

Ph.D. Thesis.

“Efficacy of bovine colostrum in short bowel syndrome”

Experimental studies in piglets and clinical studies in infants and children.

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Preface:

This PhD. thesis presents results, obtained from experimental and clinical studies. The trial was conducted at Copenhagen University (KU-LIFE) and Hans Christian Andersen Children's Hospital, Odense University Hospital while I was employed as PhD. fellow from June 2009 to May 2012 and research assistant from June 2012 to September 2012 at The Paediatric Research Unit, Hans Christian Andersen Children's Hospital.

The experimental studies included development and use of a model of short bowel syndrome in neonatal piglets and studies on enteral nutrition in children with short bowel syndrome and infants after extensive small intestinal resection.

The thesis is based on following papers:

- 1) Bovine colostrum to children with short bowel syndrome: A randomized, double-blind cross-over, pilot study. (Accepted for JPEN December 2012)
- 2) Effect of bovine colostrum on adaptation after intestinal resection in newborn pigs and infants.(Manuscript ready for submission to Paediatric Research November 2012)
- 3) Prematurity at birth reduces adaption to intestinal resection. (Under revision for JPEN April 2012)

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Summary in English:

The aims of this thesis were:

1. To participate in the development of a model of neonatal SBS with a jejunostomy in preterm and term piglets (Study I).
2. To test the effect of minimal enteral nutrition to term piglets with SBS (Study II)
3. To test the effect of bovine colostrum on intestinal adaptation in neonates after intestinal resection (Study III)
4. To test the effect of bovine colostrum on intestinal function in children with short bowel syndrome (Study IV).

Background: Short bowel syndrome (SBS) is a rare and complex disease caused by congenital gastrointestinal diseases, volvulus or necrotising enterocolitis (NEC) in infancy and additionally thrombosis, trauma, complications to surgery or inflammatory bowel diseases in childhood. Anatomical or functional loss of small intestine characterises the disease. A definition of SBS has been a matter of debate, so precise incidences have been difficult to obtain. Increasing survival rates following preterm birth lead to higher incidences of NEC subsequently leading to extensive surgical resections, which may increase incidence of SBS. Subsequent to intestinal resection the process of adaptation aims to ensure sufficient nutritional status, growth and development of the child. Adaptation is characterised by morphological and functional changes and is promoted by luminal nutrients, systemic hormonal mediators e.g. IGF's and GH, locally released hormones e.g. EGF, and hormones stimulated by enteral feeding e.g. gastrin. Bovine colostrum contains in high concentrations many of the bioactive factors known to promote intestinal adaptation. Previously it has been shown that bovine colostrum improved adaptation of the small

intestine in piglets after surgically induced SBS. In humans, adults with SBS increased addition of bovine colostrum muscle strength and lean body mass.

Material and Methods: The research group has developed an experimental model of neonatal SBS in preterm and term piglets. In the present experiments SBS was induced on Day 2 of life and acute adaptation was assessed by histology, enzymatic response and by intestinal permeability tests. In term piglets with SBS the effect of colostrum and formula fed as minimal enteral nutrition (MEN) was compared to controls given total parenteral nutrition (TPN). The trophic response was evaluated by growth response, histology, enzyme activity, permeability test and nutrient and wet weight balance study as well as tolerance to colostrum.

In human newborns with congenital gastrointestinal malformations, a randomised case-control study was performed after extensive abdominal surgery. After surgery the infants were subsequently fed 50% of their total enteral intake as colostrum, in an attempt to improve intestinal adaptation. Efficacy was evaluated by growth, duration of parenteral nutrition (PN), hospital stay, circulating insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3 (IGF-BP3). Tolerance to colostrum was evaluated by specific immunoglobulin E for milk (IgE for milk) and gastrointestinal symptoms of allergy (diarrhoea, constipation, and emesis). Furthermore, the potential positive effect of colostrum on intestinal function was tested in a randomised, double-blind cross-over study of stable children with SBS. Efficacy was evaluated by growth, circulating IGF-1 and IGF-BP3 and nutrient and wet weight balance studies. Using energy and wet weight balance analysis the children with SBS were categorised into SBS-intestinal insufficiency (SBS-II) and SBS-intestinal failure (SBS-IF).

Results: It was possible to establish a model of SBS with an jejunostomy in term as well as in preterm piglets. In both, enteral feeding stimulated trophic responses in terms of morphological

changes whereas functional changes were most pronounced in term piglets. Bovine colostrum administered as MEN was significantly better at stimulating adaptation compared to formula or in controls not given enteral feeding. The data were confirmed by an improved absorption of wet weight and Na^+ . Colostrum was well tolerated.

The initial results of a larger ongoing study of infants after extensive abdominal surgery revealed that randomisation seems to distribute infants equally and that colostrum was well tolerated with no provocation of acute allergy symptoms. In children with SBS, colostrum did not improve intestinal function, but nutrient and wet weight balance studies provided information of the function of intestine in the individual child, useful for disease management.

Conclusions: Bovine colostrum did to some extent improve adaptation in the experimental piglet model. Bovine colostrum was well tolerated in infants and children. No effect of colostrum was seen in children with established SBS. The methodology used provided valuable information of the intestinal function and may be used in a clinical setting and in future studies.

Summary in Danish:

Formålet med afhandlingen var:

1. At deltage i udviklingen af en dyremodel af spædbarns SBS på præ-terme og terme grise (Studie I).
2. At teste effekten af minimal enteral ernæring (MEN) i en term dyremodel af SBS (Studie II).
3. At teste ko-colostrum's effekt på tarm adaptation hos spædbørn efter større tarm kirurgi (Studie III).
4. At teste ko-colostrum's effekt på tarm funktionen hos børn med SBS (Studie IV).

Baggrund: Korttarms syndrom (SBS) er en sjælden og kompleks sygdom forårsaget af medfødte mavetarm sygdomme, volvulus eller nekrotiserende enterocolitis (NEC) hos spædbarnet og trombose, traume, følge af kirurgi eller inflammatorisk tarmsygdom hos ældre børn. Anatomisk eller funktionelt tab af tyndtarm karakteriserer sygdommen. En klar definition af SBS har været debatteret, hvorfor præcise tal for incidens har været svære at fastsætte. Stigende overlevelse efter præterm fødsel har ført til stigende incidens af NEC, som kan medfører omfattende tarm resektion, hvilket kan føre til stigende incidens af SBS. Efter tarm resektion er det nødvendigt, med en adaptation proces for at sikre ernæringsstatus, vækst og udvikling af barnet. Adaptation er karakteriseret ved morfologiske og funktionelle forandringer, og fremmes via luminale næringsstoffer, systemiske hormonelle mediatorer ex. IGF's og GH, lokalt udløste hormoner ex. EGF og hormoner stimuleret af enteral indtag ex. gastrin. Ko-colostrum indeholder i høj koncentration flere bioaktive faktorer, som stimulerer og fremmer tarm adaptationen. Tidligere er det vist, at ko-colostrum kan fremme tarm adaptationen hos nyfødte grise efter kirurgisk påført

SBS. Hos voksne med SBS er det vist, at ko-colostrum som tilskud til habituel diæt kan øge muskel styrken og muskelmassen.

Materiale og metoder: Forskningsgruppen udviklede en eksperimentel model af spædbørns SBS med stomi, i præ-terme og terme grise. SBS blev induceret på dag 2 efter fødslen og akut adaptation blev bedømt ud fra histologi, respons af fordøjelses enzymer og test af tarmens permabilitet. I terme grise med SBS, blev effekten af colostrum og modermælks erstatning givet som MEN, sammenlignet med kontroller der udelukkende blev ernæret med total parenteral ernæring (TPN). Det trofiske respons blev evalueret ud fra vækst respons, histologi, enzym aktivitet, permabilitet test og energi og væske balance studier. Ligeledes blev tolerance overfor colostrum vurderet.

Et randomiseret case-kontrol studie blev udført, på spædbørn der havde gennemgået større gastrointestinal kirurgi. Efter operationen blev 50 % af deres orale indtag erstattet af colostrum, i forsøg på at forbedre tarm adaptationen. Effekten blev vurderet ud fra vækst, varighed af parenteral ernærings tilskud (PN), indlæggelses tid, cirkulerende insulin-like growth factor-1 (IGF-1), insulin-like growth factor bindende protein-3 (IGF-BP3). Tolerancen overfor colostrum blev evalueret ud fra specifik immunoglobulin E for mælk (IgE for mælk) og symptomer på allergi fra mavetarm kanalen (diarre, obstipation eller opkastninger). Desuden blev den potentielle positive effekt, af colostrum på tarmens funktion testet i et randomiseret dobbeltblindet overkrydsnings forsøg på børn med SBS. Effekten blev evalueret ud fra vækst, cirkulerende IGF-1 og IGF-BP3 samt energi og væske balance studier. Ud fra disse balancestudier var det muligt at kategorisere børnene med SBS som henholdsvis SBS-tarm insufficiente (SBS-II) og SBS-tarm svigt (SBS-IF).

Resultater: Det var muligt at udvikle en model af SBS i både terme og præ-terme grise. I begge grupper førte enteral ernæring til stimulation af trofisk respons ved morfologiske

ændringer, hvorimod funktionelle ændringer var mest udtalte i terme grise. Ko-colostrum givet som MEN forbedrede vitale funktioner (væske og elektrolyt optag) sammenlignet med modernælks erstatning og kontroller. Terme grise med SBS tolererede colostrum godt.

De præliminære resultater fra det igangværende studie af spædbørn efter større marvetarm kirurgi viser, at randomiseringen fordeler børnene ensartet samt, at colostrum tolereres og ikke fremprovokerer allergiske symptomer. Hos børn med SBS fremmede colostrum ikke tarmfunktionen, men energi og væske balance studierne gav information om den enkeltes tarmfunktion, hvilket er vigtigt for den enkeltes behandling.

Konklusioner: Ko-colostrum førte i nogen grad til forbedret adaptation i den eksperimentelle grise-model. Ko-colostrum blev tolereret af spædbørn og børn. Der var ingen effekt af colostrum hos børn med SBS. Den anvendte metode, gav værdifuld information om tarmfunktionen, er brugbar i den daglige klink og fremtidige studier inden for området.

Abbreviations and definitions:

AA: absolute absorption

BF: bolus feeding

BFR: basal fluid requirement

BMC: bone mineral content

BMD: bone mineral density

BMI: Body Mass Index

BMR: basal metabolic rate

CEF: continues enteral feeding

CF: Complementary feeding

DXA: Dual-energy X-ray Absorptiometry

EEI: enteral energy intake

EFG: epidermal growth factors

GHRF: releasing factor

GIT: gastrointestinal tract

GLP-2: glycogen-like-peptides 2

HC: Head circumferences in centimetres (cm)

HIV: human immunodeficiency virus

HRQoL: health-related quality of life

ICD-10: WHO-International Classification of Diseases (2010)

IBD: inflammatory bowel disease

IEA: intestinal energy absorption

IGF-1: Insulin growth factor-1

IGF-BP3: Insulin growth factor-binding protein-3

INF- γ : interferon- γ

IUGR: intra uterine growth retarded

IWA: intestinal wet weight absorption

L: length in centimetres (cm)

MEN: Minimal enteral nutrition

MJ: Mega Joule

MM: Mother's milk

NEC: Necrotizing enterocolitis

PDGF: platelet-derived growth factor

PN: parenteral nutrition

RA: relative absorption

S-IgE: Allergen Specific Immunoglobulin E

SBRL: short bowel remnant length

SBS: Short bowel syndrome

SD: Standard deviation

SEM: Standard error of the mean

TES: total energy supply

TGF- α : transforming growth factor α

TGF- β : transforming growth factor β

TNF- α : tumor necrosis factor- α

TPN: total parenteral nutrition

UO: urine output

VEGF: vascular endothelial growth factor

W: weight in kilo (Kg)

Ww^a: wet weight absorption

WWI: wet weight intake

Ww^o: wet weight of oral intake

Ww^s: wet weight of stools

Introduction:

Extensive intestinal resection in infancy and childhood, due to congenital diseases, necrotising enterocolitis (NEC), trauma, intestinal thrombosis or surgical complications, is associated with early and late comprised intestinal function that may lead to short bowel syndrome (SBS). The incidence of SBS in children is increasing due to improved survival rate after intestinal resection in infancy and optimised postoperative therapy. The challenges are numerous, sufficient nutritional regime is the cornerstone as well as maintenance of water and electrolyte balance in order to allow growth and maturation of the child. Furthermore, infections and side effects to parenteral nutrition (PN) should be kept to a minimum.

Intestinal adaptation after resection is important and determines the subsequent degree of intestinal insufficiency. It has been shown that adaptation is promoted by internal factors e.g. epidermal growth factors (EGF) and growth hormone (GH) as well as external factors such as luminal nutrients. Previously, experimental as well as human studies have focused upon the process of adaptation to improve intestinal function. Only few studies have been performed on children and none on the efficacy of bovine colostrum.

Colostrum is thought to be an optimal nutrition for newborns, as it in high concentrations contains bioactive factors known to facilitate maturation and growth of the intestine, and as such these factors might also promote adaptation and improve function of the comprised intestine after intestinal resection. A model of SBS in neonatal piglets was developed and the efficacy of minimal enteral nutrition with colostrum compared to formula was performed. From these results a randomised double-blind cross-over trial was performed to study the efficacy of colostrum in children with SBS and a randomised controlled trial in infants after extensive intestinal surgery.

Background:

Embryology of the intestine:

There are three major milestones in the formation of the gastrointestinal tract (GIT): 1) differentiation of the endoderm, 2) formation of the tube and 3) the organogenesis with the outgrowth of the different organs from the tube, which forms the gastrointestinal tract to an organ with an entry and exit. This process is completed by the end of the 12th week. Animal studies has identified numerous gene families that influence these milestones e.g. the transforming growth factor β (TGF- β) superfamily, sonic and Indian hedgehog signalling proteins ¹.

Late in the organogenesis, the morphogenesis commences proceeding in a cranial-caudal direction. It comprises of three stages: i) morphogenesis and cell proliferation, ii) cell differentiation and iii) functional maturation. The end product is a well-known multiple layer configuration which include; tunica mucosa, lamina propria, telasubmucosa, tunica muscularis and tunica serosa (Fig. 1).

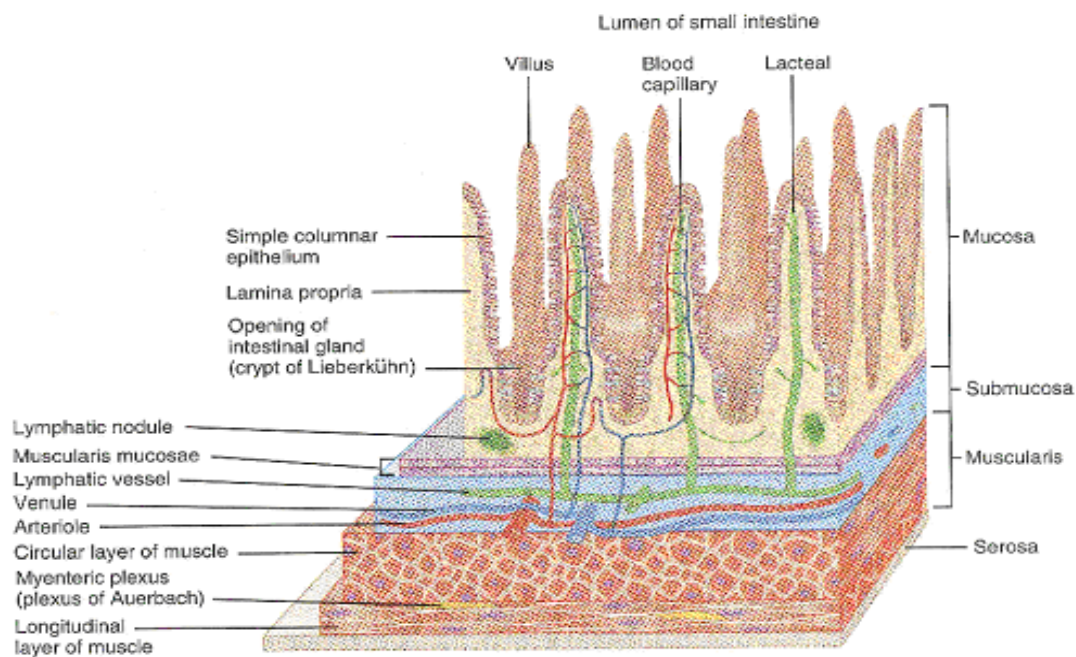


Figure 1: Intestinal anatomy (Tortora and Grabowski, 1996).

Columnar epithelium develops from polarised enterocytes on the brush border membrane and villi arise from the basolateral membrane during 9-10th gestational week. Initially, the proliferative cells will be located along the villi. As the crypts develop, the location of the proliferative cells will change and from the 12th week of gestation mitotic cells will be located in the crypts. A mucosal remodelling occurs in the crypt-villus area and absorptive cells, goblet cells, enteroendocrine and Paneth cells develop. M-cells, associated with Peyer's patches, will be present in human intestinal mucosa from 17th week of gestation and during the subsequent weeks (17-20th week) the underlying mucosa muscularis is formed¹. The onset of enzyme activity takes place as enterocytes develop. In humans, several of these enzymes are in function at term birth and responsible for digestion of human milk, such as lactase, sucrase and lipase. The enzymatic activity increases with time².

Intestinal growth is genetically regulated and additionally stimulated by different growth factors e.g. epidermal growth factor (EGF), TGF- β , insulin-like growth factor I and II (IGF I, II) and insulin. These factors induce cell proliferation and migration in addition to enzymatic activity³⁻⁵. During the last trimester the development of the intestine is intensified, lengthening and maturation occurs simultaneously with motility. Foetal swallowing is detectable from 16th week of gestation and mature motility from the 36th week. In humans and other species, the maturation of the GIT occurs in clusters at birth and at weaning due to dietary changes, endocrinology, microbiology and immunity. The human small intestine is completely developed and mature 52 weeks after term birth. Other species has a different pattern of development e.g. in the piglet onset of maturation commence later and terminates earlier (12 week after term birth)⁶.

Milk:

The foetal intestine of all species is, designed to consume milk from term birth. It is generally accepted that, breast milk is the optimal nutrition of the newborn mammalian. Colostrum is the first

milk produced during the postpartum period, and the majority of bioactive factors reach maximum concentration within the first 24-48 hours after birth^{7;8}. The composition is species dependent e.g. human colostrum contains high concentrations of EGF and somewhat lower concentrations of IGF I and IGF II whereas the reverse is true for bovine colostrum. TGF α and β , platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), lactoferrin and growth hormone (GH) as well as its releasing factor (GHRF) are other bioactive factors found in colostrum. These provide intra- and extra luminal growth activity stimulation. Besides, colostrum contains micro- and macronutrients essential for the newborn, immunoglobulins (IgG, IgM and IgA), β -casomorphin, cytokines, interleukins, tumor necrosis factor- α (TNF- α) and interferon- γ (INF- γ)⁹⁻¹⁵. The concentrations of these bioactive factors have been measured in bovine as well as human colostrum and milk^{3;11;16-18}. In bovine colostrum, the concentrations of bioactive factors are affected by e.g. breed, herd, number of lactation⁹. The concentrations were up to 40 times higher in bovine colostrum than ordinary cow-milk whereas in human colostrum the concentrations were only up to five times higher. Thus in theory, bovine colostrum would more potent than human colostrum⁷ improve immunity, and stimulate maturation and growth of GIT. A number of animal studies have been performed to test the effects of colostrum. In preterm piglets, colostrum protects against development of NEC¹⁹⁻²². In humans, efficacy of colostrum on intestinal immunity was studied in both adults with human immunodeficiency virus (HIV) associated diarrhea^{23;24} and children with acute gastroenteritis^{25;26}, with a positive effect on numbers of stool in adults and time to recovery in children with rotavirus. Intake of 60 g of freeze-dried bovine colostrum reduced the incidences of upper respiratory tract infections in adult athletes²⁷. Muscle mass and exercise performance was improved by intake of 60 or 20 g of freeze-dried colostrum, in adult active persons, with no increase in circulation IGF-I^{28;29}, others found no effect on performance³⁰.

When mother's milk is unavailable it will be replaced by infant formulas. They are based on mature bovine milk and designed to match breast milk. It contains a similar concentration of micro-and macronutrients. The bioactive factors will still be present after processing, but variability in concentrations will be considerably why formulas is deemed as non-comparable to fresh milk and colostrum⁹.

Growth:

Human growth can be divided in to linear and ponderal growth, the first refers to statuary growth and the latter to body composition. Growth occurs in phases; foetal growth (from fertilisation to birth), infant growth (the first year of life), childhood (the second year of life till early puberty) and adolescent (puberty). All phase of growth are dependent on hormonal regulation, nutritional need and response to skeletal stimulation. The foetal growth is defined by genetic predisposition, the placenta, gender, multiple pregnancy and hormones especially insulin and IGF's. Infant and childhood growth depends mainly on thyroid and growth hormones, where as growth in puberty mainly depends on sex and growth hormones. In addition, growth is affected by metabolic and environmental factors including nutrition. To ensure an adequate growth at different ages, recommendations for daily intake has been established for both genders^{31;32}.

Anthropometric measurements as weight, length, head circumference, Body Mass Index (BMI) etc. are recognized methods of assessing growth. More specific methods used to assess growth and body composition are skin-fold thickness, limb circumferences, Dual-energy X-ray Absorptiometry (DXA) and knemometry. DXA evaluates the body composition of bone mineral density (BMD), water, soft tissue and fat with high accuracy but involves radiation why repeated use will be restricted especially in children³³. Knemometry, a measure of lower limb length, provides information of short time growth defined as growth within weeks up to six months³⁴. In infancy and

young childhood, knemometry can be assessed using a portable knemometer, later in childhood a specialised knemometer, first described by Valk must be used. Knemometric measuring has been validated by many studies within different age groups and has have shown to be valid and reproducible³⁵⁻⁴⁰. In infancy the growth velocity range between 0.41 mm/d to 0.52 mm/d^{36;41}, in early childhood (1 – 3 years of age) velocity ranged between 0.85 mm/d to 0.92 mm/d^{37;42} whereas in later childhood velocity was found to be 0.40 mm/week⁴³.

Short Bowel Syndrome:

SBS, is a complex disease frequently caused by anatomically loss of small intestine after resection or by functional loss of the small intestine⁴⁴. The incidence of short bowel syndrome has previously been estimated to 1.7 per 10⁵ births per year in Denmark⁴⁵, in other countries the incidence was 24.5 per 10⁵ births in Canada⁴⁶ and 120 per 10⁵ births per year in United States of America and increasing at decreasing gestational age⁴⁷. In general, incidences have been difficult to estimate, since definitions of SBS vary. Different anatomically definitions of SBS have been suggested: 1) Less than 75 cm (30 %) remnant intestine⁴⁸ or 2) Divided in type of resection (large or extensive), with a subsequent remnant at a) 40 – 100 cm or b) less than 40 cm⁴⁹. From a functional point of view SBS, may be defined as a condition where inadequate absorptive surface of the intestine leads to necessity of PN in order to maintain growth and development⁵⁰. The most common causes of SBS are intestinal resection due to congenital diseases e.g. atresia, gastroschisis, volvulus and NEC in infancy. Thrombosis, complications to surgery, trauma and inflammatory bowel disease (IBD) are the most common causes in childhood⁵¹. The later consequences of SBS depend on age at onset, site of resection, in combination with capacity of remnant intestine. The absorptive capacity with regard to nutrient and wet weight divides SBS into intestinal insufficiency (SBS-II) and intestinal failure (SBS-IF) respectively. SBS-II may be characterised by inability to maintain macro- and micronutrient, fluid, protein and electrolyte balance when on a normal diet whereas SBS-IF is

characterised by inability to maintain the above mentioned balances irrespective of dietary intake. In practical terms, the child with SBS-II needs nutritional support in addition to normal diet e.g. enriched meals or enteral formulas, whereas the child with SBS-IF needs nutritional support in form of PN⁴⁴. Management of paediatric SBS aims to accomplish intestinal autonomy to avoiding the numerous complications to PN. The most common complications are central venous catheter infections, catheter related thrombosis, liver related complications to PN (cholestasis, steatosis, cirrhosis)^{52;53}. There are few available long-term studies on SBS. The survival rate range between 70-89% depending on aetiology (cause for surgery), length of remnant intestine, gestational age (GA) and weight at birth^{50;54;55}. Results on growth vary from normal target height⁵⁶ to short stature, low bone mineral content (BMC) but normal weight for height⁵⁷. Normal cognitive function, assessed by IQ tests were achieved if children were weaned of PN⁵⁸. Children with a history of infantile SBS and their families report lower health-related quality of life (HRQoL) compared to healthy controls⁵⁹. Cost of care depends on duration of hospital admission, duration of PN and co-morbidity. In USA, these have been estimated to US\$ 1.6 million per patient over the first 5 years after onset⁵⁴.

The overall outcome of SBS depends on intestinal adaptation after resection, the medical and nutritional management aims to promote this. The adaptive process begins within the first 48 hours after resection and includes morphological and functional changes characterised by increased villus height and crypt depth due to proliferation and elevated nutrient uptake by enterocytes⁶⁰. The process of adaptation may reach a plateau within the first months, and within two years of age the process terminates⁶¹. The morphological changes results in increased area of absorptive surface and is promoted by systemic hormonal mediators e.g. IGF's, GH, local released hormones e.g. EGF, and hormones stimulated by enteral feeding e.g. gastrin as well as luminal nutrients. The increased uptake of nutrients arises from increased absorptive surface in both small intestine and proximal

colon as well as from decreased intestinal transit time⁶². Because of this, early enteral feeding is important, whether it should be continues enteral feeding (CEF), bolus feeding (BF) as minimal enteral nutrition (MEN) or not, is debated⁶³.

Experimental studies of SBS:

Experimental studies of SBS were initially performed on adult rats or pigs but new techniques have made it possible to perform studies in newborn animals which make the resemblance to infants even more realistic. Rats has been preferred, however, the development of the intestine in the piglet is more homologous to infants, especially if preterm, with regard to gastrointestinal development and function, metabolism and body composition⁶. A model of infant SBS has previously been developed in term piglets^{64;65}. Using this model, adaptation was characterised and it was possible to distinguish between short- and long-term adaptations⁶⁴⁻⁶⁶. In anticipation of promoting adaptation, investigations have focused upon treatment with diets and bioactive factors (GH, EGF, IGF-1, and glycogen-like-peptides 2 (GLP-2)) as well as the impact of PN treatment on the intestine, with primary focus upon metabolism and change in body composition. To initiate adaptation by nutrition, at least 40 % of total nutrient intake has to be enteral feeds. To obtain normal growth of intestine and gain mucosal proliferation, 60 % of total nutrient intake must be enteral, whereas circulating levels of IGF-1 was not increased until 80 % of total nutrient intake was enteral⁶⁷. Studies of the colostral effect on adaptation in SBS piglet models suggests a positive effect on muscle hyperplasia, increased villus height and crypt depth in jejunum as well as increased levels of IGF-1 and GLP-2 in blood and jejunal tissue, even though results were conflicting⁶⁸⁻⁷¹. In SBS rats and piglets, it was shown that IGF-1 and GLP-2 stimulates the classical features of adaptation⁷²⁻⁷⁶.

Studies of preterm piglets with SBS are scarce due to difficulties in keeping them alive (Premature piglets show reduced adaptation to intestinal resection, Aunsholt et al, in progress). At 90 % gestation the piglets can survive, this resembles the age ranging from 29 to 31 weeks of gestation in preterm infants. The intestinal tract is at this age very sensitive to enteral feeding, infections and change in circulation. Studies in animals have shown that type of diet and volume of feeds may provoke development of NEC^{19;20}.

Human studies:

Studies of SBS in humans have focused on adaptation and improvement of intestinal function. Adult studies have revealed, that the process of adaptation terminates approximately 2 years after intestinal resection, depending on function and length of the remnant intestine. It is likely to be the same or even longer in children. Studies on the process of adaptation in adults have shown that patients with colonic continuity have improved adaptation compared with patients with a jejunostomy^{77;78}. Hormones that effect intestinal adaptation have been tested in terms of efficacy on improving intestinal function. Especially GH and GLP-2 and its analogue was studied and evaluated by nutrient and fluid balance studies, body weight and lean body mass by DXA⁷⁹⁻⁸¹. High-dose GH in combination with oral and parenteral glutamine and a high carbohydrate diet had effect on wet weight absorption. Low-dose GH improves energy absorption but the effect ceases with cessation of treatment and the reported side effects were severe. Furthermore, the initial positive effects of GH could not be reproduced in subsequent studies^{82;83}. GLP-2 and especially the analogue teduglutide likewise improve wet weight absorption and teduglutide promoted growth of villus and crypts with $38 \pm 45\%$ and $22 \pm 18\%$ respectively. Adverse effects were stoma swelling and leg oedema^{79;80}. In addition to adaptive studies, different nutrition and feeding regimes, adverse effects to PN (e.g. liver associated side effects, central venous catheter related side effects), quality of life (QoL) in patients dependent on home-PN and surgical procedures (transplantation and

lengthening of remnant intestine) have been of focus. The overall conclusions were that systemic short chain fatty acids in combination with enteral induces adaptation but PN has numerous side effects. Surgical treatment such as transplantation may be the only solution in some and with a relative good long-term outcome. QoL in long-term home-PN patients is depressed.

Postsurgical, infants gained weight after administration of GH, even though resting energy expenditure was increased. An increase in polyamine concentrations, in red blood cells and jejunal mucosa, was observed and interpreted as an expression of GH influence. None of the 12 participating infants were weaned of PN after 10 days of treatment⁸⁴. In older children, one study reported PN weaning in two children after long-term treatment with GH in addition to enteral administered glutamine⁸⁵. Treatment with high-dose GH solely for 12 weeks was able to wean 6 out of 8 children of PN, energy intake was improved, concentrations of circulating IGF-1, IGF-BP3 and plasma-citrulline increased, but body mass index decreased with weight loss. After 1 year follow-up only 2 of 8 were able to remain weaned from PN, energy intake was stable but IGF-1 and IGF-BP3 levels decreased⁸⁶. In a study comprising 14 children, no effect were found after treatment with GH, in a similar dose and study design⁸⁷. In children, efficacy of GLP-2 or teduglutid treatment remains to be studied. Secreting levels of GLP-2 after intestinal resection in infants have shown correlation with later PN weaning (concentrations >15 pmol/L predicts possible PN weaning), as well as length of remnant intestine and the absorptive capacity⁸⁸. A pilot study of enteral treatment with EGF to four neonates and one child indicated improved carbohydrate absorption that disappeared after cessation of treatment⁷². It may be concluded that for children with SBS, that knowledge as to the effect of nutritional regimes, complications and co-morbidity is lacking.

Hypothesis:

We hypothesised that enteral feeding with bovine colostrum would promote intestinal function in neonatal piglets with SBS, in human neonates after extensive intestinal resection and in children aged 1 to 15 years with SBS.

Aims:

We aimed to:

1. Establish a model of neonatal SBS in preterm and term piglets:
Study I.
2. To test the effect of minimal enteral nutrition to term piglets with SBS: Study II.
3. To test the effect of bovine colostrum on intestinal adaptation in human neonates after intestinal resection: Study III.
4. To test the effect of bovine colostrum to children with SBS: Study IV.

Materials and Methods:

Bovine colostrum:

The colostrum used in all four studies was supplied by Biodane Pharm A/S, Vejen, Denmark and processed as described below. The raw colostrum was delivered when needed. Bovine colostrum was collected from cows (Danish Holstein) in six different herds in southern Denmark (within the first 24 hours after calving). All suppliers were registered dairy farmers and subject to institutional veterinarian inspection and control. In each batch, the content of immunoglobulin G (IgG) was measured with radial immunodiffusion kits (VMRD, Pullman, USA) and colostrum containing less than 3.5 % IgG was discarded. Containers with maximum 130 grams of colostrum were irradiated with electron beam β -irradiation (10 kGy, Sterigenics, Esbjerg, Denmark). Sterility was controlled (Eurofins, Steins Laboratory, Holstebro, Denmark) and repetitive testing never showed bacterial contamination. The nutrient content was: 0.011 kg/L of protein, 0.005 kg/L of fat, 0.004 kg/L of carbohydrate and 0.546 MJ/L of energy.

Throughout the study period it was not possible to use the same batch of colostrum, but each batch was evaluated identically. We chose not to use powdered colostrum since this had a significant taint which could create problems with patient and parent compliance.

Nutrient and wet weight balance study:

In experimental study II and human study IV nutrient and wet weight balance studies were performed. Analysing faeces, food and beverages was similar in the studies duration of the balance studies were different as explained. Energy content was analysed using bomb calorimetry, carbohydrate content using Englyst's method⁸⁹, fat content by van de Kamer titration⁹⁰ and nitrogen content by Kjeldahl's method⁹¹ as previously described. Weight of oral intake, faecal loss and urinary output was measured. Absolute wet weight absorption (Ww^a) was calculated by subtracting

wet weight of stools (Ww^s) from oral intake (Ww^o) and the relative wet weight absorption expressed as the percentage of total wet weight intake $((Ww^o - Ww^s)/Ww^o) \times 100$. Absolute intestinal energy, fat protein and micronutrient absorption was calculated in the same way, equivalent to the difference between ingested and excreted energy and the relative absorption as a percentage of total energy intakes.

Prior to study IV a faecal recovery study was performed in 10 adults with a stoma. One part of the stoma output was analysed as above, the other part was poured on to cotton diapers and frozen. Later, faecal material was dissolved, lyophilized and analysed as previously described. Energy was recovered by $79 \pm 10 \%$, fat by $78 \pm 11 \%$, carbohydrate by $75 \pm 14 \%$ and protein by $81 \pm 13 \%$.

Table 1: Energy and macronutrient content in colostrum, formulas and milk-mix used in study IV.

	Protein g/100 ml	Fat g/100ml	Carbohydrate g/100ml	Energy KJ/100ml
Colostrum	11,27	5,23	4	545,67
Milk-mix	10,8	3,7	5,13	467,75
Infantrini	57	194	175	422
Neocate	66	175	202	430
Blackcurrant				
Neocate advance	65	118	274	444
Neocate	83	217	270	568
Pregestemil	41	144	114	277
Ketocal	78	486	22	722
Peptidite	60	122	206	401

Experimental studies:

Piglets used in experiment I were of Large White x Danish Landrace, whereas in experiment II they were large white x landrace x duroc. Gestational age and ways of delivery is described in details in each relevant section of material and method.

Surgical procedures:

All piglets were fitted with a single lumen vascular catheter and an orogastric tube (6F; Portex, Kent, UK) that was passed through the cheek and secured in the neck to prevent chewing. Still under the guise of anaesthesia from caesarean section piglets in study I were fitted with a vascular catheter (infant feeding tube 4F; Portex) which was inserted into the dorsal aorta via the transected umbilical cord and. In study II anaesthesia was induced and maintained with a combination of zolazepam+tiletamin (Zoletil 50 Vet, Virbac, Kolding, Denmark), xylacin (Narcoxyl 20mg/mL, MSD Animal Health, Ballerup, Denmark), ketamin (Ketaminol 100 mg/mL, MSD Animal Health, Ballerup Denmark) and butorphanol (Torbugesic 10mg/mL, ScanVet, Fredensborg, Denmark). Local infiltration with Lidocain 2% ensured analgesia in the skin around sulcus jugularis and a single lumen jugular catheter was inserted and exteriorized on the dorsal part of the neck. Antibiotic treatment was initiated after this procedure in both studies and continued for 3 days (Ampicillin: 50 mg/kg, Pentrexyl, Bristol-Myers Squibb, Bromma, Sweden and Gentamicin: 5 mg/kg, B. Braun Melsungen AG, Melsungen, Germany, both i.m. in study II into the jugular vein (silastic tube).

After fasting, all pigs were re-anaesthetized using isoflurane 1-3% inhalation for both induction and maintenance. Analgesia was secured with Butorphanol (Torbugesic: 10mg/mL, ScanVet) and Lidocain 2% was applied subcutaneously in the abdominal midline before incision. Through a 5 cm celiotomy incision the caecum and plica iliocaecalis were identified. The entire length of the small

intestine was predicted from the equation: total intestinal length (cm) = in study I: 280 cm/body weight (kg)^{0.60} and in study II: 300 x body weight (kg)^{0.65} which was based on observations from a pilot study with pigs of the same age. Starting from the ileocecal junction, 50% of the small intestine was excised using an ultrasonic scalpel. When the isolated intestine had a length of predicted 50% of the entire length, it was transected and the remnant intestine was exteriorized on the abdominal wall as a jejunostomy, created a.m. Turnbull. A silastic tube was inserted 5 cm into the stoma and secured with a suture. The abdomen was closed by interrupted sutures including peritoneum, muscle and fascia in one layer, and the skin was close by a running suture. Antibiotic regime was continued for three days.

Sample collection and analysis:

Intestinal absorption:

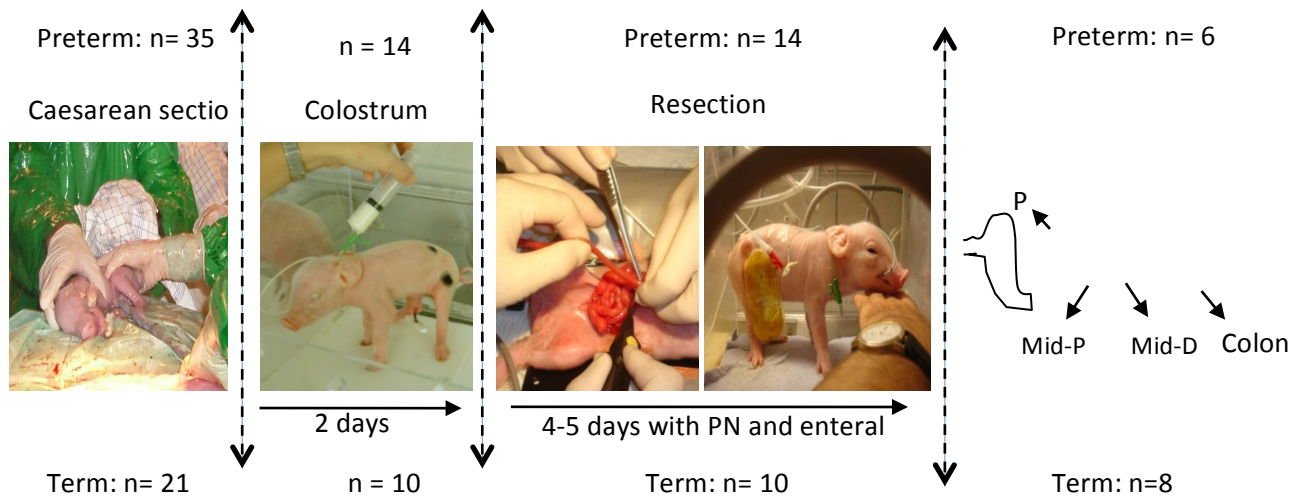
In study I, a D-galactose test was performed in vivo as an indicator for intestinal absorptive capacity. Each pig received 15 mL/kg of 0.9% saline with galactose and mannitol (50 and 20 g/L, respectively) via the orogastric tube. Arterial blood samples were collected before and at 20, 40, and 60 min after the galactose/mannitol/saline bolus was administered, as previously described⁹². The plasma was separated, deproteinized using perchloric acid, and analyzed for concentrations of mannitol by a modification of a method based on reduction of nicotinamide adenine dinucleotide (NAD) using mannitol dehydrogenase⁹³ and for galactose using a commercially available kit (Boehringer Mannheim, Darmstadt, Germany).

In study II, a lactulose-mannitol test was performed and the lactulose-mannitol ratio served as an indicator of gut permeability. Three hours prior to euthanasia, an oral bolus of a 2% mannitol, 2% lactulose solution (15 mL/kg) was given and concentrations in urine collected by cystocentesis at euthanasia were spectrophotometrically analysed.

Mucosal evaluation:

Intestinal samples were collected at birth (four sample sites), at resection (Mid-D and colon) and at euthanasia (Mid-P and proximal jejunum, P), Figure 1.

Figure 1:



A 10 cm segment from the proximal part of the remnant intestine, the distal part of the intestine in close proximity to the stoma, and at resection the transaction close site, were isolated and weighed. The segments were slit along their side longitudinally so the mucosa was exposed. The mucosa was gently scraped off and weighed separately. The segments were then dried for 76 hours at 60°C and weighed again.

Other samples were placed in 4% buffered formaldehyde, later embedded in paraffin, trimmed and placed in cassettes by standard techniques. Five-micrometer cross sections from blocks obtained from Mid-d, Mid-p or P were stained for histological procedures. Standard hematoxylin-erosin staining was performed for evaluation of villus height and crypt depth. Proliferative cells in crypts were identified with a Ki67 rabbit monoclonal antibody.

Microscopy and stereology was in study I performed by the author. A Leica DMR microscope (Leica Microsystems GmbH, Wetzlar, Germany) was used and images of the HE-stained cross sections were photographed with a 2.5 X lens. Initially, a pathologist specialised in intestinal histology supervised the stereology interpretations. Randomly selected slides were analysed until an intrapersonal variance below 10 % was achieved. The length of all villi and crypts were measured in each slide, if they could be visualised in their full length and depth, respectively. The measurements were made in μm after calibration of the system and performed using ImageJ (National Institutes of Health, USA). For obtaining the crypt proliferation index, Ki67 positive cells in the crypts were counted by random stereological principles using the newCAST software module (Visiopharm, Hørsholm, Denmark). A total of 150 nuclei were counted in the lamina mucosa of each sample. The counting was performed on whole slide scanned images obtained by NanoZoomer 2.0-HT (Hamamatsu, Japan). The proliferation index was calculated by dividing the number of Ki67-labeled nuclei by the total number of nuclei and the results were expressed as a percentage.

In study II, analyses were performed by Lars Mecklenburg (Nycomed, Germany) using the analySIS docu software system (Olympus Soft Imaging Solutions GmbH). Briefly, two tissue samples per animal were photographed using a 1.25 objective. The lengths of villi and crypts (6 to 25 villi/crypts per sample) were measured in each sample, and an average length per animal was calculated.

Digestive enzyme activity:

For intestinal enzyme measurements frozen intestinal tissue was homogenized in 1.0% Triton X-100 (6 mL per g tissue). Homogenates were assayed spectrophotometrically for disaccharidase (lactase, maltase, and sucrase) and peptidase (Aminopeptidase N, Aminopeptidase A, Dipeptidylpeptidase IV) activity using specific substrates. Sucrose (0.01 mol/L; no. 194018, INC,

Aurora, OH) and lactose (0.12 mol/L; L-3625, Sigma) dissolved in sodium maleate buffer (50 mmol/L, pH 6.0) were substrates for sucrase-isomaltase (EC 3.2.1.48-10) and lactase-phloridzin (EC 3.2.1.23-62), respectively. Maltose (0.0112 mol/L; L-5885, Sigma) was used for maltase activity representing a combination of maltase-glucoamylase (EC 3.2.1.20) and sucrase-isomaltase. Dipeptidyl-peptidase IV (EC 3.4.14.5), aminopeptidase N (EC 3.4.11.2) and aminopeptidase A (EC 3.4.11.7) activities were analysed using three peptidase-specific substrates i.e., 15 mmol/L glycyl-L-prolin-4-nitroanilide (Bachem, Bubendorf, Switzerland) in 50 mmol/L Tris-HCl, pH 8.0, 10 mmol/L L-alanine-4-nitroanilide (Merck, Darmstadt, Germany) in 50 mmol/L Tris-HCl, pH 7.3 and 10 mol/L α -L-glutamic acid 4-nitroanilide (synthesized at the Institute of Protein Chemistry, Hørsholm, Denmark) in 50 mmol/L Tris-HCl, pH 8.0, respectively⁹⁴. Enzyme activities were expressed per gram of wet intestinal tissue and one unit of activity (U) represented 1 μ mol of substrate hydrolysed/min at 37°C. The change in enzyme activity from resection to euthanasia was evaluated by dividing values of Mid-P samples with Mid-D. This ratio reflects the degree of adaptation from before resection to 4-5 days after resection (1.0 = no change).

All procedures in the piglet studies were approved by The National Committee on Animal Experimentation, Denmark.

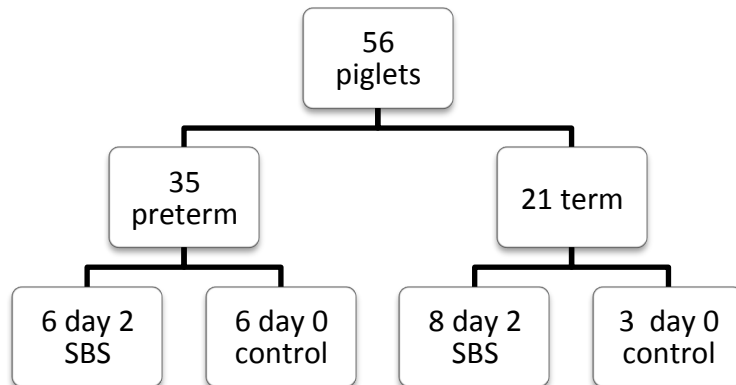
Study I:

In this study a model of neonatal SBS was developed in order to study effects of intestinal resection and in vivo as well as in vitro developmental responsiveness to resection. From previous studies it was known that bovine colostrum feeding to preterm piglets was protective against inflammatory insults (NEC) why all piglets were feed this optimal nutrition²².

Design:

Fifty-six piglets from three sows (Large White x Danish Landrace), were delivered by caesarean section either prematurely (92% gestation, n=35) or at full term (n=21), as described in detail elsewhere⁹⁵. The piglets were stratified to euthanasia for tissue collection immediately after birth (< 4 hours of age) and before any feeding (Day-0: n=6 preterm and 3 term), or to undergo intestinal resection at age 46 – 54 hours after birth (Day-2: n = 14 preterm and 10 term). Intra uterine growth retarded piglets were excluded (IUGR: n = 8, 4 preterm and 4 term). For testing the surgical procedures, six preterm and 3 term piglets were used 5 preterm and 1 term died prematurely. 135-155 hours after birth the piglets were euthanatized (Day-6/7: n = 6 preterm and 8 term). After delivery, piglets were transferred to heated incubators (Air-Shields, Hartboro, PA). Numbers of surviving piglets available for analysis are shown in figure 2.

Figure 2:



To ensure optimal immunity, 20 ml of sow plasma was infused intravenously the first 12 hours after delivery, in piglets stratified for day 2 surgery. They were post-partum feed 5 meals of porcine colostrum (15 mL/kg), manually collected from Large White x Landrace sows and containing 6.8 MJ/L energy and 0.146 kg/L protein with an osmolality of 344 mOsm/L. Until resection on day 2 piglets were feed bovine colostrum (15mL/kg/h). After surgery Enteral feeding with bovine colostrum was resumed within three hours of surgery at 2mL/kg/2h increasing to 5mL/kg/h on day 5. The bovine colostrum was diluted 50% with tap water. Intravenous PN was commenced within 3 h of surgery with an infusion rate of 5 mL/kg/h, on day five it was terminated. PN solutions were based on Nutriflex lipid plus (Braun, Melsungen, Germany) and Vamin (Fresenius Kabi, Uppsala, Sweden) with the complete mix containing energy (3.123 kJ/L) amino acids (45 g/L), glucose (72 g/L), lipids (31 g/L), as described previously⁹⁵.

Complementing the analysis described above, measurements of blood-glucose before, during and after surgery as well as acid-base before and after surgery were performed.

The piglets were daily weighed and clinical status was assessed at each feeding (feeding intolerance, volume and consistency of stoma output, abdominal distension and signs of circulatory distress). The piglets were euthanized if signs of discomfort or illness occurred (hemodynamic

instability, hypothermia, small intestinal dysmotility, dehydration, respiratory distress and peritonitis). At euthanasia standard autopsy was performed.

Statistics:

Statistical analyses were performed using non-parametric analysis, Mann Whitney Rank sum test. Data are presented as mean \pm standard error of mean (SEM) if not otherwise stated. Statistics was performed using Sigma Stat for Windows Version 3.0 (Jandel Corporation, Erkrath, Germany). A probability of $p < .05$ was considered significant.

Study II:

The effect of MEN with formula or bovine colostrum was tested in neonatal term piglets as a model of SBS. The concept of MEN is minute volume of feeds enough to stimulate mucosal growth, promote peristaltic movement and stimulate endocrine effect. MEN did not provide enough calories to cover basal metabolic rate (BMR) why supplementation with PN was inevitable. A mixed formula (Energy 4.461 MJ/L Protein 0.054 Kg/L, carbohydrate (CHO) 0.054 Kg/L and Fat 0.071 kg/L) or colostrum (Energy 5.28 MJ/L, Protein 0.013 Kg/L, CHO 0.042 Kg/L and Fat 0.068 Kg/L) enteral feed as MEN, was tested to purely TPN feeding.

Design:

Thirty, two day old piglets were delivered to the animal research unit, in a fasting state ready for surgery. After insertion of jugular catheter and orogastric tube they were allowed to acclimate. Enteral feeding with a milk replacer was given via the orogastric tube (15 ml/kg/3hour), PN was commenced (4 ml/kg/hour) six hours before surgery while piglets were fasting. Three day old they had an intestinal resection performed. The piglets were subsequent stratified to either formula (PN-FORM, n = 9), colostrum (PN-COL, n = 5) or a purely TPN nourished control group (TPN, n = 10), according to birth weight (Figure 3).

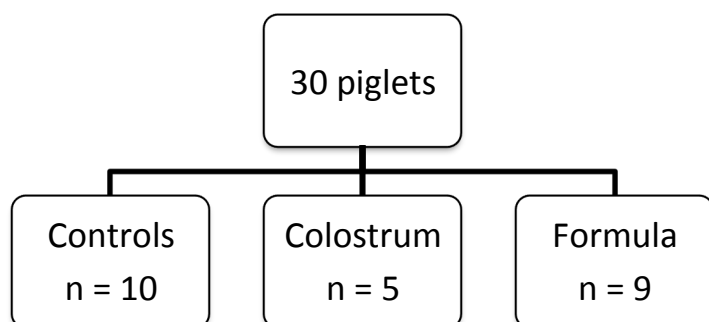


Figure 3: Surviving piglets available for analyses after stratification.

Oral feeding was commenced 1-3 hours after surgery, initially 2 ml/kg/3hour increasing to 4 ml/kg/3hour the second day, 8 ml/kg/3hour from the third day onwards. At day 5 after resection, a nutrient and wet weight balance study was performed and concurrently oral feeding was increased to 10 ml/kg/2hour. TPN and PN-FORM piglets were fed formula during the balance study and PN-COL piglets were feed colostrum. PN infusion was continued at 4 ml/kg/hour after resection, increasing to 6 ml/kg/hour after 12 hours and further to 8 ml/kg/hour after 24 hours and onwards. Saline infusions was initiated 12 hours after surgery and continued at a rate of 2 ml/kg/hour apart from the day of balance study where it was increased to 4 ml/kg/hour.

Statistics:

Statistical analyses were performed using one way ANOVA on Ranks (Kruskal-Wallis). Data are presented as mean \pm standard error of mean (SEM) if not otherwise stated. Statistics was performed using Sigma Stat for Windows Version 3.0 (Jandel Corporation, Erkrath, Germany). A probability of $p < .05$ was considered significant.

Human studies:

The human studies were approved by The Danish Regional Committee on Biomedical Research Ethics (No: S-20090089 and No: S-20090081). The randomised controlled trial (study I) was commenced April 2010 and is still ongoing, the randomised double-blind cross-over trial (study II) was conducted between December 2009 to February 2012. All procedures were conducted in accordance with the Helsinki Declaration II. After verbal and written information, parents consented, their child, to participate in the study. The medical treatment and provision of parenteral support was managed according to local departmental standards.

Anthropometric measurements:

In both studies, short time growth was assessed in children (0 - 4 years of age), using a portable knemometer. Prior to enrolment of the participants, the author trained the assessment method, and an intrapersonal error of 0.2 mm was accepted. Embedded in the left lateral position, 5 measurements on the right lower leg were performed, between the hours of 01:00 to 03:00 pm, the lowest and highest value was excluded and the mean value of the remaining was used for calculations. Measurements of weight were performed using the same equipment each time. (Young children: SECA model 717; older children: SECA model 701, both by SECA Germany). Head circumference was measured in children younger than 5 years of age. If the child was unable to stand, a standardised measurement gauge was used to measure length while they were lying on their back; otherwise it was measured in an upright position.

Laboratory analysis:

In both studies III and IV, analysis of haemoglobin, leucocytes, C-reactive protein, erythrocyte-sedimentation-reaction (ESR), platelet count, urea, creatinine, sodium, potassium, magnesium, glucose, alanine-transaminase, alkaline-phosphatase and acid-base balance was measured. Each

measurement was done according to Odense University Hospital laboratory standards. IGF-1 and IGF-BP3 analyses were performed at the Section of Growth and Reproduction, Copenhagen University Hospital-Rigshospitalet, Denmark⁹⁶.

Study III:

Design:

Infants were after major abdominal surgery invited to participate in a randomised case-control study testing bovine colostrum to enteral nutrition after standard procedures. Inclusion criteria were: children older than one week and younger than two months with a need of PN for more than 1 week after intestinal surgery. Children who were predisposed to cow milk allergy were excluded.

During a study period of 8 weeks, children randomised to bovine colostrum had 50% of their enteral nutrition replaced with colostrum for four weeks while children randomised to the control group had enteral nutrition after standard procedures (mother's milk or formula). The following four weeks colostrum replacement was discontinued (figure 4).

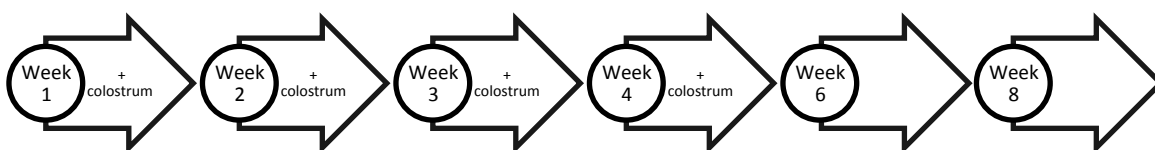


Figure 4: Duration of study III. Cases were supplemented with colostrum the first four weeks. All infants were followed for 8 weeks.

Anthropometric measurements and blood analysis were described above. In addition, three samples of faeces per week and two plasma samples were stored at -20°C for later bacteriological of faeces in plasma, p-citrulline analysis.

Duration of PN, incidences of vomiting and infection were noted as well as duration of hospital admission.

Statistics:

A power calculation was performed. A power of 80% and a level of significance at .05 was used to assess sample size, using Altman's monogram (Altman 1981). A desired weight gain of 200 g per week and a reduction of mean duration of PN supply from 7 to 5 days required 70 and 60 participants respectively. We chose to aim at inclusion of 80, 40 in each group, and leaving room for dropouts. With this sample size, we assumed that infants would be equally dispersed in terms of GA, weight and underlying condition.

Study IV:

Design:

All Danish children older than one year of age with SBS (WHO ICD-10 diagnosis of SBS, DK91.2B) were invited to participate in a randomised double-blind, cross-over trial testing bovine colostrum compared to a locally prepared milk-mixture. Inclusion criteria were: primary surgery more than one year prior to inclusion and a need for nutritional supplementation, with PN, enteral nutrition or both. Children with documented cow milk allergy or enlisted for intestinal transplant surgery were excluded.

During a study period of 12 weeks, 20 % of the children's basal fluid requirement (BFR)³² was replaced with colostrum or milk-mix in two 4-week periods in a random and double-blind order. The two 4-week periods were separated by a 4-week wash-out period. The children were hospitalised four times (figure 5): at first baseline (1), after first replacement (2), at wash-out (3, baseline 2) and after second replacement (4). Information on parental observed changes in faecal composition, significant changes in nutritional composition and medication were obtained on admissions. Blood sampling, knemometry and anthropometric measurements were performed together with a 48-hour nutrient, fluid and electrolyte balance assessment at each hospital stay.

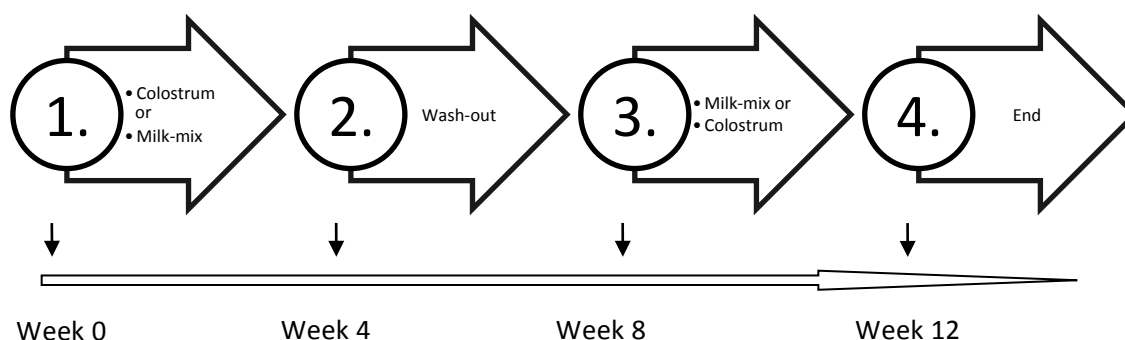


Figure 5: Design of study IV.

Each 48-hour wet weight and energy balance study commenced at 1 pm with measurement of body weight, changing of diaper and initiation of nutrient and fluid registration. Children were allowed free oral intake and to continue their usual basic enteral feeding. Children with oral intake were provided with an electronic weight (measuring in grams) and a container, in which they were instructed to collect a duplicate of all oral intakes (both liquids and solid foods). In children with a naso-gastric or a percutaneous endoscopic gastrostomy tube (PEG-tube) oral intake was weighed, and a batch of the nutritional supply was collected as a control. Any additional intake of energy containing fluids (e.g. milk products or juices) was collected as a duplicate. Foods and beverages comprising the total wet weight were stored at -20°C . In children wearing diapers, stools were collected in cotton diapers, weighed and stored at -20°C . One child was able to defecate in a pot. Prior to analysis, the collected faeces were washed out in minimal amount of distillate water and lyophilized. Urine output was collected in stoma bags (ID 2240 Coloplast, Humlebæk, Denmark).

Calculations: Weight of the 48-hour oral intake, faecal loss and urinary output was measured. Absolute wet weight absorption was calculated by subtracting wet weight of stools from oral intake and the relative wet weight absorption as a coefficient expressed as a percentage of total wet weight intake. Absolute intestinal energy absorption was calculated as equivalent to the difference between ingested and excreted energy and the relative absorption as a coefficient expressed as a percentage of total energy intake. Basal metabolic rate (BMR)³¹ was calculated and energy content of parenteral nutrition was calculated from the information given by the hospital pharmacies. Energy content of formulas was analysed in our laboratory as previously described. Creatinine clearance ($\text{ml}/\text{min}/1.73 \text{ m}^2$) was calculated using p-creatinine, age, gender and height⁹⁷.

The colostrum used was previously described in this thesis. The milk-mix was prepared from semi-skimmed milk, cream and whey protein (Lacprodan* DI-9224, Arla Food

Ingredients, Aarhus, Denmark) ensuring that the ratio of the contents of protein, fat and energy were comparable (0.011 kg/L of protein, 0.004 kg/L of fat, 0.005 kg/L of carbohydrate and 0.468 MJ/L of energy) to the contents in the colostrum (0.011 kg/L of protein, 0.005 kg/L of fat, 0.004 kg/L of carbohydrate and 0.546 MJ/L of energy). The mixture was frozen and stored at -20°C in containers identical to colostrum containers. The daily intake of replacements was noted in a diary and kept identical in both of the two times 4-week periods.

Statistics:

Statistics: Descriptive data were expressed as mean \pm standard deviation (SD). Wilcoxon Signed Rank test was used to analyse effect of colostrum and milk-mix compared to baseline 1 and 2. Mann Whitney Rank Sum test was used to compare the two diets and SBS-IF with SBS-II patients. Statistics was performed using Sigma Stat for Windows Version 3.0 (Jandel Corporation, Erkrath, Germany). A probability of $p < .05$ was considered significant.

Results:

In the following section, the presented results represent a selection of results from the four studies. The remaining results could be found in the appended papers/ paper manuscripts. Study III remains in progress why presented data are descriptive.

Study I:

In the study of a novel SBS model in preterm and term piglets with a jejunostomy 33 piglets were included. Of the 33 piglets, 14 preterm and 10 term piglets were stratified for day 2 SBS surgery while 6 preterm and 3 term piglets were stratified for day 0 controls. Pigs that showed clinical signs of distress, severe dehydration or irritated skin due to leakage from the jejunostomy were euthanized, while the remaining piglets were euthanized for tissue collection at 6-7 days (n = 6 preterm, n = 8 term). In general, preterm piglets were more sensitive to anaesthesia, surgery and SBS. If piglets were euthanized ahead of study termination, this was due to clinical complications including hemodynamic instability, hypothermia, SI dysmotility, dehydration, respiratory distress and peritonitis.

Two days of enteral nutrition stimulated a trophic response and mucosal dimensions were significantly improved in terms of villus height and crypt depth as well as proliferative cells (Ki67 positive nuclei) in preterm and term. In both groups, these results were affected when inducing SBS. Villus height decreased significantly from day 2 to day 6-7 in both preterm (853 ± 25 to $352 \pm 26 \mu\text{m}$) and term piglets (886 ± 35 to $687 \pm 24 \mu\text{m}$), Ki67 index decreased in term piglets (0.51 ± 0.02 to 0.45 ± 0.02) whereas crypt depth increased in term piglets (65 ± 3 to $78 \pm 4 \mu\text{m}$). Both crypt depth and Ki67 were unaffected in preterm piglets after resection. When compared, intestinal adaptation after resection was not as improved in preterm as in term piglets evaluated by morphology, stereology and activity of digestive enzymes (figure 6).

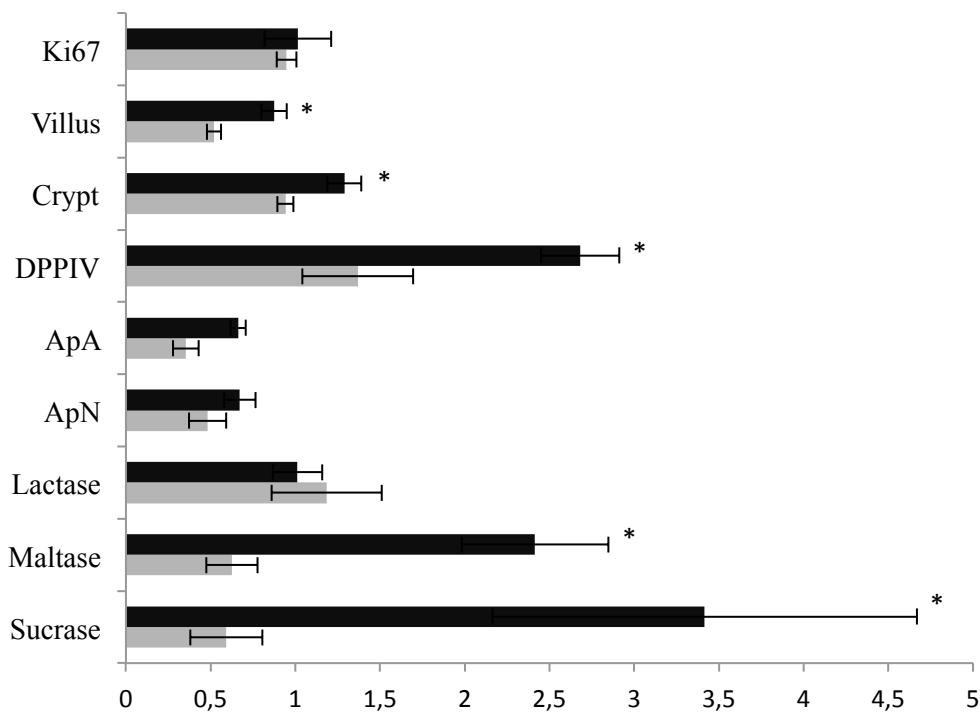


Figure 6: Responses of the mid small intestine to resection on day 2 followed by 4-5 days of parenteral/enteral feeding in preterm (light gray bars) and term (black bars) pigs. Values for intestinal morphology (villus height, crypt depth), cell proliferation (Ki67) and digestive enzyme activities in the Mid-P sample at 6-7 days were divided by the corresponding value in the Mid-D sample at the time of resection at 2 days. 1.0 = no change in mean value during the 4-5 days after resection, *P < 0.05 for term versus preterm pigs.

In conclusion, resection of terminal ileum depresses intestinal adaptation in term and preterm piglets, although most pronounced in preterm. An animal model of preterm as well as term infant SBS was successfully developed.

Study II:

In the study of ability to stimulate intestinal adaptation following intestinal resection with MEN of colostrum and formula, 24 of the 30 piglets completed the study (TPN n = 10, PN-COL n = 5 and PN-FORM n = 9). None of the colostrum fed animals experienced vomiting, increased stoma output, infections related to surgery, casein plugs in the gastrointestinal tract and no one showed signs of discomfort during the study period.

The relative absorption of Na^+ was significantly higher than in PN-COL compared to PN-FORM and TPN piglets. Relative wet weight absorption was significantly higher in PN-COL compared to TPN piglets and with no difference between PN-COL and PN-FORM (figures 7 and 8).

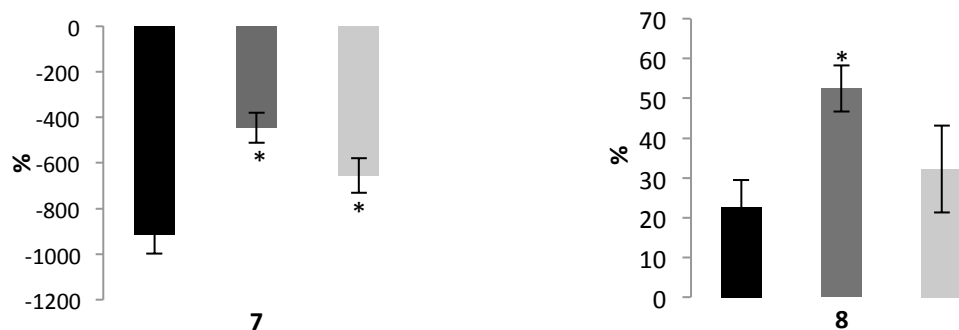


Figure 7: Relatively Na^+ absorption in %. Black bars: TPN group, dark grey bars: PN-COL and light grey bars: PN-FORM. * Above bars indicate $P < 0.05$ when compared to the TPN group.

Figure 8: Relative wet weight absorption in %.

In conclusion, colostrum was well tolerated in piglets with induced SBS when feed as MEN. Efficacy of MEN on intestinal adaptation, evaluated by nutrient and wet weight balance studies, activity of digestive enzymes and morphology stimulated intestinal adaptation and colostrum improved some of the cornerstones in management of infant SBS; maintenance of fluid and electrolyte balance.

Study III:

In the randomised controlled study of colostrums effect on intestinal adaptation after major intestinal surgery, evaluated by duration of PN need, time to exclusively enteral nutrient intake, growth, and duration of hospital stay, a consecutive series of 10 infants was evaluated. Five of the ten were randomised to colostrum replacement of these, one showed clinical symptoms of allergy (emesis and diarrhea without fever) why replacement was prematurely terminated and one dropped out on parent's request. All five control infants completed the study. Mean GA was slightly different within the groups, causes for surgery were similar, and subsequent severity was reasonably uniform, indicating that the block randomisation allocated the infants uniformly (table 2).

ID	Gender	GA	BW (g)	BL (cm)	HC (cm)	Protocol
1	Girl	34+1	1820	45	31,5	Control
2	Boy	32+0	1731	44	31	Control
3	Girl	33+2	2147	48	34	Control
4	Girl	33+3	3350	50	34	Colostrum
5	Girl	38+0	2800	49		Control
6	Boy	39+4	3600			Colostrum
7	Girl	34+0	2105	42		Control
8	Girl	27+0	740			Colostrum
9	Boy	39+4	3500	57		Colostrum

Table2: Demographics at birth of the consecutive 10 infants included in the randomised case-control study. Data were descriptively presented when evaluation the tolerance to colostrum.

Premature termination of colostrum, in the one infant with clinical suspicion of allergy, did not lead to cessation of symptoms. Subsequent examination revealed no cause of the gastrointestinal symptoms and later they disappeared without treatment or explanation. In this case, specific IgE for milk remained stable and at levels below cut-off value compatible to allergy. Neither of the remaining infants who received colostrum experienced increase in specific IgE for milk. One of the control infants, who were feed non-hydrolyzed formula, experienced increasing IgE at termination of the study.

In conclusion, a randomised controlled study with at least 80 infants testing efficacy of colostrum with regard to PN weaning and improved growth seems safe.

Study IV:

In the randomised double-blinded cross-over trial testing efficacy of colostrum on improvement of intestinal function nine of 12 possible patients (four girls and five boys) accepted to participate and completed the study. They all had colonic continuity. Eight patients had undergone small intestinal resection during the neonatal period, due to intestinal atresia (n= 2), gastroschisis (n=3) or necrotizing enterocolitis (n=3), one patient had undergone resection at 9 years of age due to mid-gut volvulus. Four of the nine children received parenteral nutrition and six received enteral supplementation with formula, in addition to habitual diets. None had dietary restrictions.

It was possible to estimate the severity of intestinal function, evaluated by nutrient and wet weight balance studies, in the nine children with SBS. They were divided by need for PN, all PN dependent children fell in the same lower left quadrant why, a cut-off value of 150% of BMR and 50% of BFR was set from this estimation (figure 9).

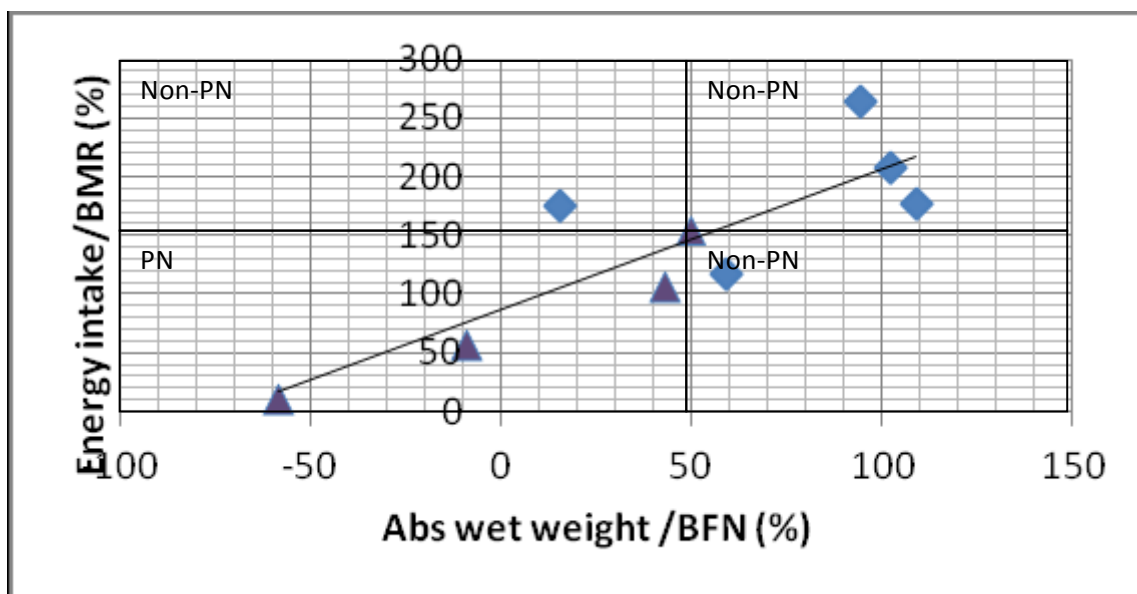


Figure 9: Intestinal function in Non-PN (diamonds) and PN (triangles) dependent children with SBS. Enteral energy intake in percents of basal metabolic requirements (BMR) was below 150% and adsorbed fluid in percents of basal fluid need (BFN) was below 50% in all PN-dependent children, with no outliers.

In conclusion, efficacy of colostrum on intestinal function, evaluated by nutrient and wet weight balance studies and anthropometrics supported by IGF-1 and IGF-BP3, did not improve intestinal function in children with SBS. Children with SBS could be categorised by intestinal function and a suggestion for a cut-off value for need of PN was estimated.

Discussion:

Study I and II:

In a model of infant SBS, surgery and the later clinical condition concomitant to induced SBS was most critical for the preterm piglet in terms of e.g. hyperglycaemia, hypothermia, dehydration, SI dysmotility and post surgery infection. There was a significant stimulation of mucosal dimensions and proliferative index after two days of enteral feeding, in both preterm and term piglets. After surgery this trophic response was affected with a subsequent decrease or stagnation, in villus height, crypt depth and proliferative index. When comparing preterm to term, mucosal dimensions as well as digestive enzyme activity was most pronounced in term piglets at all times. In conclusion, prematurity reduced adaptation after intestinal resection.

Using the SBS jejunostomy model, efficacy of MEN with colostrum and formula was tested in term piglets and controlled to purely TPN nourished SBS piglets. Colostrum feeding was well tolerated in piglets, there was no negative impact on intestinal transit time (diarrhoea or constipation) and incidence of emesis was low. Colostrum fed piglets had significantly improved wet weight absorption and less Na^+ secretion from the intestine. Absorption of other micro- and macronutrients was without difference between the groups. After resection, colostrum fed piglets had significant longer villi while formula fed piglets had deeper crypts. Enzyme activity increased from resection to euthanasia in two out of six evaluated, but with no significant difference between groups. In conclusion, MEN did stimulate intestinal adaptation and colostrum improved essential elements of the maintenance of fluid and electrolyte balance.

A model of preterm and term SBS with a jejunostomy was the novelty of study I. Previously a model of infant SBS has been developed in both term neonatal or juvenile piglets as well as juvenile rodents^{64;65;98;99}. After infliction of SBS, a subsequent intestinal adaptation is vital

to preserve ability to digest and absorb nutrients and fluid. Supply of enteral nutrients, aims to promote maturation and growth of the small intestine in the newborn.

At birth, villus height corresponded to half the final height (approximately 800 μm) in preterm and term piglets, as shown in study I. Crypt depths were approximately one third of final depth, though somewhat deeper in term compared to preterm. These results corresponds the results of others studies as does the results of trophic responds to two days of enteral feeding^{19;67;100;101}. Subsequent to resection there was little or no change in mucosal dimensions. The intestine tends to flatten when not stimulated but responds fairly rapid to nutrient stimulation^{100;102}. In the neonatal piglet, villus height and crypt depth in the jejunum achieves normal configuration after few hours of enteral stimuli with 60 % or more of total nutrient intake. When provided less than 60% of total nutrient intake, enteral nutrient stimulation seems to promote little or no response, comparative to intestinal conditions of a piglet purely given intravenous nutrients⁶⁷. This may explain why the mucosal dimensions after resection in study II, did not correspond to the results of the term piglets in study I. Previous studies of infant SBS have shown that subsequent to resection, there was an increase in villus height and crypt depth. These piglets had colonic continuity and primarily ileum was preserved, in piglets without ileum, the results were less pronounced^{64;65}. These results were much similar to ours, indicating that length of preserved ileum correlates to later outcome.

Enteral feeding promoted activity of digestive enzymes in 6/6 investigated (sucrase, maltase, lactase, ApN, ApA and DPP-IV) in preterm and term piglets. These results corresponds to the results of others^{100;101;103}. Subsequent to resection this activity increased further in 4/6 and 2/6 investigated in term and preterm piglets, respectively. In study II, activity of sucrase and maltase increased in all groups, though most pronounced in TPN nourished piglets. Sucrase and maltase are present from birth and enables digestion of other carbohydrates than lactose. Initially activity is low, but total activity increases after few hours of feeding¹⁰⁴. Others have found that disaccharidase

increased following resection and that this response could be stimulated by colostrum^{68;70;105}. In neither of the studies I and II did lactase increase remarkable when measured across the mid part of the intestine. Lactase activity increases towards the end of foetal life and is abundant in the proximal part of the intestine⁹⁴ and throughout the perinatal period it serves as the most important disaccharidase responsible for degradation of lactose why activity decreases after weaning¹⁰⁵. Previously it has been shown that parenteral nutrition diminish digestion of lactose¹⁰⁶ and that enteral intake below 80% of total nutrient intake does not stimulate lactase activity sufficiently⁶⁷. This might explain why lactase activity did not change after resection in both study I and II.

In attempt to improve adaptation, effects of e.g. colostrum, GLP-2, Butyrate and EGF have been investigated. Though results were not consistent, these factors improve adaptation in respect of morphology, circulating hormones that stimulate intestinal growth (GLP-2, IGF-1 and binding proteins) and growth of intestine^{66;68;69;71;107}. Improved gut function may also be assessed by nutrient balance studies, a laborious method. An alternative method could be 3-0-methylglucose (3-0-MG) uptake. In rats, these methods have been performed when assessing the absorptive capacity after intestinal resection and induced SBS while lactulose-mannitol ratio (lac/man ratio) assessed permeability. 3-0-MG correlated well to energy, protein and carbohydrate uptake as well as surface area¹⁰⁸. When GLP-2 was administered to juvenile rodents, intestinal dimensions improved at different sites, 3-0-MG increased while lac/man ratio decreased, indicating improved absorptive capacity with impaired permeability¹⁰⁹. When applied to piglets these results were essentially confirmed^{75;107}.

Evaluation of study I and II:

The two experimental studies were designed as stratified studies to take into account, an equitable balance within the respective groups. This balance could be modified if uneven dropout occurred. When using preterm piglets this risk increases due to greater vulnerability. In study I, two litters of preterm piglets was used and compared to one litter of term in attempt to achieve an equal number of observations in the two groups and to avoid the “litter effect”. In study II the piglets were obtained from 3 different litters and piglets were stratified according to birth weight, for the purpose to take confounding into account. Unfortunately, there was an unequal dropout within the groups, and thus a smaller number of observations of colostrum supplemented piglets. The initial statistical analysis took the 3 litters into account (two-way ANOVA), but found no litter effect, why subsequent analysis were performed as ANOVA on Ranks.

The strengths of the two experimental studies were the possibility to develop and test a model of infant SBS and a treatment in a controlled and uniform setting. It provided knowledge of the impact of the condition and the treatment on an anatomical as well as a physiological level. It is well known that piglets resemble infants in terms of anatomy and physiology to a greater extent than rodents^{6;110}. Using a 92% preterm piglet simulates the clinical situation of a preterm infant subjected to surgery, where as the term piglet resembles an infant, with a minor risk of need for surgery. As such, the information obtained from these preterm piglets, contributes to the sparse knowledge we have about the preterm infant with SBS.

Due to vulnerability of the preterm piglets there was a considerable loss of material but the experience gained from study I have enabled the group and perhaps others to reproduce the model. In study there was an unequal dropout why stratification became unbalanced and numbers of observations in one of the groups nearly made statistics worthless. In general, confounding could

only be avoided if multiple litters were included in both studies, which would be unethical. We could have included other analysis. In study II, morphology tests could have provided information of the conditions before resection and analysis proliferative nuclei (Ki67) to further provided information of the changes and adaptation to resection.

Study III and IV:

In the comparative human study III, no infants experienced increasing specific IgE to milk after intake of colostrum and colostrum was well tolerated. Mean weight at inclusion and increment during the study period equalled in the two groups, as did causes for surgery (NEC, atresia and gastroschisis).

In the randomised double-blind cross-over study comprising apparently stable SBS patients, a possible effect of colostrum could not be demonstrated using nutrient and wet weight balance study. Results from the nutrient and wet weight balance study showed that PN patients absorbed less than 150% of their BMR and less 50% of their BFR. As demonstrated in figure 5, all non-PN were located outside the lower left quadrant why it seems reasonable to use the results as cut-off values for PN need.

Numbers of studies in children with SBS is sparse, partly due to relatively low prevalence in most countries and partly because the disease and the related treatments had to be uncovered by studies in animals and adults at first. Most studies in children have focused on ways to promote adaptation and as such improve intestinal function. Treatment with high dose of GH reduced need for PN during treatment but weaning of PN was only possible in few case after cessation of GH (n=14, n=8 and n=12). Children gained weight⁸⁴, energy intake and relative energy absorption increased during treatment and stayed improved⁸⁶ and IGF-1, IGF-BP3 as well as p-citrulline concentrations increased during GH treatment with a subsequent drop to baseline

values^{86;87} following termination. Orally administered EGF in children with SBS for six months following primary surgery (n=5) improved tolerance to enteral feeds and intestinal absorptive capacity⁷². In this study, absorptive capacity was evaluated by 3-O-MG, a method that correlates well to nutrient balance study results in humans¹¹². Bovine colostrum contains high concentration of GH, IGF-1 and 2 but lower concentrations of EGF^{9;113-115}. In study III there was an increase in IGF-1, without an increase in IGF-BP3, in study IV neither of the two was affected. The concentration of the different growth factors were not assessed in the colostrum used in the studies, but it would unlikely match the concentrations used in the above mentioned studies.

Serum-Urea increased within the first week of supplementation in study III, which may be explained by the increased protein intake that colostrum provides in these infants. Mean growth increment was greater than in non-colostrum replaced infants and these observations correspond well to one previous study of fortification with cow milk based fortifier to preterm, This reported increased values of s-urea to correlate to improved catch-up growth¹¹⁶ with no increased incidence of specific cow milk allergy¹¹⁷. week of colostrum replacement in study III, the overall growth increment during the study was greater than in control children, but due to small sample size, statistical analysis were inconclusive.

In adults, efficacy of bovine colostrum was assessed using nutrient and wet weight balance studies, DXA-scan, measurements of hand strength and lung function in addition. Colostrum increased muscle strength and lean body mass but had no effect on nutrient or wet weight balance¹¹⁸. Latest research have shown improved intestinal function in regards of reduced stoma output, maintenance of electrolyte balance at lower intake, increasing lean body mass, villus height and plasma-citrulline levels when treated with GLP-2^{119;120} and in 2012 FDA recommended GLP-2 as a standard treatment to adults with SBS.

In respect of the new definitions recently suggested by a panel of experts⁴⁴, we characterised the nine children with SBS participating in study IV by using nutrient and wet weight balance studies. They were grouped according to dependency of PN and their ability to absorb energy and fluid in relation to basic need and a cut-off value for need of PN was suggested. Children with values below the cut-off values could be characterised as SBS-IF patients and the remaining as SBS-II. A cut-off value of 84% of BMR and 1.4 kg/day of wet weight was found in adults using a similar method¹¹¹. This study comprised 89 patients and our cut-off value was based on results obtained from nine patients. Anyhow, it seems fairly reasonable to rely on these, since they were consistent in all PN-dependent patients.

Evaluation of study III and IV:

Both human studies were designed using block-randomisation conducted before study commencement. Comparable groups were ensured and confounding prevented by randomisation in study III, while information bias was avoided by adding a double blinding in study IV. In study III, parents were not informed of which group their infant would belong to ahead of decision. Age at inclusion took into consideration that mothers' colostrum was preferred, before replacing half part of enteral intake with bovine colostrum. It was not possible to blind this study because the parents, in most cases, were responsible of feeding the infants themselves. In study IV, the sequence of replacements was randomised and double-blinded. The two different replacements were marked and extradited by the assisting nurse, decoding occurred when all participants had completed their study period.

Based on hospital records from the previous 5 years (2003-2008) it was expected, that the desired number of patients, needed for study III, could be included within 2 years. Unfortunately, the actual

number of infants born with a subsequent need for major intestinal surgery did not reach the expected number. In addition, recruitment was hampered, primarily due to the parental stress at time of inclusion, along with their reticence towards bovine colostrum. Reticence was partly explained by, the mother's fear of reducing her milk production and partly by recommendations from the Board of Health regarding cow milk intake before six months of age.

The prevalence of SBS children in Denmark is limited by low incidence. The aim of the study was to improve intestinal function, and taking this into account, calculations of sample size should be based on the recovery study. With a power of 0.80 and a risk of type 1 error of 0.05, a change to be detected of 0.20 and SD=0.20, a sample size of 18 would be required, using Sigma Stat for Windows Version 3.0 (Jandel Corporation, Erkrath, Germany). The strength of study IV was the cross-over design within a limited period of time and the fact it was double-blinded. In attempt to avoid a carry-over effect a wash-out period was incorporated. In regard of relative absorption of energy, results were controlled for carry-over effect. Though not precisely balanced there was no difference whether one replacement was given before the other. Was formula given at first, there were a negative difference of 3%, though not statistical significant.

Evaluation of nutrient and wet weight balance studies:

Nutrient and wet weight balance studies has been well implemented in the clinical evaluation of intestinal function in adults with SBS and provides important knowledge of how treatments affect the capacity of the intestine. This analytical method was previously found to be of high precision and reproducibility¹²¹. Rarely adult SBS patients have colonic continuity, why collection of output can be with little waste and food intake can be uniform at each time why the balance study can be conducted completely precise. To perform a 100% controlled balance study would not be possible in children wearing a diaper as demonstrated in the recovery study.

Nutrient balance studies have been used to assess correlation of remnant intestinal length to GLP-2 levels in infants after major abdominal surgery. In this study a non absorptive diaper was used⁸⁸. We were not able to use a diaper for older children that were totally non absorptive. In studies of infants and children with cystic fibrosis that was wearing diapers a dental bib was used as a non-absorbent diaper^{122;123}. There were no indications of recovery studies in neither of these why assumed these were conducted much similar to our piglets study. In study II, piglets wore stoma bags during the nutrient and wet weight balance study but, adherence of the bags was difficult to secure and they were often leaking. Upon the bags, they wore body stockings with a known weight. Any leakage of output was caught in the body stockings, and by adding the subsequent weight difference to volume obtained from the stoma bags, the total volume was known. The study was therefore thought of as 100% controlled.

Both experimental and human studies, could have supplemented the nutrient balance studies with 3-0-MG and indirect calorimetry¹²⁴, but nutrient balance study was believed to provide the most correct and detailed information of intestinal function. It should have been complemented by measuring intestinal transit time, using a Brilliant Blue marker at baseline.

Evaluation of anthropometrics:

Knemometry is well known as a research tool used to assess short-time growth in both infants and children. In study III and IV, a portable knemometer was used to assess short-time growth and measurements were only performed by the author of this thesis, to ensure a uniform result. To optimise these, a knemometer with an electronic scale could have been used and measurements performed in a blinded set-up.

To complement the other anthropometric assessments, implementation of DXA-scans was tried. In the youngest children, this was abandoned in more cases, since the children could not be brought to rest.

Evaluation of intestinal adaptation and function:

Studies in both adults and children with SBS has shown that p-citrulline correlates positively to post surgical remnant intestinal length, predicts duration of PN dependency and by monitoring it provides information of ongoing adaptation as it reflects the functional enterocyte mass¹²⁵. Plasma-Citrulline could have been a complement to our results in all four studies. In study III and IV, biopsy of the intestine could have provided information about the morphology of the intestine in infants and children. This could have provided information of adaptation and intestinal function of the intestine in neonates and as a consequence to SBS.

A general evaluation:

This thesis presents results from a translational study. The strength was the possibility to test a hypothesis in an experimental model before the human infant and child. The animal model provided valuable information of anatomical and functional intestinal adaptation that must be assumed to resemble this process in an infant. Using similar methods in both experimental and human studies makes comparison more reliable. Safety of colostrum was found and a rigorous design was used to assess efficacy. Weaknesses were primarily found within the human studies, in study III inclusion could not be terminated and in study IV, the available population with SBS (n=9) was not the appropriate number to detect a significant difference (n=18).

Conclusions and Perspectives:

A model of infant SBS in both preterm and term piglets was successfully developed. It provided valuable information about the adaptive process in preterm and term piglets, and results indicated that presence of ileum is important for this process. This result has clinical relevance for the later outcome after intestinal resection. When nourished with colostrum as MEN, wet weight balance was improved and loss of Na^+ by the intestine was decreased. Enteral nutrition

corresponding to 50% of total nutrient intake did stimulate adaptation, in terms of morphometry, digestive enzyme activity and improved nutrient uptake. In children with SBS, nutrient and wet weight balance studies provided information of the intestinal function. Bovine colostrum did not improve intestinal function in regards of improved uptake of energy and nutrients in children with SBS, under the present regime. But the methodology used was possible and should be used in future studies of the subject. Bovine colostrum was tolerated in infants after major abdominal surgery and none of the children developed allergy.

Future studies should focus on improved adaptation in infants and children as well as intestinal function in children with SBS as a consequence of anatomical or functional loss. The methodology used in the present study should be used when evaluating efficacy, but ought to be supplemented by some of the previously suggested methods. Clearly one should aim for a larger sample size e.g. by multicenter studies. Enteral nutritional stimuli will probably not provide enough change in intestinal function to make any significant difference. One should therefore aim for investigating factors, with known adaptive effect.

Recently U.S. Food and Drug Administration (FDA) have recommended GLP-2 treatment for adults with SBS. It would be of great interest to test GLP-2 in both infants and children with SBS. Prior to this, a dose response study should be performed in piglets before application of GLP-2 to children and infants. Initially children dependent on PN would be of interest, but infants in the initial adoptive phase would most likely also benefit of GLP-2 treatment. Other intestinal growth mediators will be of interest as well.

As a consequence of my close contact with the participating families, particularly in study IV, it was natural for me to consider whether our current organization was the optimal. I learned that, being a parent to a child with SBS, without a doubt put the families under a lot of

pressure from time of surgery to stagnation of the disease, and even then do they only just trust their child will survive. There is a lack of knowledge about the quality of life within these families. Of course we are aware of their situation, since we know these families well, due to long duration of hospital admissions and their subsequent myriad of hospital contact. In attempt to optimize these families everyday life our organization should consist of a team of experts, including representatives from several groups of personnel: paediatricians, surgeons, nurses, dietitians, psychologists and social workers, all with an interest and an understanding of the complexity of their situation. Education, dedication and number of patients would be important. In a country with very few patients there should probably be only one expert team with the overall responsibility for all children. It should be possible to interact with the families in their own home, either by visits or contact in other forms (cyber space, telephone), in attempt to maintain daily life as normal as possible. Families should be able to meet, to share experiences and concerns in a human environment, also including e.g. grandparents since these have the same concerns but were rarely taken care of.

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