Intestinal adaptation after massive intestinal resection

A R Weale, A G Edwards, M Bailey, P A Lear

Patients with short bowel syndrome require long term parenteral nutrition support. However, after massive intestinal resection the intestine undergoes adaptation and nutritional autonomy may be obtained. Given that the complications of parenteral nutrition may be life threatening or result in treatment failure and the need for intestinal transplantation, a more attractive option is to wean patients off nutrition support by optimising the adaptive process. The article examines the evidence that after extensive small bowel resection adaptation occurs in humans and focuses on the factors that influence adaptation and the strategies that have been used to optimise this process. The review is based on an English language Medline search with secondary references obtained from key articles. There is evidence that adaptation occurs in humans. Adaptation is a complex process that results in response to nutrient and non-nutrient stimuli. Successful and reproducible strategies to improve adaptation remain elusive despite an abundance of experimental data. Nevertheless given the low patient survival and quality of life associated with other treatments for irreversible intestinal failure it is imperative that clinical research continues into the optimisation of the adaptation.

A more attractive option is to wean patients off parenteral support. After extensive gut resections it has been observed that the need for permanent support is difficult to predict. Such patients requiring home parenteral therapy continue to take fluids and nutrition orally. Over time, the intestine seems to adapt to the extent that some patients with short bowel lengths can autonomously maintain acceptable fluid, electrolyte, and nutritional balances.

The aim of this article is to evaluate the direct and indirect evidence that adaptation occurs in humans after extensive small bowel resection, review the factors that influence adaptation, and assess the strategies that have been used in attempts to optimise this process.

EXPERIMENTAL EVIDENCE FOR ADAPTATION (SEE BOX)

In rodent systems, animals subjected to extensive (>70%) intestinal resection undergo a pattern of well described morphological and functional changes. The remaining intestine changes macroscopically with dilatation, thickening, and an increase in length. There is an increase in villus height and diameter, and an elongation of the crypts. An increase in epithelial cellular proliferation, coupled with a decrease in apoptosis, produces increases in intestinal RNA, DNA, and protein content. The changes are increased with more extensive resections and the most pronounced changes occur in the ileum.

Functionally there is an increase in absorption per unit length of carbohydrates, proteins, water,...
and electrolytes. Indeed within six hours there is upregulation of the sodium-glucose cotransporter, the dominant mechanism by which fluid and electrolytes are handled by the small intestinal enterocyte.

### Changes associated with experimental adaptation

#### Morphological
- **Macroscopic**
  - Dilatation
  - Thickening
  - Increase in length
- **Microscopic**
  - Villus: increase height and diameter
  - Crypt: elongation
  - Epithelial cell life cycle: increase proliferation; decrease apoptosis
- **Protein content**
  - Increase RNA content
  - Increase DNA content

#### Functional
- **Absorption**
  - Carbohydrate: increase absorption per unit length
  - Protein: increase absorption per unit length
  - Electrolytes: upregulation of sodium-glucose transporter

### HUMAN EVIDENCE FOR ADAPTATION

The direct evidence for such changes in humans is limited. Although the macroscopic changes of intestinal hypertrophy and lengthening have been described, the changes in villus/crypt architecture described in rodents have not been reproduced by most studies.

Functional changes have been shown to occur in humans, both in the small and large intestine. Xylose and calcium absorption increases per unit length after resection and continues to increase for at least two years. The oligopeptide transporter, PepT1, the H^+ dependent mechanism for transport of di-peptides and tri-peptides in the gastrointestinal tract, has been shown to be up-regulated in colon but not the small intestine.

Indirect evidence that adaptation of the intestine occurs comes from the fact that patients with very short bowel lengths can become independent of the need for parenteral nutrition after a period of months or even years. Whether a patient can be weaned from parenteral nutrition is dependent on a number of factors. The length of small bowel and the presence of colon are particularly important. Other factors that are useful in predicting whether intestinal failure is permanent are the time on parenteral nutrition (>2 years) and the amount of energy the patient can derive from enteral feeding.

Other indirect evidence comes from patients who receive live related segmental intestinal transplants, in whom only 180–200 cm of ileum is transplanted. These grafts do undergo morphological and functional adaptation, with an increase in villus area of up to 50% and normal carbohydrate and fat absorption tests by six months.

### NORMAL INTESTINAL EPITHELIAL HOMOEOSTASIS

The intestinal epithelium is a continually renewing single cell layer sheet, containing four different columnar cell types, which is folded into invaginated crypts and finger-like villi. The cell lineages include the absorptive enterocyte (columnar lineage), which is the majority cell (>96%) and three types of secretory cell (mucus lineage): the goblet cell; the entero-endocrine cell, and the crypt Paneth cell. All types of epithelial cell within the intestine are derived from multipotent stem cells found near the base of the crypts.

Mouse studies have shown that in steady state conditions the absorptive enterocyte, the goblet cell, and the entero-endocrine cell all migrate and differentiate over a period of two to five days from the crypt upwards to the villus tip, whereupon they are lost. The Paneth cells complete their differentiation and remain within the crypt for around 20 days, at which point they are removed by phagocytosis. Each crypt contains 250–300 epithelial cells, and it is estimated in the intestine of an adult mouse that there are about 10^9 crypts. In these animals, stem cells produce equivalent to 1 g (10^9 cells) of new epithelial cells every five days.

An increase in proliferation of crypt cells, coupled with a decrease in apoptosis, increases the villus height and a total increase in DNA, RNA, and protein content. Proliferation therefore requires a supply of polyamines, putrescine, spermidine, and spermine, which are organic cations, influencing DNA, RNA, and tissue synthesis. Nutrient and non-nutrient factors are purported to have a role in intestinal epithelial cell turnover in both steady state conditions and after extensive resection.

### NUTRIENT FACTORS INVOLVED IN ADAPTATION

#### Amino acids

Enteral feeding is the primary source of amino acids for intestinal tissue as, apart from glutamine, there is little or no arterial uptake of any amino acids. Glutamine, rather than glucose, is the major fuel for mitochondrial respiration in enterocytes. Glutamine is used for protein synthesis either directly or as a result of catabolic pathways.

Within 24 hours of 80% small bowel resection in the rodent, glutamine and total amino acid uptake per gram of tissue is increased. However, with the decrease mass of tissue, overall glutamine consumption in the long term is less than controls and muscle stores of glutamine remain unchanged. The addition of glutamine or arginine to enteral feeds after extensive resection does not seem to produce a consistent effect between studies, indeed there is little evidence that either amino acid increases adaptation and some groups have reported lower protein and DNA levels than controls. Supplementation of enteral feeds with ornithine κ ketoglutarate (OKG), the soluble ornithine salt, does seem to have a positive effect on intestinal morphology and mucosal polyamine synthesis.

#### Carbohydrates

Luminal enzymes, such as amylase, digest polysaccharide carbohydrate into oligosaccharides and disaccharides. Oligosaccharides and disaccharides are hydrolysed to monosaccharides by intestinal membrane brush border enzymes, such as disaccharidases. Disaccharidase activity increases significantly after resection.

The monosaccharides are absorbed by the enterocyte by facilitative and active dependent transport. Glucose absorption is by sodium dependent transporters both actively, via SGLT 1, and down concentration gradients, via GLUT 2 and GLUT 5. The expression of SGLT 1 is transiently increased after experimental resection.
Short chain fatty acids (SCFA), such as butyrate, are produced by bacterial hydrolysis and fermentation of carbohydrates and proteins that reach the colon undigested. SCFA can be absorbed by colonocytes and provide energy. Complex carbohydrates such as fibre are one such source of SCFA in the diet. Diets high in fibre and butyrate have been shown to increase the content of DNA, RNA, and protein per unit weight of small intestine mucosa after resection in rodents compared with controls. 57

In humans, fermentation of non-digested carbohydrate in the colon to SCFA can result in a decrease in luminal pH and an overgrowth in D-lactate producing bacteria, such as Lactobacillus acidophilus, Lactobacillus fermenti, and streptococcus. This may result in D-lactic acidosis, 54 which presents severe metabolic acidosis. Management requires low carbohydrate diets and supportive treatments, such as dialysis. 68

Lipids
The main mechanism of lipid absorption is by passive diffusion, although the evidence for protein facilitated transfer is accumulating. 49–51 Fatty acid binding protein (I-FABP) 52 and fatty acid translocase (FAT/CD36) 53, 54 are expressed in the intestine and upregulated after resection in rodents. However, this is not achieved by the increased intestinal uptake of long chain fatty acids (LCFA). 55 Essential fatty acids may be required for optimal adaptation after resection. Rats fed fatty acid deficient diets had reduced mucosal hyperplasia compared with controls. 56 However, a low fat normo-caloric diet, reduces but does not abolish the adaptive response. 56–59 LCFAs, such as arachidonic acid and eicosapentaenoic acid also contribute towards the adaptive response. 60 Supplementation of the diet with linoleic acid or medihen oil both produce increases in adaptive changes compared with controls. 61–63 LCFAs, may exert their effects by arachidonic acid metabolites such as prostaglandin. 64 Inhibition of prostaglandin by administration of aspirin, or cyclo-oxygenase inhibitors only reduces the expected adaptive response of the distal ileum. 65–67 Unfortunately, patients with extensive distal small bowel resections have reduced bile salt concentrations in the duodenum as re-absorption normally occurs in the ileum. This leads to a decrease in micellar solubilisation, and malabsorption of LCFAs in particular. 68

Effects of Parenteral Nutrition (see Box)
In normal rodents maintained on parenteral nutrition with no luminal nutrition there is significant mucosal hypoplasia, with lower mucosal protein and DNA content, and increases in apoptosis accompanied by decreases in mitoses in the villus and crypt. 70–74 After extensive experimental gut resection, the adaptive response is limited but not abolished when no nutrition is provided orally. 75–79 Scarcce data exist regarding the effects on healthy human subjects. In eight volunteers, nutrition was provided parenterally for 14 days. The investigators found subtle mucosal changes, with an increase in intestinal permeability and a decrease in jejunal biopsy mucosal thickness attributable solely to a decrease in villus cell count. 77 A small randomised human study showed that short term addition of glutamine to TPN prevented the increase in permeability and loss of villus height. 72

Diet Types
Elemental diets are liquid feeds containing protein as free amino-acids, carbohydrates as glucose or simple sugars, fat as small quantities of defined essential fatty acids, plus vitamins and minerals. 73 The elemental diet is theoretically more easily absorbed. In patients with short bowel syndrome, studies have found no 76 or little 77 difference in protein, energy, or fluid absorption between complex or elemental diets. Indeed providing nutrition to rodents only as an elemental diet produces mucosal hypoplasia. 76, 77

Non-Nutrient Factors Involved in Adaptation
Table 1 gives a summary of the factors involved in adaptation.

Growth hormone and insulin-like growth factors
In rodent systems growth hormone has been shown to promote adaptation by increasing bowel length and function per unit length. 78–79 Human trials have been performed and these are discussed later.

Growth hormone mediates its trophic effects by insulin-like growth factor-I (IGF-I). 80–84 IGF-I produces its main biological actions through the type 1 insulin-like growth factor receptor, 85 which is distributed uniformly on epithelial cells through the small intestine, but is present to a higher degree in the colon. 86 In serum, IGF-I is bound to circulating binding proteins (insulin like growth factor binding proteins (IGFBP)). The IGFBPs can also have independent effects on cell growth; for example IGFBP-3, the predominant binding protein, has pro-apoptotic activity. 87

Growth hormone stimulates IGF-I production in the liver and locally within the intestine, leading to an increase in serum and intestinal IGF-I. 80–84 Normal small bowel epithelium expresses little of no IGF-I. 85 Treatment of human duodenal biopsy specimens with IGF-I significantly increased crypt cell proliferation rate, 86 while administration of IGF-I and glutamine to rodents increases total ileal DNA content after resection, suggesting that IGF-I induces proliferation in vivo. 87 Exogenous IGF-I given to rodents strongly increases IGFBP-5 mRNA in the jejunal lamina propria and muscularis, while growth hormone produces modest increases in IGFBP-5 mRNA in the muscularis only. 88–89 IGFBP-5 stimulates proliferation in isolated human intestinal smooth muscle cells, both independent of IGF-I, and by potentiating the effect of IGF-I-receptor interaction. 87

The role of IGF-I in human short bowel syndrome in vivo is yet to be evaluated. Several studies have reported a link between raised serum IGF-I, in the presence of low levels IGFBP-3, with increased risks of breast, 90 prostate, 91 colorectal, 92 and lung 93 cancers. As such human trials of IGF-I may prove ethically difficult to perform.

Insulin-like growth factor II (IGF-II), which is important for normal development and growth, has also been studied. However there is little evidence that parenteral administration of IGF-II produces significant changes in villus height, crypt depth, or small bowel weight after experimental resection. 94 Indeed rats given IGF-II after resection lose weight compared with baseline and controls. 92

Epidermal Growth Factors
The family of epidermal growth factors, includes epidermal growth factor (EGF), transforming growth factor α (TGFα), and heparin binding epidermal-like growth factor (HBEGF). 95 All bind the epidermal growth factor receptor (c-erb B-1) 96 that is expressed on the basolateral surface of intestinal epithelial cells. 97 EGF is produced by the Brunner glands of the duodenum and in salivary glands. 98 Increases in EGF improve the normal adaptive response after extensive rodent small bowel resection. 99–102 Reduction of circulating EGF or inhibition of EGF receptor reduces adaptation in rodent models. 98–99

TGFα is produced by the gastrointestinal epithelium, mainly by fully mature villus enterocytes 100, 101 and stimulates epithelial cell proliferation in vitro. Exogenous TGFα increases the adaptive response after experimental resection,
with increased epithelial cell proliferation and decreased apoptosis.103

HBEGF, a powerful mitogen for epithelial cells, is expressed in a number of tissues, including the intestinal epithelium itself.103–106 In vitro the expression of HBEGF mRNA is upregulated in injured culture intestinal epithelial cells.105 In vitro stimulation of pre-adipocyte cells via the IGF receptor produces selective upregulation of HBEGF mRNA.104 HBEGF may therefore play a part in the mitogenic effect of IGF-I.

**Glucagon-like peptides**

Proglucagon derived peptides are secreted from the intestinal mucosa after ingestion of food and may be important in adaptation.107 Nude mice with subcutaneous proglucagon producing tumours show signs of intestinal epithelium proliferation. Glucagon-like peptide 2 (GLP2) was proposed to be the derivative of proglucagon responsible, as parenteral administration of GLP2 produced an increased bowel length, and villus height in both jejunum and ileum.108 GLP2 stimulates intestinal epithelial proliferation in vitro.109 After resection of >75% of the intestine in rodents, parenteral GLP2 increased the intestinal diameter, crypt-villus ratio, sucrose activity, total protein, and DNA per centimetre of jejunum but not the ileum when compared with controls.110

The role of endogenously produced GLP2 has been studied in experimental resection models. It is secreted by L-type entero-endocrine cells on nutrient stimulation. These cells are situated mainly in the distal small intestine and colon.111 In rodents, resection followed by oral feeding produces increased expression of proglucagon mRNA in the ileum, with an associated sustained increase in plasma GLP2, and a large increase in observed crypt cell mitoses. Resection, followed by parenteral nutrition alone, produced a transient increase in plasma GLP2 and an increased expression of proglucagon mRNA of the colon. Resection produced a significant adaptive response in both groups; however in the resection/parenteral nutrition group there was only a modest increase in crypt mitoses.70

In mice, the mRNA encoding the receptor for GLP2 (GLP2R) is expressed in the intestine. However, it seems that GLP2R is expressed only on enteric neurons27 and not entero-endocrine cells as previously reported.111 These neurons lie in the submucosal plexus and most of their projections are in the sub-epithelial region around the crypts. Blocking enteric neurons using tetrodotoxin suppresses the proliferation of epithelial cells in response to GLP2.27 The evidence for the influence of GLP2 on the enteric nervous system is added by the finding it may also increase gut transit time.112

**Other factors**

A number of other factors have been shown to increase adaptation but not studied extensively. Hepatocyte growth factor (HGF) and its receptor c-Met are expressed in many tissues including the intestine.113 After massive experimental resection, intestinal mass and function are increased with administration of HGF compared with controls.114 There is evidence that HGF produces increases in the gene expression of glucose transporters, SGLT1 and GLUT5.114

Keratinocyte growth factor induces epithelial cell proliferation and up-regulates antiapoptotic factors in the intestine.115 It is produced locally in the gut by intraepithelial lymphocytes and stromal cells in the lamina propria. Parenteral administration of KGF has been shown to increase adaptation in a rat116 and a mouse115 model of short bowel syndrome.

Neurotensin, a 13-amino acid peptide is produced mainly by N-type entero-endocrine cells in the ileum,117 and released normally after fat ingestion. When given exogenously, neurotensin has been shown to increase the villus height but increases in intestinal mass compared with control animals after massive intestinal resection have not been reproduced between studies.119,120 The effects of neurotensin may be mediated by pro-glucagon derived peptides.120

Leptin is produced by adipocytes, and regulates thermogenesis and appetite.121 The initial study that located leptin receptors in the small intestine, concluded that, in normal

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rats, administration of leptin inhibits sugar absorption.\textsuperscript{122} However, other studies have shown that leptin seems to increase carbohydrate absorption in the intestine of normal\textsuperscript{210} and massively resected rats.\textsuperscript{123} DNA content and mucosal mass were not increased by leptin in the short bowel syndrome model.

Interleukin 11 (IL11) is a bone marrow derived cytokine that has been shown to have effects on numerous tissues including the blood, central nervous system, reproductive organs, and the gut.\textsuperscript{211} It has been shown experimentally to reduce gastrointestinal mucosal injury caused by chemotherapeutic agents and radiation.\textsuperscript{124} by increasing mitoses in crypt cells. In a resection model of short bowel syndrome in rats, IL11 administration increases mucosal thickness, with proliferation of enterocytes,\textsuperscript{125} but only at high doses can it increase carbohydrate absorption.\textsuperscript{126}

**OPTIMISING ADAPTATION IN CLINICAL PRACTICE**

Attempts to maximise the number of patients who can be weaned off PN have been made by optimising the adaptive process using strategies based on experimental short bowel systems described previously.

**Growth hormone and glutamine**

Uncontrolled cohort studies have shown that 40% of patients with short bowel syndrome, were independent of HPN within one year of treatment with 28 days high dose growth hormone (0.09 mg/kg/day), continual glutamine (30 g/day) and high fibre diet supplementation.\textsuperscript{209} A more recent report has found that this strategy was more successful in patients with a preserved colon.\textsuperscript{113}

Three small, randomised, controlled trials using growth hormone have been performed. Scolapio and colleagues\textsuperscript{114} randomised eight patients with short bowel syndrome, dependent on HPN (>3 years), to receive a 21 day course of either active treatment or placebo. Active treatment involved growth hormone (0.14 mg/kg/day), glutamine and a high carbohydrate, low fat diet (1500 kcal/day). Patients crossed over to the other treatment arm at three weeks. There were no significant differences in basal metabolic rate, carbohydrate absorption, villus/crypt morphology, or crypt cell proliferation. There was however a significant increase in sodium and potassium absorption, and a delay in gastric emptying in the treatment group. All patients gained weight because of oedema, but any positive effects were not sustained.\textsuperscript{115}

A second trial\textsuperscript{116} examined the effect of four weeks high dose growth hormone (0.12 mg/kg/day) and glutamine, with no change in diet, in eight patients, all of whom had been dependent on HPN for at least one year. No significant changes in absorption of energy, wet weight, carbohydrate, sodium, potassium, calcium, magnesium, or nitrogen were found five days after withdrawal of treatment. All patients complained of side effects during the treatment arm of the study, including peripheral oedema, carpal tunnel syndrome, and gynaecomastia.

The most recent randomised cross over trial\textsuperscript{117} has found a positive effect with three weeks treatment of low dose growth hormone (0.05 mg/kg/day) in combination with an unrestricted hyperphagic diet (>onefold estimated basal metabolic rate, with at least 1 g protein/kg/day). Twelve adult patients with short bowel syndrome, dependent on HPN for at least one year, were included, and patients assessed for five days after treatment. Significant increases in lean body mass and absorption of energy, nitrogen, carbohydrate, and fat were found after treatment compared with controls. The mean (SD) increase in intestinal absorption corresponded to 37% (16%) of total HPN energy delivery. Although this study only examined the short term results, the potential longer term effects of this treatment may have an impact on parenteral nutrition dependence.

**Glucagon-like peptide**

The normal rises in plasma concentrations of GLP2 after a test meal, are reduced in size and duration in HPN patients who have undergone massive small bowel resection and colectomy.\textsuperscript{118} However, patients with intestinal failure who had a preserved colon have increased fasting GLP2 and an increased meal stimulated response compared with age match controls.\textsuperscript{119} Administration of GLP2 may improve nutrient absorption in patients with no colon. Eight patients, with short bowel syndrome, all of whom had undergone ileal and colonic resection were given 400 \(\mu\)g of GLP2 subcutaneously twice daily for five weeks. Improvements with treatment compared with controls were seen in intestinal absorption of energy, wet weight, and nitrogen, as well as increases in lean body mass and overall body weight. There were no changes in transit time. There were minimal adverse effects described and patient compliance was good.\textsuperscript{120}

However, only four patients in this study actually required home PN.

**CONCLUSION**

Adaptation is a complex process that results in response to nutrient and non-nutrient stimuli. Successful and reproducible strategies to increase adaptation remain elusive despite an abundance of experimental data. Given the small number of patients with irreversible intestinal failure, conducting trials with sufficient power in appropriate populations is difficult. Unless multicentre studies are undertaken the evidence for the effectiveness of treatments designed to increase adaptation is likely to remain weak. Nevertheless given the low patient survival and quality of life associated with other treatments for irreversible intestinal failure it is imperative that research continues into the optimisation of the adaptation.

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**REFERENCES**

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49 McIntyre PB, Fitchew M, Lennard-Jones JE. Patients with a high jejunojunostomy do not need a special diet. Gastroenterology 1986;91:25–33.


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Am J Physiol

factor.

Surgery

growth factor.

Cytokine Growth Factor Rev

peptide 2.

morphology and in vivo absorption of water.

and function of rat small intestine: comparison of the effects of two diets on

in vitro.

(IGFBP-5) stimulates growth and IGF-I secretion in human intestinal smooth

cancer risk in women.

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:229–36.


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