein breakdown and so the independent effects of nutrition support cannot be determined.

Stable isotope tracers are typically glucose or amino acid molecules that have been labeled by naturally occurring, stable, non-radioactive isotopes (^{2}H, ^{13}C), which differ from the unlabeled molecule only in their mass and not in their chemical behavior. Flux rates of amino acids and glucose can be followed in vivo by using stable isotope tracers since dilution of the tracers in plasma are inversely proportional to substrate flux. Using stable isotope tracers to measure protein turnover and its components (protein synthesis, protein breakdown and amino acid oxidation) illustrates the dynamic changes in protein metabolism. By tracking

the movement of the labeled molecule, the movement of the unlabeled molecule can be followed as well. There are a number of advantages associated with using stable isotope tracers in metabolic research. Firstly, stable isotopes such as ^{13}C are naturally occurring, non-radioactive isotopes and are safe for use in human research (101, 102). Previous research has shown that the small amounts of tracer administered during research do not pose a risk to human subjects (101, 102). Further, when using the stable isotope tracer L-[1-^{13}C]leucine, and administering a priming dose, it is possible to begin collecting samples at plateau within 90 minutes of the start of the infusion (101), which helps to minimize subject burden.

1.5.1. Amino acid kinetics

According to the single-pool model of whole-body protein metabolism, amino acids enter the free pool from dietary intake and protein breakdown while amino acids leave the free pool by amino acid oxidation and amino acid uptake for protein synthesis (103). 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 (104).

To determine whole body protein metabolism, an isotope dilution technique has been developed using the tracer L-[1-^{13}C]leucine (104). Using a single amino acid tracer at isotopic steady state, the rate of appearance (R_a) equals the rate of disappearance (R_d) and can be used to determine whole body protein turnover or flux (Q). During constant infusion of the stable isotope tracer at steady state, the R_a of L-[1-^{13}C]leucine represents the total movement of leucine into the plasma pool from dietary intake and protein breakdown. The R_d represents the total movement of amino acids from the plasma pool due to incorporation into body proteins and oxidation.

The reciprocal-pool stochastic model of amino acid kinetics was used to calculate whole body protein metabolism in the present study. The L-[1-^{13}C]leucine tracer is administered to the plasma pool and is sampled from plasma (103). According to this model, plasma leucine is in equilibrium with intracellular leucine (103). The intracellular space is assumed to be one compartment within

which α-ketoisocaproate (α-KIC) is synthesized from intracellular leucine. Following its synthesis from leucine, α-KIC can move freely into the plasma (105). Thus, plasma α-KIC enrichment reflects intracellular leucine enrichment (105). Intracellular α-KIC can also undergo oxidation, during which the first carbon is irreversibly released to form CO_2. Knowing this, the rate of leucine oxidation can be quantified from the $^{13}CO_2$ enrichment in the breath samples collected during plateau. Since the oxidized carbon label must pass through the body's bicarbonate pool before it is released in exhaled breath, a correction factor must be applied to the leucine oxidation rate to account for the amount of $^{13}CO_2$ that is retained by the body's bicarbonate pool (101). Finally, whole body protein balance can be calculated, assuming that leucine comprises 8% of body protein (106).

1.6 Rationale

Colorectal surgery frequently requires a protracted period of perioperative fasting, which exacerbates the stress response to surgery. The period immediately following bowel surgery is characterized by a net protein catabolic state (2, 3), which may result in a loss of body protein. Body protein loss is associated with reduced muscle strength and endurance, impaired immune function and increased risk of morbidity after surgery (2, 23). Postoperative glucose metabolism is characterized by hyperglycemia due to impaired glucose utilization together with inappropriately high hepatic glucose production. Since counter-regulatory hormones induce an insulin resistant state (1, 6, 107), even moderate rates of glucose administration are associated with hyperglycemia (45). Hyperglycemia is associated with a number of postoperative complications (18, 107) so controlling blood glucose concentration is very important during recovery after colorectal surgery. Nutrition support is one of the modalities used to minimize postoperative protein breakdown and hyperglycemia. The fundamental goals of perioperative nutrition support are to decrease protein breakdown and amino acid oxidation and maintain normoglycemia through optimal nutrient delivery.

Previous studies in colorectal patients receiving epidural analgesia have established that administering parenteral amino acids with or without glucose, but not glucose alone, can achieve a net protein anabolic state while maintaining normoglycemia (42, 44, 45). However, enteral nutrition can also achieve protein anabolism (62, 65) while avoiding the costs and potential complications associated with parenteral nutrition (55, 56). The primary goal of the present study was to examine whether postoperative oral protein and glucose can not only prevent protein catabolism and stimulate protein synthesis but also facilitate the maintenance of normal blood glucose concentrations. Pressurized whey protein isolate was provided as the protein source since previous human trials have demonstrated that whey protein is highly bioavailable and easily digested (83, 84).

The proposed oral nutrition regimen based on whey protein (with or without glucose) was based on extensive parenteral feeding studies (44, 45, 54). The parenteral studies infused amino acids to supply 2.9g/(kg.d), theoretically given over one day. The three-hour infusion of 0.12 g/(kg.h) was intended be high enough to stimulate protein synthesis if given in absence of glucose and to simulate the quality of amino acids that would be absorbed after a meal. This oral feeding study was designed to allow for retrospective comparisons to previous parenteral nutrition studies. Thus, the oral nutrition regimen was devised so that the drink was sipped at the same rate as previous parenteral infusions (44, 45, 54). However, the feeding period was extended from three hours to four hours to account for some delay for digestion and absorption in achieving isotopic steady state in the tracer pools.

1.7 Objectives and Hypothesis

A prospective, randomized and controlled study was proposed to establish whether an oral nutrition support regimen based on pressurized whey protein and glucose improved the postoperative utilization of amino acids compared to glucose alone. Kinetics of protein metabolism (protein breakdown, protein synthesis and amino acid oxidation) was investigated using stable isotope

methodology before and after surgery in patients undergoing colorectal resection. Stable isotope infusions were conducted one week before surgery and on the second postoperative day for two hours in the fasted state and for four hours while sipping the oral nutrition support regimen. Patients consumed one of two oral nutrition support regimens consisting of a drink containing either pressurized whey protein and glucose or glucose alone.

The following hypothesis was tested: an oral nutrition support regimen based on pressurized whey protein with glucose, compared to glucose alone, promotes positive protein balance through increased protein synthesis or reduced protein breakdown while maintaining normoglycemia.

II. METHODS

2.1 Patients

Seventeen patients undergoing elective colorectal surgery were recruited between January 2010 and August 2010 from the preoperative clinic at Montreal General Hospital in Montreal, Quebec, Canada. The surgical techniques/medical devices/reproductive technologies research ethics board of the McGill University Health Centre (MUHC) in Montreal, Canada approved the study protocol. Written informed consent was obtained from all patients (Appendix A and Appendix B). Inclusion criteria were: age older than 18 years; ASA class I to III, colorectal surgery for non-metastatic disease (including right, transverse, left, sigmoid, subtotal, total and hemicolectomy and low anterior resection); body mass index >17 and <30 kg/m^2; stable weight over the preceding three months (<10 % body weight loss); serum albumin >35 g/L. Exclusion criteria were: severe cardiac, renal or hepatic failure; diabetes, hyper and hypothyroidism; active inflammatory bowel or diverticular disease; musculoskeletal or neuromuscular disease, anemia (hematocrit <30 g/L) and albumin < 25 g/L; pregnancy, use of steroids and intolerance of milk products. The patients were randomly allocated by a computer-generated blocked randomization method (2 patients per group per

block x 5 blocks) to receive one of two oral nutrition regimens: glucose only or pressurized whey protein and glucose.

2.2 Surgical and Perioperative Care

Patients underwent bowel preparation on the day before surgery and were allowed to drink clear fluids until midnight. Patients underwent surgery between 8:00 AM and 2:00 PM by three colorectal surgeons at Montreal General Hospital site of the MUHC in Montreal, Quebec, Canada. On the first postoperative day, patients were allowed to drink clear fluids unless contraindicated. Clear fluids consisted of a small portion of apple juice (approximately 110 kcal) and Jell-O® (Kraft Foods, Northfield, Illinois) (approximately 70 kcal). Patients were also allowed to drink water or received intravenous 0.9% normal saline overnight until the postoperative infusion study the following morning.

The present study only accepted patients who expected to receive perioperative epidural analgesia. However, four patients received intraoperative systemic analgesia with opioids and postoperative PCA with opioids at the discretion of the anesthesiologist. The remaining patients received continuous epidural analgesia with local anesthetics initiated prior to surgery and maintained until the end of postoperative day 2.

2.3 Oral Nutrition Support Regimens

2.3.1 Composition and preparation of the oral nutrition support regimens

The oral nutrition support regimens were each formulated as a drink, based on previous parenteral nutrition support regimens (44, 45, 54). The glucose source was anhydrous beet dextrose (Avebe, Foxhol, Holland), which has a low ^{13}C natural abundance, and would therefore be unlikely to disturb the $^{13}CO_2$ enrichment in expired air. Dextrose was dissolved in water to create a 200 g/L stock solution.

The protein source was whey protein isolate (InPro 90, Vitalus Nutrition Inc., Abbotsford, British Columbia) dissolved in water as a 10% stock solution (100 g/L) in sealed plastic bottles designed for individual use. The bottles containing whey protein were submerged in water in the pressure chamber and pressurized with an Avure High Pressure Processing System model QFP 215L-600 (Avure Technologies, Columbus, OH) using one cycle of pressurization at 550 MPa with 1 min holding time.

Subjects had a choice of the flavor of the oral nutrition regimen (lemon lime, strawberry-orange-banana, pink lemonade and tangerine-grapefruit) (Crystal Light®, Kraft Foods, Northfield, Illinois). The drinks were prepared individually for each subject from stock solutions in the food preparation kitchen at Montreal General Hospital, according to Table 2. The final composition of each drink was: glucose-based: 200 g/L of glucose; whey protein-based: 200 g/L of glucose, 100 g/L of protein.

2.3.2 Nutrient intake

Patients in the glucose-only group sipped the oral nutrition regimen at a rate approximating 1.2 mL/(kg.h) over 4 hours to simulate a meal (Table 2). Patients in the glucose-whey group sipped the drink at a rate approximating 2.54 mL/(kg.h) over four hours (Table 2). The patients were asked to sip a small aliquot from a small cup every 30 min for 4 h. This amino acid intake over the 4 h corresponds to approximately 73% (0.48 g/(kg.4h)) of the daily Estimated Average Requirement (EAR) of protein as well as a minimum of 95% and an average 144% of the EAR of each indispensable amino acid (Table 1).

2.4 Experimental Protocol

One week prior to surgery, following an overnight fast, patients underwent a 6-h stable isotope infusion starting at 7:00 AM. Each infusion study began and continued for 2 h while the patient was in the fasted state. The infusion continued for a further 4 h while the patient sipped the test oral nutrition regimen. The study was repeated starting at 7:00 AM on the second postoperative day (Figure 1).

Blood and expired air samples were collected at time zero to determine baseline enrichments. The bicarbonate pool was primed with an oral dose of sodium bicarbonate ($NaH^{13}CO_3$, 1 μmol/kg) prior to the start of the stable isotope tracer infusion. A cannula was inserted in a forearm vein to provide access for the infusion of sterile, pyrogen-free L-[1-^{13}C]leucine tracer solution (Cambridge Isotope Laboratories, Cambridge, MA). A priming dose of L-[1-^{13}C]leucine (4 μmol/kg) was administered intravenously followed by a continuous infusion of L-[1-^{13}C]leucine (0.06 μmol/(kg.min)) over the first 2 h while the patient was fasted. Blood and breath samples were collected at 90, 100, 110 and 120 min to determine protein kinetics of the fasted state. At the end of the first 2 h, the patient began to sip the test oral nutrition regimen according to a specified sipping protocol. The tracer infusion continued for another 4 h during which time, the L-[1-^{13}C]leucine infusion rate increased to 0.12 μmol/(kg.min). Blood and breath samples were collected at 330, 340, 350, 360 min to determine protein kinetics in the fed state. Additional blood was drawn at 0 min and 330 min to analyze serum cortisol, glucose, insulin, total protein and albumin. Each blood sample was transferred to a vacutainer (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ), centrifuged at 4°C (2,400 x g for 15 min) and the plasma was stored at -70°C. Breath samples were collected in a 4 L bag and transferred immediately to 10 mL non-coated evacuated tubes (Kendall, Covidien, Mansfield, MA).

2.5 Measurements and Analytical Methods

2.5.1 Analysis of metabolite isotopic enrichment

Isotopic enrichment of [1-^{13}C]α-KIC in plasma, representing intracellular leucine was used as the basis for calculating both flux and oxidation of leucine (108). From each blood sample, 200 μL of plasma was combined with 250 μL of both tetrabutyl ammonium hydrogen sulphate (TBA) solution and pentafluorobenzyl bromide (PFB-Br) solution, vortexed for 1 min and refrigerated overnight (109). The organic acids were extracted by adding 900 μL of hexane and 200 μL of ethanol, vortexing the sample for 1 min and centrifuging the sample for 10 min at 1500 x g (109). The organic layer was transferred to an auto

sampler vial. [1-^{13}C]α-KIC enrichment was analyzed as its pentafluorobenzyl ester derivative using methane negative chemical ionization gas chromatography-mass spectrometry (GC/MS) (Agilent 5975, Agilent Technologies Inc., Santa Clara, CA) (110). Isotopic enrichment was measured as the increase above the baseline measurement in molecules percent excess (MPE) using the equation MPE = (R_s-R_b.100)/(1+R_s-R_b). According to this equation, R_b is the isotope ratio at baseline; accounting for the naturally occurring ^{13}C and R_s is the isotope ratio at steady state; accounting for both naturally occurring and enriched ^{13}C (101). Steady state conditions were established for [1-^{13}C]α-KIC if the coefficient of variation of the four samples in each study period was less than 5% during each of the fasted and fed period.

$^{13}CO_2$ enrichment in expired breath was determined by isotope ratio mass spectrometry (Analytical Precision AP2003, Analytical Precision Ltd., Manchester, United Kingdom) (108). Isotopic enrichment was calculated as atoms percent excess over baseline. Steady state conditions were established for $^{13}CO_2$ if the coefficient of variation of the four samples in each study period was less than 5% during each of the fasted and fed period.

2.5.2 Calculation of leucine kinetics

Whole-body leucine kinetics was calculated by conventional isotope dilution practice using a two-pool stochastic model during steady-state conditions (111, 112). At isotopic steady state, the R_a (μmol/(kg.h)) of unlabeled substrate can be derived from the plasma enrichment (MPE) calculated by R_a = (MPE_{inf} / MPE_{pl} 1)·F where F is the infusion rate of the labeled tracer, MPE_{inf} is the tracer enrichment in the infusate and MPE_{pl} is the tracer enrichment in plasma at steady state (112). The MPE value used in this calculation represents the mean of the MPE values determined during each isotopic plateau. Under steady state conditions, leucine flux (Q) is defined by the formula: $Q = S + O = B + I$, where S is the rate at which leucine is incorporated into body protein, O is the rate of oxidation of leucine, B is the rate at which unlabeled leucine enters the free amino acid pool from endogenous protein breakdown, and I is the rate of leucine intake, including tracer and dietary amino acid intake. When the subjects are in the fasted

state, or consuming glucose only, leucine intake equals zero and $B = Q$. When amino acids are fed, the rate of leucine intake (I) was subtracted from the total leucine flux to calculate the rate of endogenous leucine release (112). The rate of exogenous leucine intake was calculated as the product of the leucine concentration in the oral nutrition support regimen (μmol/mL) and the oral leucine intake rate (mL/(kg.h)). Leucine balance (μmol/(kg.h)) is calculated as protein synthesis (S) minus protein breakdown (B). Since leucine comprises 8% of body protein, leucine balance was used to calculate whole body protein balance (g/(kg.d)) (106).

In the calculation of oxidation, a factor of 0.76 was used in the fasted state to account for the fraction of ^{13}C-carbon dioxide released from leucine but retained within slow turnover-rate pools of the body (113). Consistent with previous work on leucine kinetics in patients receiving parenteral nutrition with amino acids and glucose (113) and patients receiving oral whey protein (87, 88, 106), a retention factor of 0.81 was used during the fed state in this study.

2.5.3 Metabolic substrates and hormones

Serum glucose concentration was measured using an oxygen rate method by the Synchron LX® system (Beckman Coulter, Fullerton, California). Serum insulin was determined using the Access Ultrasensitive insulin assay, a one-step immunoenzymatic ("sandwich") assay (Beckman Coulter, Fullerton, California). Serum cortisol was determined using the Access Cortisol assay, a competitive binding immunoenzymatic assay (Beckman Coulter, Fullerton, California). Serum total protein concentration was measured using a rate biuret method by the Synchron LX® system (Beckman Coulter, Fullerton, California). Serum albumin concentration was determined using a biochromatic digital endpoint methodology using bromcresol purple agent by the Synchron LX® system (Beckman Coulter, Fullerton, California).

2.5.4 Gaseous exchange

Indirect calorimetry (Vmax 29N; SensorMedics, Yorba Linda, CA) was performed for 20 min in the last hour of the fasted and fed states. During this

time, subjects were asked to lie in a semi-recumbent position and breathe room air in a ventilated hood. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured. Average oxygen consumption and carbon dioxide production values and the respiratory quotient were determined by indirect calorimetry, assuming a coefficient of variation of less than 10% over 15 min. Resting energy expenditure (REE) and respiratory quotient (RQ) were also calculated.

2.6 Statistical Analysis

The primary outcome variable was whole body leucine balance (protein synthesis minus protein breakdown as represented by leucine kinetics values) on the second postoperative day. The sample size was determined based on previous studies in colorectal surgery patients receiving parenteral nutrition at the same rate as the oral nutrition in the present study (45, 54).

Statistical analyses were conducted using SAS 9.2 for Windows (SAS Institute Inc., Cary, NC). A Kolmogorov-Smirnov test at a 0.1 significance level was used to determine whether the data was normally distributed. An F-test was used to ensure homogeneous variances. A 3-factor repeated measures ANOVA (Proc GLM) was used to determine the effect of feeding (fasted vs. fed), the effect of the type of nutrition (glucose-based vs. whey protein-based), and the effect of surgery (preoperative vs. postoperative). Significance was postulated if $P < 0.05$.

III. RESULTS

3.1 Patients

The two groups were similar in terms of the sex, age, height, weight, BMI and the duration of surgery (Table 3). Four patients were ineligible to participate in the postoperative study due to postoperative complications. Thirteen patients were included in the final analysis (glucose-based: n=6, whey protein-based: n=7) (Figure 2).

3.2 Leucine Kinetics

A plateau in the isotopic enrichment of [1-^{13}C]α-KIC and expired $^{13}CO_2$ was achieved during the fasted and fed states of each study period (coefficient of variation < 5%) (Figure 3 and Figure 4). Preoperative leucine kinetics in the fasted state was comparable between the two groups (Table 4). Oral nutrition affected leucine R_a (P <0.0001) and fed state leucine R_a was greater in the whey protein-based group (P <0.0001). Net protein breakdown decreased in both groups in the fed state (P <0.0001) and protein breakdown was lower in the whey protein-based group (P <0.0002). Oral nutrition affected leucine oxidation (P <0.0001) and fed state leucine oxidation was greater in the whey protein-based group (P <0.0001). Protein synthesis did not change in the fed state (P <0.2737) and there was no difference between the groups (P <0.1124). Leucine balance increased in both groups in the fed state (P <0.0001) and leucine balance was significantly greater in the whey protein-based group (P <0.0001). It is noteworthy that only the whey protein-based group achieved positive leucine balance (Table 4).

During the postoperative study, oral nutrition affected leucine R_a (P <0.0005) (Table 5) and fed state leucine R_a was greater in the whey protein-based group (P <0.0001). Net protein breakdown decreased in both groups in the fed state (P <0.0001) but net protein breakdown was suppressed to a greater extent in the whey protein-based group (P <0.0001). Oral nutrition affected leucine oxidation (P <0.0001) and fed state leucine oxidation was greater in the whey protein-based group (P <0.0001). Protein synthesis was not different in the fed state (P <0.1094) and there was no difference between the groups (P <0.4827). Leucine balance increased in both groups in the fed state (P <0.0001) leucine balance was greater in the whey protein-based group (P <0.0001). Only the whey protein-based group achieved positive leucine balance. The effect of feeding on leucine kinetics was not different following surgery and there was no interaction between surgery and type of nutrition (Table 5). Finally, the fasting R_a of leucine (P <0.0078), protein breakdown (P <0.0075) and protein synthesis (P <0.0037) were all significantly higher after surgery with no differences between groups.

3.3 Hormones and Metabolites

During the preoperative study in the fasted state, serum cortisol, glucose, insulin, total protein and albumin were not different between the two groups (Table 4).

During the postoperative study, serum glucose (P <0.0118) and insulin (P <0.0001) increased in the fed state. Cortisol (P <0.0001) and total protein (P <0.0219) decreased in the fed state (Table 6). However, cortisol, glucose, insulin, total protein and albumin were not significantly different between the groups (Table 6). Finally, fasting cortisol (P <0.0069), total protein (P <0.0010) and albumin (P <0.0063) decreased significantly after surgery in both groups (Table 8).

3.4 Gaseous Exchange

During the preoperative study in the fasted state, VO_2, VCO_2, or RQ were not significantly different between the groups (Table 4).

During the postoperative study, VO_2 (P <0.0058), VCO_2 (P <0.0001) and RQ (P <0.0001) increased significantly in the fed state (Table 7). However, VO_2, VCO_2 and RQ were not significantly different between the groups (Table 7).

Table 1. The amino acid profile of whey protein (InPro 90) compared to the amino acid profile of a standard sample of egg protein. Also, the amino acid intake of whey protein (InPro 90) corresponding to the 4-hour study compared to the Estimated Average Requirement (EAR) of indispensible amino acids for adults 19 years and older.

Amino Acid	Amino acid profile of InPro 90 (mg/g)[a]	Amino acid profile of Egg (mg/g)[b]	Amino acid intake of InPro 90 (mg/(kg.4 h study))[c]	EAR of indispensible amino acids (mg/(kg.d))[b]	Intake as a percentage of EAR[b]
Isoleucine	63	63	30	15	203%
Leucine	123	88	59	34	174%
Valine	56	72	27	19	141%
Lysine	89	70	43	31	139%
Methionine + Cysteine	46 (23 + 23)	56	22 (11 +11)	15	148%
Phenylalanine + Tyrosine	71 (35 + 36)	98	34 (17 +17)	27	126%
Threonine	56	49	27	16	168%
Tryptophan	9	16	4	4	103%
Histidine	22	24	10	11	95%
Arginine	24		12		
Glycine	15		7		
Alanine	54		26		
Aspartic Acid	112		54		
Glutamic Acid	143		69		
Proline	51		25		
Serine	68		33		
Total Essential Amino Acids (mg)	533	536			

[a]InPro 90, Vitalus Nutrition Inc., Abbotsford, BC.

[b]Data from FAO/WHO/UNU. Energy and protein requirements. Technical report series no. 724. Geneva: World Health Organization, 1985.

[c]Amino acid intake corresponding to a consumption rate of 2.9 g/(kg.day).

Figure 1. Overview of the study protocol.

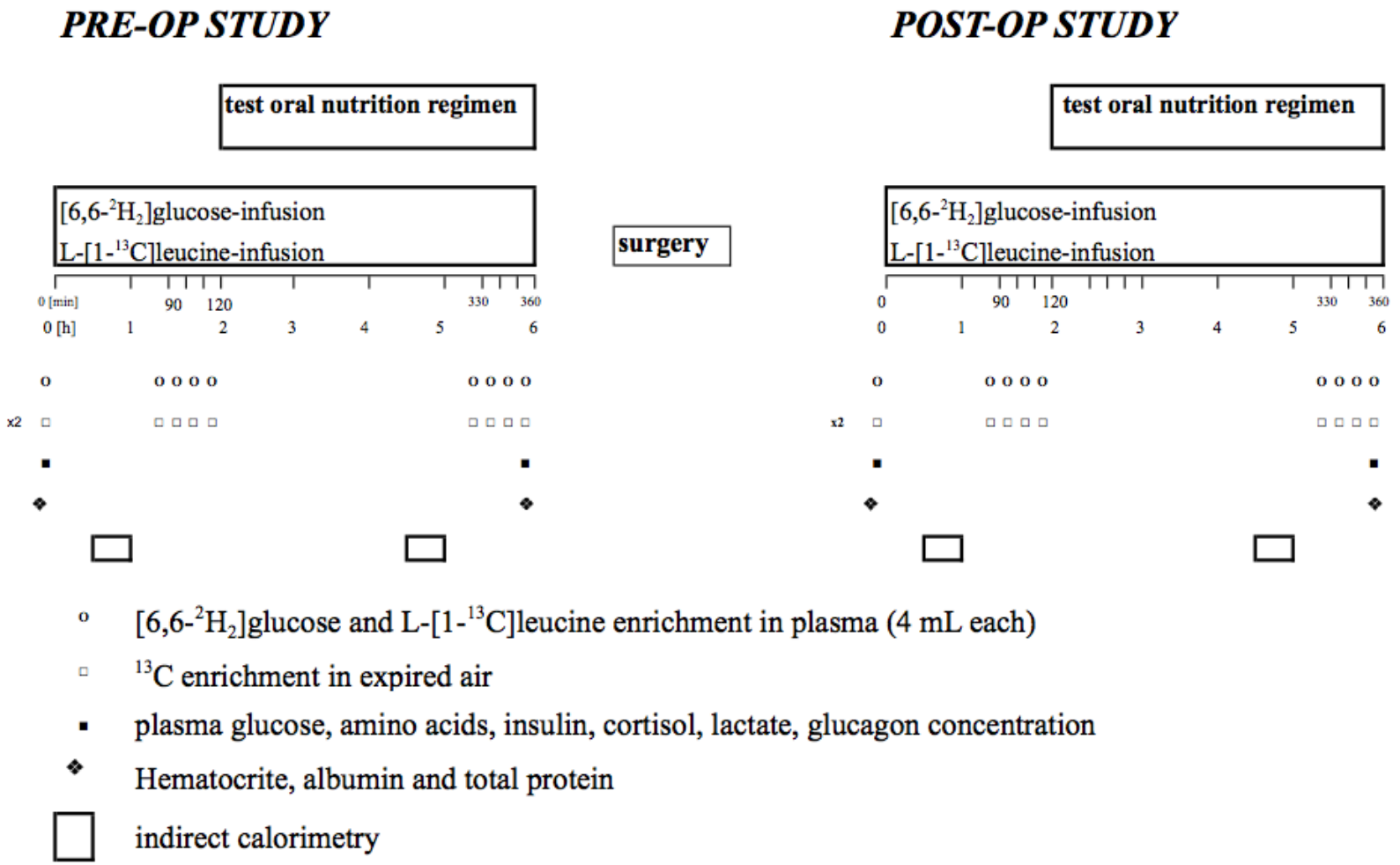


Table 2. Nutrient intakes for the two oral nutrition support regimens prepared from stock solutions of glucose (200 g/L) and whey protein (100 g/L).

	Oral Glucose-based	Oral Whey Protein-based
Preparation		
Glucose Stock[1] (g/L)	200	200
Whey Stock[2] (g/L)	0	100
Crystal Light® (g)	2.7	2.7
Nutrient Composition of Drink		
Glucose (g/mL)	0.2	0.2
Whey (g/mL)	0	0.1
Nutrient Intake		
Glucose (mg/kg.min)[3]	4	4
Protein (mg/kg.min)[4]	0	2
Glucose (g/70 kg.h)	16.8	16.8
Protein (g/70 kg.h)	0	8.4
Volume Intake		
(mL/kg.h)	1.2	2.5
(mL/70 kg.h)	84	177.8

[1]The calculation of glucose content includes dextrose plus lactose.

[2]Pressurized whey solution is 92% protein, 3% lactose (InPro 90, Vitalus Nutrition Inc., Abbotsford, BC).

[3]Oral glucose intake corresponds to approximately 22 μmol/(kg.min)

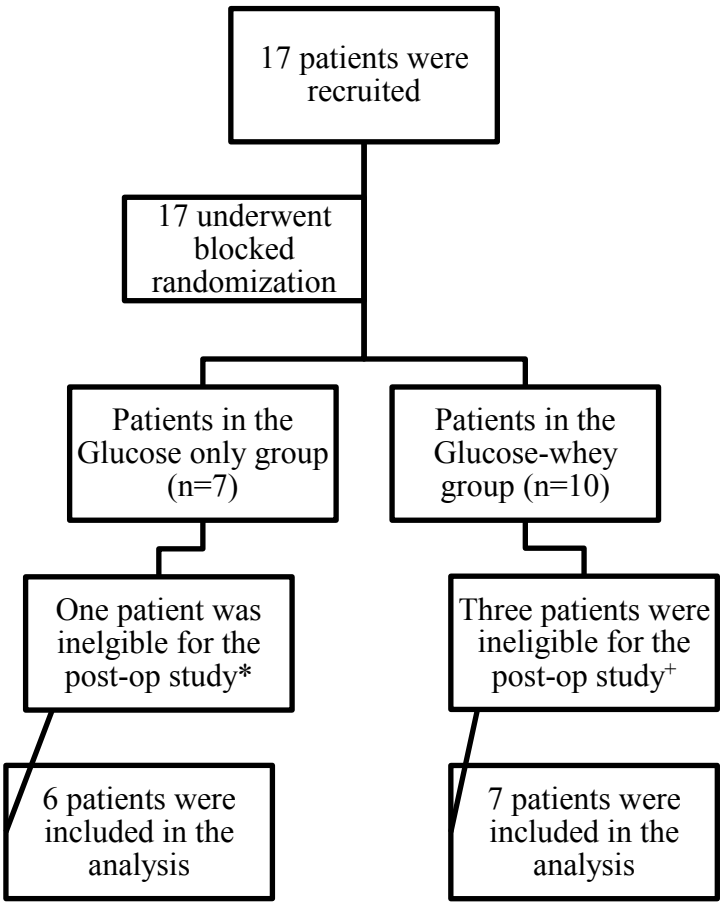
[4]Oral protein intake corresponds to a leucine intake of approximately 2 μmol/(kg.min)

Table 3. Characteristics of the patients.

Variable	Oral Glucose-based	Oral Whey Protein-based
Sex (n, male:female)	4:2	4:3
Age (yr)	67 ± 8	67 ± 12
Weight (kg)	73 ± 13	70 ± 9
BMI (kg/m^2)	25.3 ± 4.5	25.7 ± 2.3
ASA physical status, I/II/III	0/6/0	0/7/0
Type of surgery (n)		
Hemicolectomy/colectomy	4	4
Sigmoid resection	0	2
Anterior resection	2	1
Duration (min)	227 ± 49	221 ± 47

Values are presented as means ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

Figure 2. Flow diagram of a randomized, parallel trial comparing glucose-based oral nutrition to the whey protein-based oral nutrition.



* Patient became ineligible for the postoperative study because the patient was discharged from the hospital on postoperative day 1.

+ Three patients became ineligible for postoperative studies for the following reasons: [1] The patient could not consume the oral nutrition regimen due to severe nausea and vomiting; [2] The patient suffered a myocardial infarction on postoperative day 0; [3] The patient was vomiting on postoperative day 2 and could not consume the oral nutrition regimen.

Figure 3. Preoperative [1-^{13}C]α-KIC enrichment (MPE) of the glucose-based and whey protein-based oral nutrition groups in the fasted and fed states.

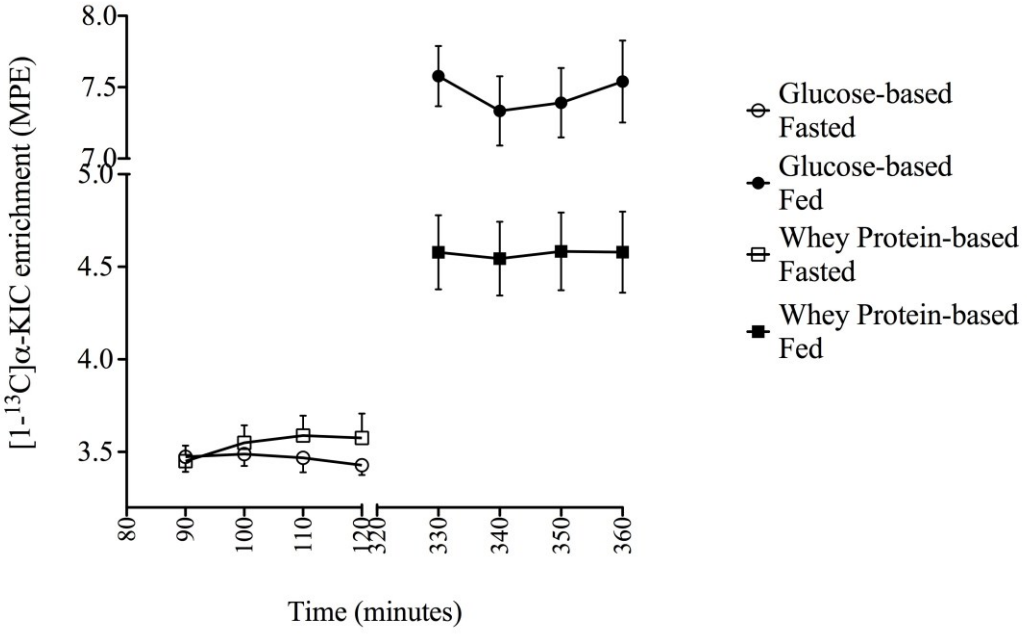


Figure 4. Postoperative [1-^{13}C]α-KIC enrichment (MPE) of the glucose-based and whey protein-based oral nutrition groups in the fasted and fed states.

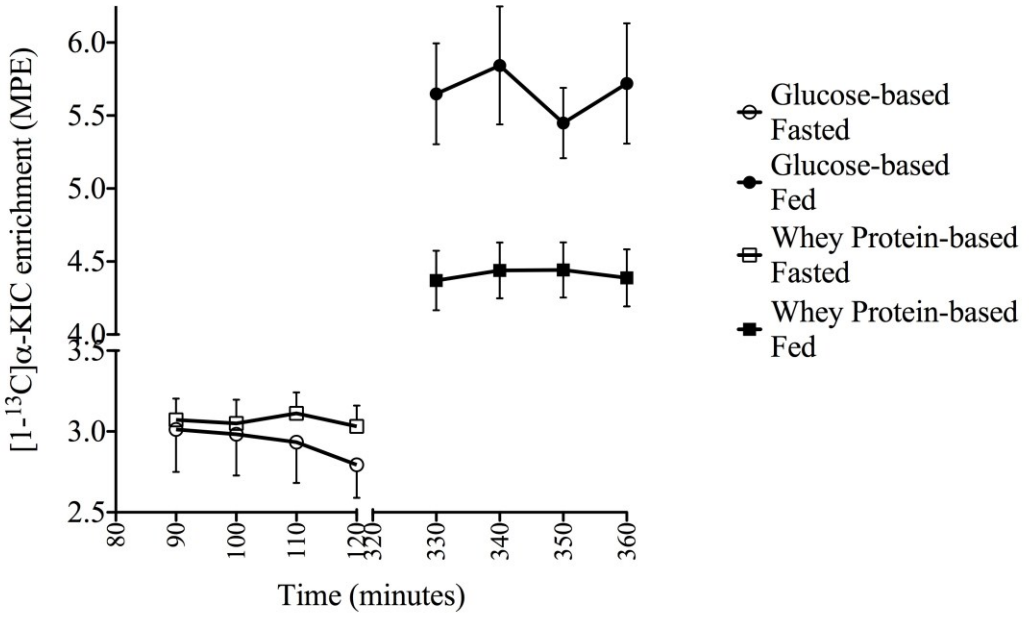


Table 4. Preoperative leucine kinetics, hormones, metabolites and indirect calorimetry of patients receiving either glucose-based or whey protein-based nutrition in the fasted and fed states.

Variable	Glucose-based		Whey Protein-based		*P* Value
	Fasted	Fed	Fasted	Fed	Fasted[a]
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
R_a leucine (μmol/(kg.h))	101 ± 12	87 ± 9	97 ± 7	146 ± 18	0.4563
Net protein breakdown (μmol/(kg.h))	97 ± 12	80 ± 9	93 ± 7	30 ± 18	0.4552
Leucine oxidation (μmol/(kg.h))	18 ± 3	13 ± 2	18 ± 8	49 ± 8	0.9290
Protein synthesis (μmol/(kg.h))	83 ± 10	74 ± 8	79 ± 8	97 ± 17	0.3809
Leucine Balance (μmol/(kg.h))	-18 ± 3	-6 ± 2	-18 ± 8	67 ± 8	0.9290
Whole body protein balance (g/(kg.h))	-0.7 ± 0.1	-0.2 ± 0.1	-0.7 ± 0.3	2.6 ± 0.3	0.9290
Cortisol (nmol/L)	543 ± 78	352 ± 68	456 ± 88	259 ± 46	0.1083
Glucose (mmol/L)	6 ± 1	6 ± 2	6 ± 1	6 ± 1	0.9299
Insulin (pmol/L)	51 ± 21	95 ± 49	61 ± 37	137 ± 70	0.6281
Total Protein (g/L)	62 ± 8	59 ± 2	60 ± 7	57 ± 4	0.6224
Albumin (g/L)	35 ± 8	35 ± 1	37 ± 4	35 ± 1	0.5307
REE (Kcal/day)	1284 ± 204		1212 ± 147		
VO_2 (L/min)	0.190 ± 0.028	0.192 ± 0.035	0.182 ± 0.024	0.201 ± 0.023	0.6039
VCO_2 (L/min)	0.138 ± 0.025	0.150 ± 0.030	0.133 ± 0.020	0.163 ± 0.026	0.6725
RQ	0.73 ± 0.03	0.78 ± 0.04	0.73 ± 0.02	0.81 ± 0.04	0.6867
Oral glucose and protein intake (g)		70 ± 12		(68 ± 9)+(34 ± 4)	
Oral glucose and protein intake (kcal)		282 ± 49		405 ± 53	
Intake as % of daily REE		22% ± 2%		34% ± 3%	

REE: Resting Energy Expenditure; VO_2: oxygen consumption; VCO_2: carbon dioxide production; RQ: Respiratory Quotient

Values are presented as means ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

[a]Probability that the fasted state values were different between the 2 groups.

Table 5. Postoperative leucine kinetics in patients receiving either glucose-based or whey protein-based oral nutrition in the fasted and fed states.

Variable	Glucose-based		Whey Protein-based		*P* Value			
	Fasted	Fed	Fasted	Fed				
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Feeding State[a]	Type of Nutrition[b]	Surgery[c]	Interaction[d]
R_a leucine (μmol/(kg.h))	119 ± 22	116 ± 18	112 ± 12	147 ± 15	0.0005	0.0001	N.S. (0.8498)	N.S. (0.1217)
Net protein breakdown (μmol/(kg.h))	115 ± 22	109 ± 18	109 ± 12	27 ± 19	0.0001	0.0001	N.S. (0.7088)	N.S. (0.0929)
Leucine oxidation (μmol/(kg.h))	21 ± 10	15 ± 6	21 ± 8	46 ± 6	0.0001	0.0001	N.S. (0.2825)	N.S. (0.3488)
Protein synthesis (μmol/(kg.h))	98 ± 16	101 ± 16	91 ± 12	101 ± 12	N.S. (0.1094)	N.S. (0.4827)	N.S. (0.7712)	N.S. (0.1274)
Leucine Balance (μmol/(kg.h))	-21 ± 10	-8 ± 5	-21 ± 8	74 ± 13	0.0001	0.0001	N.S. (0.1699)	N.S. (0.2076)
Whole body protein balance (g/(kg.h))	-0.03 ± 0.02	-0.01 ± 0.01	-0.03 ± 0.01	0.12 ± 0.02	0.0001	0.0001	N.S. (0.1699)	N.S. (0.2076)

Values are presented as means ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

[a]Probability of an effect of the oral nutrition regimens.

[b]Probability of an effect of the type of oral nutrition regimen.

[c]Probability of an effect of surgery.

[d]Probability that the effect of the type of oral nutrition regimen and the effect of surgery are different between the 2 groups.

Table 6. Postoperative hormones and metabolites in patients receiving either glucose-based or whey protein-based oral nutrition in the fasted and fed states.

Variable	Glucose-based		Whey Protein-based		*P* Value			
	Fasted	Fed	Fasted	Fed				
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Feeding State[a]	Type of Nutrition[b]	Surgery[c]	Interaction[d]
Cortisol (nmol/L)	503 ± 92	337 ± 111	293 ± 97	253 ± 78	0.0001	N.S. (0.3392)	0.0153	N.S. (0.2593)
Glucose (mmol/L)	6 ± 1	7 ± 2	5 ± 1	6 ± 2	0.0118	N.S. (0.3248)	N.S. (0.2858)	N.S. (0.8436)
Insulin (pmol/L)	59 ± 31	145 ± 105	31 ± 14	138 ± 72	0.0001	N.S. (0.9496)	N.S. (0.4841)	N.S. (0.9621)
Total Protein (g/L)	50 ± 7	47 ± 6	51 ± 8	45 ± 11	0.0219	N.S. (0.3993)	N.S. (0.5799)	N.S. (0.4233)
Albumin (g/L)	30 ± 5	28 ± 5	30 ± 5	26 ± 5	N.S. (0.0772)	N.S. (0.7956)	N.S. (0.4550)	N.S. (0.9427)

Values are presented as means ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

[a]Probability of an effect of the oral nutrition regimens.

[b]Probability of an effect of the type of oral nutrition regimen.

[c]Probability of an effect of surgery.

[d]Probability that the effect of the type of oral nutrition regimen and the effect of surgery are different between the 2 groups.

Table 7. Postoperative indirect calorimetry in patients receiving either glucose-based or whey protein-based oral nutrition in the fasted and fed states.

Variable	Glucose-based		Whey Protein-based		*P* Value			
	Fasted	Fed	Fasted	Fed				
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Feeding State[a]	Type of Nutrition[b]	Surgery[c]	Interaction[d]
REE (Kcal/day)	1304 ± 251		1277 ± 189					
VO_2 (L/min)	0.192 ± 0.028	0.198 ± 0.028	0.191 ± 0.028	0.196 ± 0.033	0.0058	N.S. (0.8792)	N.S. (0.9691)	N.S. (0.0672)
VCO_2 (L/min)	0.136 ± 0.021	0.151 ± 0.028	0.137 ± 0.018	0.159 ± 0.023	0.0001	N.S. (0.5452)	N.S. (0.9792)	N.S. (0.1526)
RQ	0.71 ± 0.03	0.77 ± 0.06	0.72 ± 0.02	0.81 ± 0.05	0.0001	N.S. (0.0825)	N.S. (0.7584)	N.S. (0.6494)
Oral glucose and protein intake (g)		70 ± 12		(68 ± 9)+(34 ± 4)				
Oral glucose and protein intake (Kcal)		282 ± 49		405 ± 53				
Oral intake as % of daily REE		22% ± 2%		32% ± 3%				

REE: Resting Energy Expenditure; VO_2: oxygen consumption; VCO_2: carbon dioxide production; RQ: Respiratory Quotient

Values are presented as means ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

[a]Probability of an effect of the oral nutrition regimens.

[b]Probability of an effect of the type of oral nutrition regimen.

[c]Probability of an effect of surgery.

[d]Probability that the effect of the type of oral nutrition regimen and the effect of surgery are different between the 2 groups.

Table 8. Comparison of preoperative and postoperative fasted state hormones and metabolites in patients receiving either glucose-based or whey protein-based oral nutrition.

Variable	Glucose-based		Whey Protein-based		*P* Value	
	Pre-op Fasted	Post-op Fasted	Pre-op Fasted	Post-op Fasted		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Surgery[a]	Type of Nutrition[b]
Cortisol (nmol/L)	543 ± 78	503 ± 92	456 ± 88	293 ± 97	0.0069	N.S. (0.1191)
Glucose (mmol/L)	6 ± 1	6 ± 1	6 ± 1	5 ± 1	N.S. (0.3092)	N.S. (0.8806)
Insulin (pmol/L)	51 ± 21	59 ± 31	61 ± 37	31 ± 14	N.S. (0.4640)	N.S. (0.1143)
Total Protein (g/L)	62 ± 8	50 ± 7	60 ± 7	51 ± 8	0.0010	N.S. (0.8655)
Albumin (g/L)	35 ± 8	30 ± 5	37 ± 4	30 ± 5	0.0063	N.S. (0.3659)

Values are presented as mean ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

[a]Probability of an effect of surgery.

[b]Probability of an effect of the type of oral nutrition regimen.

IV. DISCUSSION

4.1 Major Findings

4.1.1. Leucine kinetics

The objective of the present study was to determine whether an oral nutrition support regimen based on pressurized whey protein isolate and glucose improves the postoperative utilization of amino acids. More specifically, this study tested the hypothesis that an oral nutrition support regimen based on pressurized whey protein with glucose, compared to glucose alone, promotes positive protein balance through increased protein synthesis or reduced protein breakdown while maintaining normoglycemia. The results of the present study demonstrated that an oral nutrition regimen based on whey protein and glucose achieved positive protein balance while an oral nutrition regimen based on glucose alone did not. Positive protein balance was established through suppression of protein breakdown, not through stimulation of protein synthesis.

One way to explain the observed changes in protein synthesis and breakdown is to examine the properties of whey protein and the structure of the oral nutrition regimen. Whey protein is digested rapidly and when healthy subjects ingest a single 30 g serving, protein synthesis increases (88). However, when healthy subjects are fed small quantities of whey protein every 20 min for 240 min, whey protein acts as a slow-digesting protein and protein synthesis is unchanged (87). In the present study, subjects were fed a small aliquot of the oral nutrition regimen every 30 min for 240 min and the results agreed with the above-mentioned study. By supplying the whey-based regimen in small amounts, the increase in plasma amino acids may not have been sufficient to stimulate synthesis (114, 115) as plasma amino acids would have to increase at least 100% above baseline to stimulate synthesis (114, 116). Instead of stimulating protein synthesis, the whey-based nutrition regimen likely suppressed protein breakdown through the slow provision of amino acids (114, 117). Small increases in plasma amino acids (20-25% above baseline) inhibit protein breakdown and trigger leucine oxidation but have little effect on protein synthesis (114). However, the

present study did not quantify plasma amino acids so it is not possible to determine the magnitude of change in the fed state.

It is important to note that the quantification of protein breakdown was dependent on leucine intake and flux. The relationship between protein breakdown and leucine intake is such that greater leucine intake results in lower protein breakdown (B = Q – I). In the present study, leucine intake was considerably higher in patients receiving oral whey protein-based nutrition because whey protein is rich in leucine and was fed at a high rate to simulate a meal over four hours. Leucine is one of several amino acids capable of regulating autophagic-lysosomal proteolysis. Although the mechanism has not been fully clarified in mammalian cells, it is believed that leucine may control hepatic autophagic proteolysis by binding a leucine receptor or sensor on the plasma membrane and triggering an intracellular signaling cascade that inhibits autophagic-lysosomal proteolysis (118). Insulin also inhibits autophagic-lysosomal proteolysis albeit through a different intracellular pathway involving mammalian target of rapamyacin (mTOR) (118). Furthermore, leucine stimulates mTOR which may enhance insulin-mediated suppression of autophagic-lysosomal proteolysis (118). Insulin can also independently inhibit proteolysis by regulating insulin degrading enzyme (IDE) and the activity of the energy- and ubiquitin-dependent proteasome pathway (119, 120). IDE is associated with the 26S and 20S proteasomes and the proteolytic activity of the 26S proteasome is greater when associated with IDE (120). The presence of insulin within the cell (121) causes IDE to dissociate from the 26S proteasome and proteolytic activity decreases (100, 120). In the present study, serum insulin increased significantly in response to oral nutrition, helping to suppress proteolysis.

4.1.2. Serum hormones and metabolites

This study demonstrated that fasting serum cortisol decreased after surgery, which was contrary to what was expected. It should be noted that the preoperative cortisol assessment was performed in the week before surgery and it is possible that the patients' anxiety about their upcoming surgery may have affected cortisol secretion. Furthermore, cortisol concentration typically peaks

during surgery and then decreases towards normal on the first postoperative day (122) so the cortisol concentrations observed in the present study were in line with previous research. The data from the present study also determined that serum cortisol dropped significantly after consuming the oral nutrition regimen, independent of the composition of the drink. The observed result could have occurred because the fasted cortisol sample was taken in the early morning when cortisol is higher while the fed sample was taken in the early afternoon, when cortisol is relatively lower (123). Further research is needed to determine whether the drink itself affected serum cortisol.

Postoperative serum albumin values were not affected by the oral nutrition regimens in the present study. Nevertheless, fasting serum albumin dropped significantly following surgery. Hypoalbuminemia is typically observed during after surgery because of the inflammatory and catabolic response to tissue injury (12, 124-126). Furthermore, previous work has shown that plasma albumin drops significantly within 1 h of the induction of anesthesia and remains significantly lower after surgery (127). Other research in surgical patients established that serum albumin was significantly lower 48 h after surgery (128). However, the mechanism by which anesthesia affects serum albumin has not yet been clarified (127) so caution should be used when interpreting this data.

4.1.3. Substrate utilization

In the present study, RQ increased significantly after patients consumed the oral nutrition regimen. However, the change in RQ from the fasted state to the fed state was not different between groups, indicating that diet group did not affect substrate utilization. Nevertheless, as expected, the mean RQ in the fasted state signified fat oxidation. Further, the mean fed state RQ indicated a shift towards carbohydrate oxidation after consuming the oral nutrition regimen. Overall, the results agreed with previous research and confirmed that nutrients from the drink were metabolized.

4.2 Comments About Whey Protein- and Glucose-Based Oral Nutrition

4.2.1. Pressurized whey protein isolate

Pressurized whey protein isolate was used in one of the present oral nutrition regimens because it contains high quality proteins (83) that are generally rapidly digested (87) and highly bioavailable (83) and as such is a desirable component of perioperative nutrition supplementation. Furthermore, high-pressure processed whey protein isolate is less resistant to pepsin hydrolysis, which enhances the digestibility of specific whey proteins compared to native whey protein isolate (95). The enhanced digestibility of pressurized whey protein isolate is associated with improved bioavailability of cysteine and consequently higher concentrations of lymphocyte glutathione (96). Moreover, peptides released during the digestion of whey protein have antioxidant properties (129). These peptides are associated with increased radical scavenging activity (130) and help decrease oxidative stress. Oxidative stress is elevated during the postoperative recovery period, and ROS activate the energy- and ubiquitin-dependent proteasome pathway, which enhances proteolysis (21). Thus, improving antioxidant status through pressurized whey protein supplementation could prevent protein breakdown.

4.2.2. Glucose-based oral nutrition

This study compared a whey protein-based regimen to a glucose-based regimen because glucose-based oral and enteral nutrition support formulas are commonly used during the perioperative period (62, 131-133). In the present study, the two groups exhibited similar changes in blood glucose in response to feeding and both groups remained normoglycemic. However, protein balance was only established in the glucose-whey group. So while it is metabolically beneficial to include protein in an oral nutrition regimen, is glucose also necessary? On one hand, surgery-induced insulin resistance makes postoperative patients prone to hyperglycemia (134). On the other hand, within an enhanced recovery protocol, avoiding preoperative fasting by consuming a carbohydrate-based drink attenuates postoperative insulin resistance (132, 133). So including glucose in a perioperative oral nutrition regimen may improve postoperative glucose

metabolism. In fact, within an enhanced recovery protocol, postoperative, glucose-based enteral feeding (either hypocaloric or complete) does not lead to hyperglycemia (62). In terms of protein metabolism, the glucose content of the whey protein-based regimen likely provoked a greater insulin response than if the drink had contained whey protein alone (87). Thus, insulin-mediated inhibition of proteolysis may not have been as great if the drink had not contained glucose. Glucose also provides energy and may help meet energy needs as part of a complete oral nutrition regimen. So while glucose alone was not capable of achieving positive protein balance, including glucose as part of a protein-based oral nutrition regimen is a safe way to provide energy during the postoperative recovery period.

4.3 Retrospective Comparisons

The oral nutrition regimens in the present study were designed to allow for retrospective comparisons to similar parenteral nutrition regimens (45, 54). Accordingly, the oral nutrition intake rate (mL/(kg.min)) in the present study was the same as the parenteral nutrition infusion rates used previously (45, 54). The present study established that patients receiving glucose-based oral nutrition were not able to achieve positive leucine balance after feeding. This result was similar to what was observed by Carli et al. in patients receiving parenteral glucose alone (54). Notably, leucine balance was comparatively less negative in patients receiving oral glucose [-8 ± 5 μmol/(kg.h) vs. -24 ± 3 μmol/(kg.h); oral nutrition vs. parenteral nutrition] (54). The current study demonstrated that patients receiving the whey protein-based oral nutrition regimen achieved positive leucine balance. Schricker and colleagues observed similar results in patients receiving parenteral amino acids and glucose (45). Notably, protein balance was relatively higher in patients receiving the oral glucose-whey nutrition regimen [74 ± 13 μmol/(kg.h) vs. 7 ± 5 μmol/(kg.h); oral nutrition vs. parenteral nutrition] (45). Other retrospective comparisons have been made regarding the changes in blood glucose in response to receiving oral versus parenteral nutrition. Patients receiving oral nutrition remained normoglycemic while patients receiving comparable

parenteral regimens had relatively higher blood glucose levels (54) or became hyperglycemic (45). Earlier work has shown that blood glucose was consistently and significantly lower within an enteral nutrition regimen compared to a similar parenteral nutrition regimen (59). The above retrospective comparisons demonstrate that substrate utilization is better when patients are given oral nutrition compared to parenteral nutrition. These latter observations support the notion that the metabolic fates of the nutrients depend on the route of administration and the gut should be used when it is available.

V. CONCLUSION

The results of this study demonstrate that an oral nutrition regimen based on pressurized whey protein isolate and glucose promoted positive protein balance through decreased protein breakdown without a concomitant increase in protein synthesis or serum glucose. It is important to note that patients receiving whey protein-based oral nutrition achieved positive protein balance through suppressed protein breakdown and not increased protein synthesis. This finding was especially novel as protein breakdown was suppressed to a far greater extent than anticipated based on previous research in colorectal resection patients receiving hypocaloric PPN based on amino acids and glucose (45) or glucose alone (54). Nevertheless, the results of the present study are supported by previous research, which demonstrated that feeding small portions of whey protein over a few hours suppressed protein breakdown without affecting protein synthesis (87).

5.1 Relevance to the Field of Research

Colorectal cancer is the second leading cause of cancer death in men and women in Canada (135). Treatment of colorectal cancer often requires surgical resection of malignancies. Surgical preparation and associated preoperative fasting can induce an unfavorable metabolic state, which can enhance the catabolic response to surgery (28, 29). The unique nutrient status of colorectal

resection patients highlights the importance of elucidating an optimal nutrition support regimen for these patients. The results of the present study demonstrated that protein catabolism was especially sensitive to nutrition support, and whey protein-based nutrition support in particular. Surgery-induced protein catabolism and subsequent loss of body protein, left unabated, puts patients at greater risk of postoperative morbidity and mortality. Positive whole body protein balance was achieved after feeding in the whey protein-based group. A typical 70 kg patient in this group could acquire 34 g of body protein over four hours. Conversely, a typical 70 kg patient in the glucose-only group could lose about 4 g of body protein in the same four-hour period. Ultimately, patients receiving whey protein-based oral nutrition could avoid complications associated with loss of body protein. Furthermore, since patients in both groups remained normoglycemic, complications caused by postoperative hyperglycemia were also avoided. The use of stable isotope tracer methodology contributed to our understanding of the physiological processes that are altered after surgical trauma, and elicited how these changes were modulated by oral nutrition. This knowledge will provide a rationale for studying future nutrition interventions in the hope that such an intervention could shorten the recovery period after colorectal surgery.

5.2 Limitations

There were a few limitations of this study. The present findings were attained using a relatively high feeding rate over four hours. While this feeding rate was designed to simulate a meal over four hours, it may not be applicable to surgical patients receiving postoperative nutrition supplementation in the hospital. Additionally, the postoperative study data were obtained on the second postoperative day during a six-hour period. The changes detected over this short time period, two days after surgery may not represent the metabolic modifications observed during the entire postoperative recovery period, especially those observed immediately after surgery.

5.3 Suggestions for Future Research

The results of the present study highlight the need for more research in this area. Firstly, future research could attempt to take advantage of the fast-digesting properties of whey protein. For example, giving one bolus-like volume of whey protein followed by smaller amounts over a longer period of time may both stimulate protein synthesis and suppress protein breakdown. Since it is currently not known whether short-term pressurized whey protein supplementation could affect inflammatory cytokines or antioxidant status, future research should assess whether pressurized whey protein supplementation affects inflammatory markers and GSH status during the postoperative recovery period. The present study did not examine the effects of oral nutrition based on native whey protein. A recent study in piglets with dextran sulfate-induced colitis determined that pressurized whey protein isolate had greater anabolic, anti-inflammatory and antioxidant effects compared to native whey protein isolate (136). Based on these results, it would be unethical to use native whey protein-based oral nutrition support during future research in surgical patients.

Future research should also focus on incorporating this oral nutrition regimen within a multi-modal, enhanced recovery protocol that aims to minimize perioperative fasting. For example, the oral nutrition regimen used in the present study could be given to patients prior to surgery to help minimize or avoid the metabolic modifications initiated by preoperative fasting. Since the present study demonstrated that the oral nutrition regimen was well tolerated on the second postoperative day, future studies should use the drink to encourage early postoperative oral feeding.

The present study was designed to allow for retrospective comparisons to previous parenteral nutrition regimens and the results indicated that protein balance is greater in patients receiving oral nutrition. However, it is not possible to formulate any conclusions without a direct comparison between oral and parenteral nutrition. Future research should compare similar oral and parenteral nutrition regimens in colorectal resection patients to demonstrate which method of nutrient delivery is best.

Finally, previous work has shown that perioperative epidural analgesia together with nutrition support improves postoperative recovery of functional exercise capacity (46). A large-scale clinical trial is necessary to correlate protein metabolism data obtained from stable isotope studies with clinical outcomes such as incidence of infectious and non-infectious complications, return of bowel function, mobility, and length of hospital stay. This type of clinical trial would indicate whether the positive results observed in this metabolic study translate to whole-body outcomes.

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APPENDIX A: Informed Consent Form- English Version

Informed Consent Form

Whey protein-based oral nutrition support to improve protein economy in surgical patients

Principal Investigator: Dr Franco Carli, Department of Anesthesia

Co-investigators:
Dr Linda Wykes, School of Human Nutrition and Dietetics
Dr Stan Kubow, School of Human Nutrition and Dietetics
Jennifer Ball, School of Human Nutrition and Dietetics
Dr Patrick Charlebois, Department of Surgery

Participating Institutions: McGill University Health Centre (MUHC)

Introduction

You are being invited to participate in this research study because you will soon undergo bowel surgery and you have agreed with your treating physician that you will receive epidural analgesia for postoperative pain relief. Epidural analgesia is achieved by inserting a little plastic tube in a space surrounding the spinal cord under the skin of the back and injecting a medication which freezes the nerves of the abdomen and relieves pain.

Before you decide to participate, it is important that you understand the contents of this consent form, the risks and benefits to make an informed decision and ask any questions if there is anything that you do not understand. Please read this entire consent form and take your time to make a decision. If you decide to participate in this study, you will be asked to sign this informed consent form.

Study Description

Losses of muscle and body proteins are the principal cause of fatigue and decreased muscle strength after bowel surgery. There is evidence that body protein loss can be influenced by providing nutrients (food) after the operation. We have previously shown that patients who are fed with glucose and/or amino acid solutions by vein over a short time period (3 hours) lose less body protein than patients not given any nutrients.

Purpose of the Study

The purpose of this study is to establish whether consuming a drink containing either glucose (sugar) and/or whey protein improves how your body uses sugar (glucose) and protein (and its building blocks amino acids) to decrease your body's loss of protein after surgery

Study procedures

This study has two parts: (1) an assessment that will take place before your surgery (preoperative) and (2) an assessment that will take place after your surgery (postoperative). The preoperative and postoperative measurements are identical. What is required of you for each measurement depends on which study group you are randomly assigned to. The three study groups are described below.

If you agree to participate in this study, and you have agreed to receive epidural for surgery, you will be randomly assigned (for example, by pulling numbers out of a hat) to one of the following three groups:

Group 1. Both before surgery and on the second day after surgery, you will be asked to consume a drink made of glucose (sugar). You will be asked to sip the drink slowly over the course of four (4) hours. Each hour you will consume approximately 84 mL of the drink.

Group 2. Both before surgery and on the second day after surgery, you will be asked to consume a drink made of whey protein and glucose (sugar). You will be asked to sip the drink slowly over the course of four (4) hours. Each hour you will consume approximately 175 mL of the drink.

Group 3. Both before surgery and on the second day after surgery, you will be asked to consume a drink made of whey protein. You will be asked to sip the drink slowly over the course of four (4) hours. Each hour you will consume approximately 91 mL of the drink.

Whey protein is a complete protein that is made from cow's milk. Whey protein is commonly used as a nutrition supplement following exercise and has been used in other patient populations to provide easily digestible high quality protein. Our whey protein, which is commercially available from Vitalus, has been specially processed to make it even more easily digestible.

No matter which group you are randomly assigned to, you will be allowed to choose the flavor of your drink from a selection of four flavors. The four flavors are: lemon lime, strawberry-orange-banana, pink lemonade and tangerine-grapefruit. The study co-coordinator, Jennifer Ball, will oversee the administration of the drinks.

The preoperative assessment will take place in a room in the Department of Anesthesia approximately one week before your scheduled surgery on the same day as your preoperative consultation with the surgeon. You are asked not to

consume breakfast on this day. The postoperative assessment will take place in a bed in the postoperative recovery unit. Both the preoperative and postoperative assessments are identical and will take six (6) hours.

Each study involves infusing a stable isotope tracer (a very small amount of glucose or an amino acid labelled with a stable non- radioactive tag) into a vein in your hand for six (6) hours. The labeled substance is commercially available from CIL, naturally occurring and commonly used in patients. This technique will determine whether oral feeding with glucose and/or protein assists the body to effectively use glucose and amino acids to minimize the loss of body proteins. Blood samples (a total of 80 mL or less than one third of a cup) will be drawn at the beginning of the study and then again after 90, 100, 110, 120, 330, 340, 350 and 360 minutes of the tracer infusion from a second plastic tube that will be inserted in one of the veins of your arm. Breath samples will also be collected at the same time. You will be asked to exhale into a bag for about 1 minute to collect each breath sample. After the first 2 hours of the study, you will be asked to sip your drink for 4 hours. Dr. Carli will oversee the administration of the labeled substance as well as the collection of blood samples.

The study will not influence your standard of care for surgery. Following surgery, you will be transferred to the recovery room, where the study doctor and nurse will monitor you until your cardio-respiratory system and sufficient pain control are stabilized. You will then be transferred to the hospital ward.

A total of thirty (30) patients undergoing elective colorectal surgery will be enrolled in this study at the Montreal General Hospital for two study periods of six hours. The overall research study will be completed within twelve (12) months.

Possible Risks and Discomforts

Blood Test: Blood sampling involves minimal risk and/or discomfort that is associated with bruising, possible fainting, infection or redness where the skin is punctured.

Local Anesthetic (bupivacaine) or freezing medication to be used through epidural catheter: The administration of local anesthetic may be associated with temporary heaviness of your legs.

Infusing the Stable Isotope Tracer: infusing the tracer involves minimal risk and/or discomfort that is associated with bruising, infection or redness where the skin is punctured to insert the catheter in your arm. The amount of tracer infused is very small and is not known to be associated with any risks.

Contraindications

If you have any of the following conditions, you will not be able to participate in this study:

- Severe heart, liver or kidney failure
- Endocrine disorders such as diabetes, hyper- or hypothyroidism
- Active inflammatory bowel disease
- Musculoskeletal or neuromuscular disease
- Anemia (low levels of hemoglobin or red blood cells in the blood) and low levels of albumin (a type of protein in blood plasma)
- Previous spinal surgery. This will limit the use of epidural analgesia during this surgery
- Use of steroids

Pregnancy risks

The risks associated with the stable isotopes are not fully known, therefore, if you are pregnant you are not eligible to participate in this study.

Possible Benefits

You should not expect any direct benefit from participating in this study. However, the information from the study will help further our knowledge of this condition, and potentially help to improve future patient care.

Alternative Treatment

If you do not take part in this study, you will be offered the standard of care. This can be discussed with your physician.

Costs and Compensation for Participation

You will not be paid to participate in this study. You will be compensated for parking fees associated with any additional visits to the hospital.

Confidentiality

All information obtained during this study is strictly confidential and kept locked in a filing cabinet in the investigator's office. The results from this study may be published, however, your identity will not be revealed in any publication. In order to verify the research study data, or compliance with institutional guidelines, research records may be reviewed by one of the McGill University Health Centre Research Ethics Boards.

By signing this consent form, you give us permission to release information regarding your participation in this study to your treating physician. Your

confidentiality will be protected in accordance with applicable laws and regulations. You will be provided with a signed copy of this consent form and a copy will be placed in your medical record

Voluntary Participation and /or Withdrawal

Your participation in this study is strictly voluntary. You may refuse to participate or you may discontinue your participation at any time without explanation, and without penalty or loss of benefits to which you are otherwise entitled. If you decide not to participate, or if you discontinue your participation, appropriate alternative therapies will be made available, and you will suffer no prejudice regarding your medical care. You will be informed of any new findings that may affect your willingness to continue your participation.

The study doctor may end your participation if you experience excessive side effects or deterioration in your health. In addition, the Royal Victoria Hospital Research Ethics Board may terminate the study.

Questions and Contact Information

If you have any questions regarding the study, you should contact Dr. Franco Carli at (514) 934-1934 local 43261.

If you have any questions regarding your rights as a study participant, you should contact the hospital Ombudsman at (514) 934-1934 local 48306.

Declaration of Consent

I have read the contents of this consent form, and I agree to participate in this research study. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. By signing this consent form, I am not giving up any of my legal rights.

Participant

_____	_____	_____
Name (print)	Signature	Date

Member of research team conducting the informed consent discussion

_____	_____	_____
Name (print)	Signature	Date

APPENDIX B: Informed Consent Form- French Version

Formulaire de consentement

SOUTIEN NUTRITIONNEL ORAL À BASE D'UNE PROTÉINE DU PETIT-LAIT AFIN D'AMÉLIORER LE NIVEAU DE PROTÉINES DES PATIENTS AYANT SUBI UNE INTERVENTION CHIRURGICALE

Chercheur principal : D^r^ Franco Carli, Service d'anesthésie

Collaborateurs :
D^re^ Linda Wykes, École de diététique et de nutrition humaine
D^r^ Stan Kubow, École de diététique et de nutrition humaine
Jennifer Ball, École de diététique et de nutrition humaine
D^r^ Patrick Charlebois, Service de chirurgie

Établissement participant : Centre universitaire de santé McGill (CUSM)

Introduction

On vous propose de participer à cette étude parce que vous subirez bientôt une chirurgie intestinale et que vous avez décidé avec votre médecin traitant de recevoir une analgésie par voie péridurale afin de soulager la douleur postopératoire. L'analgésie par voie péridurale consiste à insérer un petit tube de plastique en-dessous de la peau de votre dos dans un espace entourant la moelle épinière et par lequel on administre un médicament qui sert à « geler » les nerfs de l'abdomen afin de soulager la douleur.

Avant de décider de participer à cette étude, il est important que vous compreniez le contenu de ce formulaire de consentement ainsi que les risques et les bienfaits de l'étude, afin de prendre une décision éclairée et de poser toutes les questions nécessaires à cette fin. Veuillez lire ce formulaire de consentement au complet et prendre votre temps avant de prendre votre décision. Si vous décidez de participer à l'étude, on vous demandera de signer ce formulaire de consentement.

Description de l'étude

La perte de masse musculaire et de protéines organiques sont les causes principales de la fatigue et de la diminution de la force musculaire à la suite d'une chirurgie intestinale. Il a été démontré qu'on peut influencer cette perte de protéines au moyen de l'administration de nutriments (aliments) après l'intervention chirurgicale. Nous avons déjà démontré que les patients qui reçoivent par voie intraveineuse des solutés de glucose ou d'acides aminés, ou des deux, durant une courte période de temps (trois (3) heures) perdent moins de protéines organiques que les patients qui n'en ont pas reçus.

But de l'étude

Cette étude a pour but d'évaluer si le fait d'absorber un liquide contenant du glucose (sucre) ou une protéine du petit-lait, ou les deux, améliore la façon dont votre corps utilise le sucre (glucose) et la protéine (et ses composantes d'acides aminés de base) afin de diminuer la perte de protéines organiques après l'intervention chirurgicale.

DEROULEMENT DE L'ETUDE

Cette étude comporte deux volets : 1) une évaluation qui sera effectuée avant votre intervention chirurgicale (préopératoire); et 2) une évaluation qui sera effectuée après votre intervention chirurgicale (postopératoire). Les tests préopératoire et postopératoire sont identiques. Ce que l'on vous demandera de faire aux fins de chaque test dépendra du groupe d'étude qui vous sera assigné au hasard. Les trois groupes d'étude sont décrits ci-dessous.

Si vous acceptez de participer à cette étude et de recevoir une anesthésie péridurale aux fins de l'intervention chirurgicale, on vous affectera au hasard (p. ex., en tirant des numéros d'un chapeau) à l'un des trois groupes suivants :

Groupe 1 : À la fois avant l'intervention chirurgicale et le deuxième jour après l'intervention chirurgicale, on vous demandera de boire un liquide contenant du glucose (sucre), lentement et à petites gorgées, au cours d'une période de quatre (4) heures. Chaque heure, vous boirez environ 84 ml du liquide.

Groupe 2 : À la fois avant l'intervention chirurgicale et le deuxième jour après l'intervention chirurgicale, on vous demandera de boire un liquide contenant une protéine du petit-lait et du glucose (sucre), lentement et à petites gorgées, au cours d'une période de quatre (4) heures. Chaque heure, vous boirez environ 175 ml du liquide.

Groupe 3 : À la fois avant l'intervention chirurgicale et le deuxième jour après l'intervention chirurgicale, on vous demandera de boire un liquide contenant une protéine du petit-lait, lentement et à petites gorgées, au cours d'une période de quatre (4) heures. Chaque heure, vous boirez environ 91 ml du liquide.

La protéine du petit-lait est une protéine complète fabriquée à partir du lait de vache. La protéine du petit-lait est employée habituellement comme additif nutritionnel après l'exercice et a été utilisée auprès d'autres types de patients afin de leur fournir une protéine de grande qualité facile à digérer. La protéine du petit-lait utilisée, qui est offerte sur le marché par Vitalus, a été traitée spécialement de façon à la rendre encore plus facile à digérer.

Peu importe le groupe qui vous sera assigné au hasard, vous aurez le choix de la saveur du liquide parmi quatre saveurs : citron-lime, fraise-orange-banane, limonade rose et tangerine-pamplemousse. La coordonnatrice de l'étude, Jennifer Ball, surveillera l'administration des liquides.

L'évaluation préopératoire aura lieu dans une salle du Service de l'anesthésie environ une semaine avant la date prévue de votre intervention chirurgicale et le même jour que votre consultation préopératoire avec le chirurgien. On vous demande de ne pas prendre de petit-déjeuner ce jour-là. L'évaluation postopératoire aura lieu dans un lit de la salle de réveil postopératoire. Les évaluations préopératoire et postopératoire sont identiques et prendront six (6) heures.

Chaque étude est effectuée au moyen d'une perfusion d'un traceur d'isotopes stables (une très petite quantité de glucose ou un acide aminé portant une étiquette indiquant qu'il est stable (non radioactif)), dans une veine de la main pendant six (6) heures. La substance ainsi étiquetée est offerte sur le marché par CIL, se présente naturellement et est couramment utilisée par les patients. Cette technique déterminera si l'administration orale de glucose ou de la protéine, ou des deux, aide l'organisme à utiliser efficacement le glucose et les acides aminés afin de réduire au minimum la perte de protéines organiques. Des échantillons sanguins (une quantité totale de sang de 80 ml ou moins d'un tiers de tasse) seront prélevés au début de l'étude et par la suite, après 90, 100, 110, 120, 330, 340, 350 et 360 minutes de la perfusion du traceur, au moyen d'un second tube de plastique qui sera inséré dans l'une des veines de votre bras. Des échantillons d'haleine seront également prélevés en même temps. On vous demandera d'expirer dans un sac pendant environ une (1) minute afin de recueillir chaque échantillon d'haleine.

Après les deux (2) premières heures de l'étude, on vous demandera de boire votre liquide à petites gorgées pendant quatre (4) heures. D[r] Carli surveillera l'administration de la substance étiquetée ainsi que la cueillette des échantillons sanguins.

L'étude n'influencera pas les soins chirurgicaux réguliers qui vous seront prodigués. Après l'intervention chirurgicale, on vous transférera à la salle de réveil où le médecin responsable de l'étude et une infirmière vous surveilleront jusqu'à ce que votre système cardiorespiratoire et la maîtrise de votre douleur soient stables. Vous serez ensuite transféré à l'étage.

Un nombre total de trente (30) patients devant subir une intervention chirurgicale colorectale élective participeront à cette étude à l'Hôpital général de Montréal pendant deux périodes de six (6) heures. L'étude dans son ensemble sera terminée dans un délai de douze (12) mois.

Risques et inconforts possibles

Prélèvements sanguins : Les prélèvements sanguins comportent des risques et (ou) des inconforts minimes qui sont associés à des ecchymoses, à la possibilité de pertes de conscience et à de l'infection ou de la rougeur au site de ponction.

Agent anesthésique local (bupivacaïne) ou médicament pour « geler » administré à l'aide d'un cathéter péridural : l'administration d'agents anesthésiques locaux peut être associée à une sensation temporaire de lourdeur dans les jambes.

Perfusion d'un traceur d'isotopes stables : La perfusion d'un traceur d'isotopes comporte des risques ou des inconforts minimes qui sont associés à des ecchymoses et à de l'infection ou de la rougeur au site de ponction à la suite de l'insertion du cathéter dans votre bras. La quantité de traceur faisant l'objet de la perfusion est très petite, et aucun risque connu ne lui est associé.

Contre-indications

Vous ne pouvez participer à cette étude si vous avez l'un des problèmes de santé suivants :

- Grave insuffisance cardiaque, rénale ou hépatique (du foie);
- Trouble endocrinien comme le diabète, l'hyperthyroïdisme ou l'hypothyroïdisme;
- Maladie intestinale inflammatoire active;
- Maladie musculosquelettique ou neuromusculaire;
- Anémie (bas niveaux d'hémoglobine ou de globules rouges dans le sang) et bas niveaux d'albumine (un type de protéine dans le plasma sanguin);
- Intervention chirurgicale de la colonne vertébrale antérieure : cela limiterait l'utilisation d'une analgésie par voie péridurale pendant l'intervention chirurgicale envisagée;
- L'utilisation de stéroïdes.

Risques associés à une grossesse

Les risques associés aux isotopes stables n'étant pas entièrement connus, vous n'êtes donc pas admissible à participer à cette étude si vous êtes enceinte.

Bienfaits potentiels

Vous ne devez pas vous attendre à retirer des bienfaits directs à la suite de votre participation à cette étude. Cependant, les informations recueillies dans le cadre de l'étude serviront à améliorer nos connaissances sur cet état et potentiellement, les soins prodigués aux patients dans l'avenir.

Options thérapeutiques

Si vous décidez de ne pas prendre part à cette étude, vous recevrez des soins de santé normaux. Vous pouvez en discuter avec votre médecin.

Coûts et rémunération associés à votre participation

Vous ne recevrez aucune rémunération pour votre participation à cette étude. Toutefois, les frais de stationnement associés à des visites supplémentaires à l'hôpital vous seront remboursés.

Confidentialité

Toutes les informations recueillies dans le cadre de cette étude sont strictement confidentielles et seront gardées sous clé dans un classeur du bureau du chercheur. Les résultats de l'étude peuvent être annoncés, mais votre identité ne sera pas dévoilée lors de leur annonce. Afin de vérifier certaines données associées à cette étude ou la conformité avec les directives de l'établissement, les dossiers de cette recherche peuvent être passés en revue par un membre de l'un des comités d'éthique de la recherche du Centre universitaire de santé McGill.

En signant ce formulaire de consentement, vous nous donnez la permission de divulguer des informations concernant votre participation à cette étude à votre médecin traitant. Vos renseignements confidentiels seront protégés conformément aux lois et aux règlements applicables. On vous remettra un exemplaire du formulaire de consentement signé et un autre sera inséré dans votre dossier médical.

Participation volontaire et (ou) retrait

Votre participation à cette étude est strictement volontaire. Vous pouvez refuser d'y participer ou vous en retirer en tout temps sans explication, et sans pénalité ou perte d'avantages auxquels vous avez droit de toute autre manière. Si vous décidez de ne pas y participer ou de vous retirer, on vous offrira des options thérapeutiques, et vos soins médicaux ne seront compromis d'aucune manière. Vous serez avisé de tout nouveau développement pouvant influer sur votre décision de continuer à participer à cette étude.

Le médecin responsable peut mettre fin à votre participation à l'étude si vous éprouvez trop d'effets secondaires indésirables ou si votre état de santé se détériore. De plus, le Comité d'éthique de la recherche de l'Hôpital Royal Victoria peut mettre fin à cette étude.

Questions et personne-ressource

Pour toute question au sujet de cette étude, vous pouvez communiquer avec D^{r} Franco Carli, en composant le 514-934-1934, poste 43261.

Si vous avez des questions concernant vos droits comme sujet d'étude, veuillez communiquer avec l'ombudsman de l'hôpital, en composant le 514-934-1934, poste 48306.

Consentement

J’ai lu le contenu de ce formulaire de consentement et j’accepte de prendre part à cette étude. J’ai eu l’occasion de poser des questions à ce sujet et je suis satisfait des réponses que j’ai reçues. J’ai eu suffisamment le temps de réfléchir aux informations données ci-dessus et de demander conseil au besoin. En apposant ma signature sur ce formulaire de consentement, je ne renonce à aucun de mes droits légaux.

Participant

_____	_____	_____
Nom (en caractères d’imprimerie)	Signature	Date

Membre de l’équipe de recherche en charge des discussions sur le consentement éclairé

_____	_____	_____
Nom (en caractères d’imprimerie)	Signature	Date

APPENDIX C: Postoperative leucine kinetics in patients receiving either glucose-based or whey protein-based in the fasted and fed states

Variable	Glucose-based		Whey Protein-based		*P* Value		
	Fasted	Fed	Fasted	Fed			
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Feeding State[a]	Type of Nutrition[b]	Interaction[c]
R_a leucine (μmol/(kg.h))	119 ± 22	116 ± 18	112 ± 12	147 ± 15	0.0165	N.S. (0.0897)	0.0121
Net protein breakdown (μmol/(kg.h))	115 ± 22	109 ± 18	109 ± 12	27 ± 19	<0.0001	<0.0001	<0.0001
Leucine oxidation (μmol/(kg.h))	21 ± 10	15 ± 6	21 ± 8	46 ± 6	0.0011	<0.0001	<0.0001
Protein synthesis (μmol/(kg.h))	98 ± 16	101 ± 16	91 ± 12	101 ± 12	N.S. (0.2495)	N.S. (0.5534)	N.S. (0.5589)
Leucine Balance (μmol/(kg.h))	-21 ± 10	-8 ± 5	-21 ± 8	74 ± 13	<0.0001	<0.0001	<0.0001
Whole body protein balance (g/(kg.h))	-0.03 ± 0.02	-0.01 ± 0.01	-0.03 ± 0.01	0.12 ± 0.02	<0.0001	<0.0001	<0.0001

Values are presented as means ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

[a]Probability of an effect of the oral nutrition regimens.

[b]Probability of an effect of the type of oral nutrition regimen.

[c]Probability that the effects of oral nutrition is different between the 2 groups.

An ANOVA for repeated measures was conducted to determine the main effect of feeding state, the main effect of type of nutrition and the interaction. This analysis confirmed the main effect of feeding on leucine R_a (P <0.0165), net protein breakdown (P <0.0001), leucine oxidation (P <0.0011), leucine balance (P <0.0001) and whole body protein balance (P <0.0001) (Appendix C). Feeding state did not affect protein synthesis. There was a main effect of type of nutrition on net protein breakdown (P <0.0001), leucine oxidation (P <0.0001), leucine balance (P <0.0001) and net protein breakdown (P <0.0001) (Appendix C). However, the type of nutrition did not affect leucine R_a and protein synthesis. There was a significant interaction between feeding state and type of nutrition in terms of leucine R_a (P <0.0121), net protein breakdown (P <0.0001), leucine oxidation (P <0.0001), leucine balance (P <0.0001) and net protein breakdown (P <0.0001).

This analysis did not drastically change the overall interpretation of the main effects of feeding state and type of nutrition on leucine kinetics. However, this analysis revealed that there was a significant interaction between feeding state and type of nutrition in a majority of the measures of leucine kinetics. This means that the type of nutrition modified the effect of feeding state on leucine kinetics. More specifically, the changes in leucine R_a and leucine oxidation in response to feeding were greater in the whey protein-based group. Furthermore, the decrease in net protein breakdown, leucine balance and whole body protein balance were greater in the whey protein-based group.

APPENDIX D: Postoperative hormones and metabolites in patients receiving either glucose-based or whey protein-based oral nutrition in the fasted and fed states.

Variable	Glucose-based		Whey Protein-based		*P* Value		
	Fasted	Fed	Fasted	Fed			
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Feeding State[a]	Type of Nutrition[b]	Interaction[c]
Cortisol (nmol/L)	503 ± 92	337 ± 111	293 ± 97	253 ± 78	0.0102	0.0346	N.S. (0.2332)
Glucose (mmol/L)	6 ± 1	7 ± 2	5 ± 1	6 ± 2	0.0419	N.S.(0.3712)	N.S.(0.4855)
Insulin (pmol/L)	59 ± 31	145 ± 105	31 ± 14	138 ± 72	0.0034	N.S.(0.5750)	N.S.(0.7527)
Total Protein (g/L)	50 ± 7	47 ± 6	51 ± 8	45 ± 11	N.S.(0.0804)	N.S.(0.6306)	N.S.(0.3817)
Albumin (g/L)	30 ± 5	28 ± 5	30 ± 5	26 ± 5	N.S.(0.0543)	N.S.(0.8593)	N.S.(0.4408)

Values are presented as means ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

[a]Probability of an effect of the oral nutrition regimens.

[b]Probability of an effect of the type of oral nutrition regimen.

[c]Probability that the effects of oral nutrition is different between the 2 groups.

An ANOVA for repeated measures was conducted to determine the main effect of feeding state, the main effect of type of nutrition and the interaction. The analysis confirmed the main effect of feeding state on the concentration of cortisol (P <0.0102), glucose (P <0.0419) and insulin (P <0.0034) (Appendix D). This analysis also revealed that there was a main effect of type of nutrition on cortisol concentration (P <0.0346) and that there was no interaction between feeding state and type of nutrition (Appendix D). This analysis did not drastically change the overall interpretation of the effects of feeding state and type of nutrition on postoperative hormone and metabolite concentrations.

APPENDIX E: Postoperative resting energy expenditure and substrate utilization in patients receiving either glucose-based or whey protein-based oral nutrition in the fasted and fed states.

	Glucose-based		Whey Protein-based		*P* Value		
Variable	**Fasted**	**Fed**	**Fasted**	**Fed**			
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Feeding State[a]**	**Type of Nutrition[b]**	**Interaction[c]**
REE (Kcal/day)	1304 ± 251		1277 ± 189				
VO_2 (L/min)	0.192 ± 0.028	0.198 ± 0.028	0.191 ± 0.028	0.196 ± 0.033	N.S.(0.1045)	N.S.(0.8640)	N.S.(0.4458)
VCO_2 (L/min)	0.136 ± 0.021	0.151 ± 0.028	0.137 ± 0.018	0.159 ± 0.023	0.0037	N.S.(0.5561)	N.S.(0.9650)
RQ	0.71 ± 0.03	0.77 ± 0.06	0.72 ± 0.02	0.81 ± 0.05	0.0014	N.S.(0.1874)	N.S.(0.2332)
Oral glucose and protein intake (g)		70 ± 12		(68 ± 9)+(34 ± 4)			
Oral glucose and protein intake (Kcal)		282 ± 49		405 ± 53			
Oral intake as % of daily REE		22% ± 2%		32% ± 3%			

REE: Resting Energy Expenditure; VO_2: oxygen consumption; VCO_2: carbon dioxide production; RQ: Respiratory Quotient

Values are presented as means ± SD (Glucose-based: n=6; Whey Protein-based: n=7)

[a]Probability of an effect of the oral nutrition regimens.

[b]Probability of an effect of the type of oral nutrition regimen.

[c]Probability that the effects of oral nutrition is different between the 2 groups.

An ANOVA for repeated measures was conducted to determine the main effect of feeding state, the main effect of type of nutrition and the interaction. The analysis confirmed the main effect of feeding state on VCO_2 (P <0.0037) and RQ (P <0.0014) (Appendix E). This analysis did not drastically change the overall interpretation of the effects of feeding state and type of nutrition on postoperative resting energy expenditure and substrate utilization.