

**“FEDERICO II”
UNIVERSITY OF NAPLES**



**PhD Program
“Human Reproduction, Development and Growth”**

**Director
Prof. Claudio Pignata**

PhD Thesis

***“Gastrointestinal motility disorders in children: ontogenesis,
patophysiology, epidemiology, clinical features and
management”***

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Academic Year 2006-2007

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INTRODUCTION

Pediatric functional gastrointestinal disorders (FGIDs) include conditions in which a variable combination of often, age-dependent, chronic, or recurrent symptoms, such as vomiting, constipation, abdominal pain, are not explained by structural or biochemical abnormalities.

As the child is programmed to develop, some functional disorders which occur during childhood accompany normal development, or may triggered by age appropriate but maladaptive behavioral response to internal or external stimuli. According to a biopsychosocial conceptualization of the pathogenesis and clinical expression of the FGIDs, early in life and genetics, in addition to environmental factors such as family influences on illness expression, abuse, major losses, or exposure to infections, may affect one's psychosocial development in terms of one's susceptibility to life stress or psychological state and coping skills, as well as susceptibility to gut dysfunction, abnormal motility, altered mucosal immunity, or visceral hypersensitivity. Furthermore, these "brain-gut" variables reciprocally influence their expression. Therefore, FGIDs are the clinical product of this interaction of psychosocial factors and altered gut physiology via the brain-gut axis (1).

Genetic factors may predispose some individuals to develop FGIDs, whereas in others, environmental factors contribute to the phenomic expression of these conditions, as well as patient attitudes and behaviours (including health care seeking) relating to it. Some infants may inherit a genetic susceptibility to FGIDs characterized by a particular gastrointestinal reactivity to stress. This temperament-sensitive reactivity seems to be associated to other biological systems such as the cardiovascular, neuroendocrine and immunologic (2). Several pathways may be involved in this genetic predisposition, including lower levels of IL-10 (an anti-inflammatory cytokine) in some patients with IBS (3) that may effect gut mucosal neural sensitivity, serotonin reuptake transporter polymorphisms that can effect levels of 5-HT neurotransmitter, or the response to 5-HT blocking agents (4,5) g-protein polymorphisms that can affect both CNS and gut-related actions (6) and 2-adrenoreceptor polymorphisms that affect motility (7). Serotonin reuptake transporter polymorphisms have effects on mood disturbances (8) and may be a genetic link to disorders of brain-gut function such as IBS. This represents an interesting area for future studies.

The aggregation of FGIDs in families (9) is not only genetic. Also environmental factors during early life may play a role in the development of FGIDs. Plasticity of the neonatal brain allows early life events to program physiologic response to stress during infancy that may be perpetuated into adulthood (10). Furthermore, what children learn from parents may contribute to the risk of developing an FGID(11, 12) In fact, children of adult patients with IBS make more health care visits (and incur more health care costs) than children of non-IBS parents (13,14), so the family should be aware about the role that psychosocial factors play in the development and perpetuation of FGIDs.

Nevertheless psychosocial factors do not define the FGIDs and are not required for diagnosis, research in this field yields three general observation: 1) psychological stress exacerbates GI symptoms; 2) psychosocial factors modify the experience of illness and illness behaviours such as health care seeking; 3) a functional gastrointestinal disorder may have psychosocial consequences on one's general well-being, daily function status, one's sense of control over the symptoms, in one word on one's quality of life.

Patients with FGIDs often exhibit sensory afferent dysfunction of the digestive tract that is manifested as altered sensitivity to luminal distention or other stimuli, and that selectively affects the visceral territory (15). Sensation and motility represent the two aspects of gut physiology most relevant to the FGIDs. In health, physiological stimuli from the gut induce motor reflexes, but these remain largely unperceived, with the exception of those related to ingestion and excretion. Depending on specific organs affected, visceral hypersensitivity may underline common symptoms in the FGIDs such as chest pain, abdominal discomfort, abdominal bloating, urgency defecation. Gut sensitivity is intimately related to gut motility. Sensory and motor functions of the

gastrointestinal tract are mediated through the enteric nervous system (ENS) and through the extrinsic nerves that connect the gastrointestinal tract to the central nervous system.

The major functions of human digestive tract motility are to accomplish propulsion along the gut, to mix gut contents with digestive secretions and expose them to the absorptive surface, to facilitate temporary storage in certain regions of the gut, to prevent retrograde movement of contents from one region to another, and to dispose of residues. Motility is controlled by reflexes, both central and peripheral, as well as by descending modulation from the brain-gut axis. Communication between various regions of the gut is facilitated by the transmission of myogenic and neurogenic signals longitudinally along the gut (16). Gastrointestinal contractions may be classified on the basis of their duration; contractions may be of short duration (phasic contractions) or may be more sustained (tone).

Tone is clearly recognized in organs with reservoir function, such as the proximal stomach (accommodation response to a meal) and the colon (response to feeding), as well as in sphincter regions.

Compliance refers to the capability of a region of the gut to adapt to its content; it is expressed as the ratio of the change in volume to the change in pressure and is obtained from the pressure-volume curve. Compliance reflects the contribution of several factors, including the capacity (diameter) of the organ, the resistance of surrounding organs, the elastic properties of the gut wall, and its muscular activity. Wall tension, related to compliance, describes the force acting on the gut wall and results from the interaction between intraluminal content and the elasticity of the wall.

Gut sensation is influenced by tonic or phasic contractions, and several observations suggest that this is mediated in part by an effect on wall tension; assessment of wall tension is therefore important in the interpretation of results of tests assessing perception of visceral stimuli. Transit refers to the time taken for intraluminal contents to traverse a specified region of the gastrointestinal tract. It reflects the combined effects of the various phenomena outlined earlier. Most measurements of transit are based on detecting intraluminal movements of an extrinsic marker labelling the luminal content. Transit depends on many factors, such as the physical (eg, solid, liquid, and gas) and chemical (eg, pH, osmolality, and nutrient composition) nature of both gut contents and the administered marker. Measurement of transit is influenced by the state of gut motility at the time of marker administration (eg, fasted vs fed motility) and any preparation of the gut (eg, cleansing of the colon). Some symptoms characteristic of the FGIDs, such as constipation and diarrhea, are suggestive of dysmotility including alteration in contractile activity, tone, compliance and transit in various regions of the GI tract.

In the context of the FGIDs, gastrointestinal dysmotility can develop through dysfunction of the control mechanisms at any level from the gut to the CNS. For example, inflammatory, immune, infiltrative, degenerative, or other processes may directly affect the muscle and/or other elements of the enteric nervous system, whereas psychosocial stressors can induce profound alterations in motility (17-26). Because patients with FGID tend to have a greater gastrointestinal motor response to stressful conditions than do healthy subjects, psychosocial stressors are particularly relevant to the symptomatic manifestations of the FGIDs (27-31).

In the FGIDs, sustained and inappropriate gut hypersensitivity, as well as gut dysmotility, are well documented. These sensory-motor dysfunctions seem related to alterations in neural processing in the brain-gut axis and in visceral reflex pathways. Their underlying causes and their relevance to symptom generation are the subject of ongoing research.

Despite recent progress in our understanding of pathophysiologic mechanisms underlying some forms of FGIDs, no biologic marker exists yet to allow a final diagnosis of FGID. Without any anatomic or biochemical labels, the FGID have required a classification based solely on symptoms.

In 1997, a pediatric working team met in Rome to standardize diagnostic criteria for various FGIDs in Children. The pediatric Rome II criteria for FGIDs were published for the first time in 1999 (32). Instead of classifying disorders according to target organs, as in the adult population, the pediatric working team divided disorders according to main complaints reported by children or their parents. The diagnoses were divided into 4 categories according to the following symptoms: vomiting, abdominal pain, diarrhea, and disorders of defecation. The Rome II criteria did not represent an end-point but a starting point to enhance new well-designed studies with the aims of 1) screening large populations to show that these diseases exist across time and cultures; 2) determining if the symptom based criteria are accurate in separating children with functional disorders from those with disease. However, although this publication generated scientific interest and contributed to the recognition of these disorders as diagnostic entities, a limited number of studies as been published since (33-42).

Recently, Caplan et al (33) developed the Questionnaire on pediatric GI symptoms (QPGS). The QPGS was designed as a both a parent report and child self-report measure based on the pediatric Rome II criteria for FGID. It was constructed in English, translated into French and pilot tested in both languages. The conclusion of the study was that the QPGS parent report is a valid and reliable measure for children 4 to 9 years old. For children 10 to 18, the QPGS child report is more reliable and should be used whenever possible. The same group of Authors examined the validity of the Pediatric Rome II criteria using the QPGS as reliable measure of GI symptoms for the different age group and found that more than half of patients classified as having a functional problem met at least one pediatric Rome II diagnosis for FGID (34). Subsequently, Di Lorenzo C et al (43) reported that the interobserver reliability of the Rome II criteria among pediatric gastroenterologist and fellow were low and that further validation of the criteria were necessary. More user friendly criteria probably needed to be developed in order to enhance their diagnostic accuracy and clinical utility. A possible explanation is that as fellows have less clinical experience, they may have adhered more closely to the criteria, while some of the specialist may have used it more loosely or may have applied their clinical experience to establish the diagnosis of the cases without consulting the criteria. This study supported the findings of previous investigations that the criteria were too restrictive and exclusive of many cases of specific FGIDs in children as reported by previous studies (35,40,41).

The above-mentioned publications have offered valid criticism of some disorders and provided preliminary validation of others and all this represented an appropriate background for the Rome III criteria (44, 45). The revised version of the Rome criteria has separated the pediatric criteria in two groups, based on an arbitrary division between infants/toddlers, and child/adolescent.

Rome III criteria has been revised in order to make diagnostic criteria more applicable to clinical practice with the following end points: better care for children; better research to understand the genetic, developmental, familial and cultural components of these disorders.

This document includes papers published or in press that have explored the interesting field of FGIDs and gastrointestinal motility in children. We started with the evaluation of the development of esophageal and gastrointestinal motility in humans in order to obtain information about the normal physiology of the gastrointestinal tract. In this way, indirectly, we aimed to better understand the possible pathophysiologic mechanisms that underline FGIDs and targets for more tailored therapeutic interventions. In the second chapter we studied the epidemiology of two specific FGIDs, the gastroesophageal reflux (GER) and the disorders of defecation, in unselected populations of children. In addition, in the first study we evaluate the natural history of GER in infants followed for the first two years of life; while in the second study we evaluate the clinical applicability and validity of the new pediatric Rome III criteria for functional defecation disorders. In the same chapter a third clinical

trial has examined the relation among the pathophysiology of two specific FGIDs, functional dyspepsia (FD) and functional constipation (FC).

On the contrary, the third chapter contains four papers addressing to the following arguments of diagnosis and therapy : 1) the validity and applicability of a new tool for the study of esophageal motility in children: the high resolution manometry; 2) the temporal correlation between chronic cough and GER and the role of esophageal pH-metry and the symptomatic indices: the traditional symptom (SI) and symptom sensitivity (SSI) indices vs the new symptom association probability (SAP); 3) the usefulness of maintenance therapy for gastroesophageal reflux disease in children and finally; 4) the correlation between migraine and FGIDs and the efficacy of flunarizine in migainous children affected by FGIDS.

-How did we get to Rome?

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J Pediatr Gastroenterol Nutr 2005;41:S28-29

How Did We Get To Rome?

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Throughout history the biomedical model of clinical practice has supported the theory that symptoms are caused by disease, and disease is defined by anatomic or biochemical abnormality. Clinician's responsibility was limited mainly to finding and curing disease; if disease was not found, the patient was (often by exclusion) considered to have a functional disorder. This approach was not cost-effective to make a diagnosis using diagnostic tests that exclude other disease, and it diminished our understanding of these conditions as true clinical entities.

In 1989, a group of investigators met in Rome and developed a consensus opinion to assist in the positive diagnosis of functional gastrointestinal disorders (FGIDs) in adults, hereafter known as "Rome Criteria". Criteria for FGIDs in childhood were firstly discussed at the consensus conference in 1997 and published in 1999 as the *Pediatric Rome II Criteria* (1). The aims of the Rome criteria were the following: 1) develop a symptom based classification system, 2) establish diagnostic criteria for research and clinical care, 3) provide a rigorous, systematic review of the literature for these conditions, 4) validate and/or modify the diagnostic criteria through an evidence based process.

FGIDs are defined as a variable combination of chronic or recurrent gastrointestinal symptoms not explained by structural or biochemical abnormalities. In adults, they affect an estimated 25 million Americans and cost the US economy billions of dollars annually in lost wages and decreased productivity.

The Rome II criteria do not represent an end-point but a starting-point to enhance new well-designed studies with the aims of 1) screening large populations to show that these diseases exist across time and cultures; 2) determining if the symptom based criteria are accurate in separating children with functional disorders from those with disease.

Recently we (2) performed a prospective survey to determine the prevalence and natural history of FGIDs in a general pediatric population using the Rome II

criteria and to evaluate their accuracy for diagnosis. Of 194 children who received a diagnosis of FGIDs, 97.5% continued to satisfy the diagnostic criteria or were improved at 12 month follow-up. We concluded that Rome II criteria provide the clinician with positive diagnosis of FGIDs in childhood, thus minimizing an exhaustive "rule out" workup. However the study put in evidence the need for validate questionnaires for patients and parents, in order to eliminate bias in data collections.

Loening-Baucke reported that the Rome II criteria for functional fecal retention (FFR) are too restrictive and do not identify many children with encopresis who have symptom of FFR (3). The author suggested revising the criteria for FFR by taking other features into consideration, such as a history of bowel movements that obstruct the toilet, chronic abdominal pain relieved by enema or laxatives, abdominal or rectal fecal mass.

Another study showed that, despite the total prevalence of functional defecation disorders evaluated comparing the Rome II criteria and the Classic Iowa criteria was similar, 16% of the patients fulfilling the pediatric constipation criteria were not recognized by the Rome II criteria (4). The study suggested the need for new validated questionnaire and for revision, including encopresis and rectal digital examination and excluding arbitrary age limits and retentive behavior.

Finally Walker et al. provided the first systematic empirical evidence that recurrent abdominal pain (RAP), as defined by Apley's, includes children with symptoms consistent with the Rome symptom criteria of several FGIDs (5). The study highlighted the difficulty of defining symptom criteria for non specific abdominal pain in children. Children's symptoms are often reported by parents and may not reflect children's own perceptions of their symptoms.

In the light of emerging scientific research and the age appropriate questionnaires, the pediatric working team for FGIDs met for the second time in 2004 and established the *Rome III criteria*. Two committees were formed, one for infants and toddlers and the second for children and adolescents. The Rome III criteria represent the sign that diagnostic criteria could be

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validate and modified in the course of the time through a constructive criticism, on the basis of an evidence-based process.

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CHAPTER 1

ONTOGENY AND NORMAL PHYSIOLOGY OF THE ESOPHAGEAL AND GASTROINTESTINAL MOTILITY.

- Annamaria Staiano; Gabriella Boccia. **Development of motility.** In: Feeding during late infancy and early childhood: impact on health. Hernell O, Schmitz J eds. Nestlè Nutr Workshop Ser Pediatr Program, 2005; 56:85-98.
- Gabriella Boccia, Annamaria Staiano. **Intestinal motility: Normal Motility and Development of the Intestinal Neuroenteric System.** In: RE Kleinman, O-J Goulet eds. Walker's Pediatric gastrointestinal disease. Pathophysiology, diagnosis, management (Fifth edition, in press).
- Annamaria Staiano, Gabriella Boccia, Gennaro Salvia, Donato Zappulli, Ray E Clouse. **Development of Esophageal Peristalsis in Preterm and Term Neonates.** Gastroenterology 2007;132:1718–1725

Hernell O, Schmitz J (eds): Feedings during Late Infancy and Early Childhood: Impact on Health.

Nestlé Nutrition Workshop Series- Pediatric Program, Vol.56,pp85-98

Development of motility

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Introduction

Advance in neonatology over the past 2 decades have resulted in survival of very preterm infants. However, the major limiting factor for survival of such infants is now the ability to initiate and maintain adequate nutrition.

Multiple maturational events are necessary for successful enteral nutrition of the infant : coordination of sucking and swallowing; effective gastric emptying; forward propagation of small intestinal contents and, finally, colonic elimination. Since normal gastrointestinal function relies on the integrated maturation of absorptive, secretory and motor function, a delay in any one of these processes will result in disturbed gastrointestinal function. Immature gastrointestinal motility manifested by vomiting, abdominal distention, delay in stooling and constipation commonly postpone the time of full enteral feeding in premature infants.

Recent advances in biomedical engineering have allowed the study of gastrointestinal motility even in very premature infants. By using miniaturized feeding catheters with an outer diameter of less than 2 mm, multiple recording sites and sleeve sensors and with rates of water infusion ranging between 0.005 to 0.04 ml/min, we have learnt a great deal about the functional ontogeny of esophageal and antroduodenal motility in humans. In contrast, due to the difficulty to study the human colon in physiologic condition, very little is known about the development of colonic motility. Placement of manometric or barostat catheters in the colon requires endoscopy and cannot be justified in healthy infants while non-invasive techniques such as scintigraphic transit studies or ultrasonographic evaluations have not been standardized yet in children.

Development of myogenic control

The fetal development of the structure and function of the gastrointestinal tract is a complex process. Throughout the intestine, three layers of muscle contract in a coordinated fashion: the muscularis mucosa, a thin layer that lies beneath the villi; the circular muscle, which lies outside of the muscularis mucosa and serves as the pacemaker for gut muscle contraction; and the longitudinal muscle, the most outer layer of the three muscles. These muscles have oscillatory membrane potentials and their contraction rate is reflective of the electrical slow waves. The slow wave has different frequency at each level of the gut (i.e., 3 to 5 times per minute in the stomach, 9 to 11 times per minute in the duodenum, 8 to 10 times per minute in the jejunum and so forth). Thus, at each level of the gut, there is an intrinsic phasic contraction rate.

The muscular layers derive from the mesenchymal tissue in the gut by the 4th to the 6th week of gestation in a rostral caudal fashion (1). The circular muscle layer appears first, followed, after 2 to 3 weeks, by the longitudinal muscle coat, while the muscularis mucosa is formed later, by 22 to 23 weeks gestation. Similarly, the contractile proteins of smooth muscle cells in animal models appear in a hierarchic manner; however, no such information is available in humans (2). Just as the developmental changes of the contractile proteins occur, the frequency of the slow waves or electric control activity (ECA) of the smooth muscle cells also changes. The frequency of ECA increases with the increasing of postconceptional age, reflecting developmental changes in the activity of membrane ion pumps or in their modulation (3).

Until recently, some investigators suggested that groups of muscle cells located in the circular layer differentiated to form the interstitial cell of Cajal (ICCs), specialized cells provided of multiple processes that project in an ascending and descending manner throughout the length of the circular muscle and to the longitudinal muscle. These cells act as pacemakers by driving the slow wave frequency and coordinate neural input to gut smooth muscle (4). The ICCs are distinct from

neurons and smooth muscle cells, and they play important roles in the regulation of gastrointestinal motility.

Anatomic studies characterizing the distribution of ICCs measure immunoreactivity to c-Kit, a proto-oncogene coding for a receptor tyrosine kinase. Six distinct ICCs population were identified in the gut, including intramuscular ICCs, ICCs within the myenteric plexus, submucosal ICCs in the colon and ICCs in the deep muscular plexus of the small intestine. A recent study has reported regional variability in colonic ICC density with the highest numbers observed in the transverse colon (5).

ICCs are present from an early stage in human gut development. Intrauterine maturation of ICCs correlates with the initiation of electrical rhythmicity, in fact in mutant mice lacking ICCs, no spontaneous pacemaker activity is seen (6). Such loss of pacemaker function leads to disruption of organized luminal propagation.

Recent studies have reported that a delayed maturation of ICCs could be involved in the pathophysiology of gastrointestinal dysmotility seen in some neonates and children (7-8) and abnormalities in the density and distribution of ICCs have been described in human Hirschsprung disease and infantile hypertrophic pyloric stenosis (9-10). However, since ICCs development continues well into postnatal life, interpretation of apparent abnormalities in their distribution as being of pathological significance should be tempered.

The finding that c-kit positive ICCs are present from 9.5 weeks when neural crest colonization of the gut is approaching completion, is consistent with a modulating effect of the fetal enteric nervous system (ENS) on ICCs development.

Development of neurogenic control

Initiation and coordination of muscle contraction is regulated by neural and hormonal input.

Extrinsic neural regulation refers to all nerves that have cell body located outside of the intestinal tract. Extrinsic neural input to the gastrointestinal tract comes from the central nervous system (CNS, the sympathetic and the parasympathetic systems. Intrinsic neural regulation refers to all nerves whose cell bodies reside in the intestine. The enteric nervous system (ENS), or gut brain, provides most of this regulation. It is capable of functioning independently of the extrinsic nervous system in animals when connections to the extrinsic nerves have been severed (1).

Components of the ENS are formed in a temporal sequence that parallels the maturation of the muscle layers. Neural crest cells migrate out to the intestine via the vagal and sacral portion of the spinal cord. The undifferentiated cells are first detected in the stomach and duodenum at 7 weeks and then in the rectum at 12 weeks. They quickly differentiate along a rostral caudal axis and establish the myenteric and submucosal plexuses by week 12 to 14. Contacts between enteric nerves and the circular and longitudinal muscle cells develop between 10 and 26 weeks (11). It appears that there is intimate cross talk between the developing muscle and nerves and if either of the two fail to develop properly, maturation of the other is arrested .

Several observations suggest that development of the enteric nervous system continues after birth and through at least the first 12 to 18 months of life. Study of the argyrophilia of neurons in the sigmoid colon of human neonates shows that, prior to term, nerves are unable to take up silver and that, during the first 6 months of life, neurons in the myenteric plexus gradually assume argyrophilia (12). Thus evidence suggests that, just the majority of CNS development takes place through fetal life and continues through the first 18 months of life, a similar pattern occurs in ENS.

Neurotransmitters are elaborated by the end of first trimester as almost all of the hormones and peptides. N-methyl-D aspartate (excitatory) and nitric oxide (inhibitory) have been shown to be neurotransmitters in animal studies and may be the most potent agents in modulating bowel motility (13).

Recent studies have indicated that nitric oxide is involved in the non adrenergic-non cholinergic (NANC) innervation of the gut, mediating its relaxation. Brandt et al. (14) reported that

the onset and place of development of nitrergic innervation are similar to adrenergic and cholinergic innervation and occur before peptidergic innervation. Bowel segments from the esophagus, pylorus, ileocecal and rectosigmoid regions of 14 fetus (gestational age range from 12 to 23 weeks) were studied with nicotinamide adenosine dinucleotide phosphate (NADPH) diaphorase histochemistry. By 12 weeks gestation, nitrergic neurons had appeared in the myenteric ganglia, at all level of the gut, and had begun plexus formation. Nitrergic innervation of the submucous plexus becomes evident after 14 weeks. By 23 weeks gestation a complete nitrergic pattern, as observed in the postnatal gut, had matured.

These NANC nerves mediate the reflex opening of sphincters in the alimentary tract and the descending inhibition during intestinal peristalsis. Defects of nitrergic innervation recently have been found in congenital gut anomalies such as pyloric stenosis and Hirschsprung's disease, which suggests that a lack of nitric oxide-mediated NANC inhibitory control may be responsible for the failure of relaxation of the pylorus and hindgut, respectively (15).

The combined maturation of the enteric and central nervous system, together with their interconnections, is likely to be responsible for many of the mayor ontogenetic changes observed in intestinal motor activity before and after birth.

Characterization of motor activity

Gastric motility

Many aspects of gastrointestinal motility appear to be less mature in the preterm infant than in the term infant, and those of the term infant less mature than those seen in the child and adult.

Although fetuses in utero are able to swallow amniotic fluid from as early as 20 weeks of gestation, the sucking mechanism does not appear until 32 to 34 weeks gestation (16). Gastric emptying of swallowed amniotic fluid into the intestine may be demonstrated in the human fetus at 30 weeks'

gestation (17). Between 28 and 38 weeks gestational age, the gastric antral contraction amplitude increases from 10 to 40 mmHg. Emptying half-time doubles when newborns of 28-34 weeks are compared with full-term neonates independent of feeding.

Contractions may occur singly, but occasionally phasic contractions may be sustained for 3 to 5 minutes. However preterm infants had fewer antral clusters coordinated with duodenal clusters than term infants (18).

Small intestinal motility

Although complete interdigestive cycles can be observed occasionally in term infants, they are very rarely seen in preterm infants. Approximately 75% of the recording obtained from neonates are occupied by a motor pattern that is not typically seen in adults: the nonpropagating cluster of contraction. This pattern consists of bursts of 11 to 13/minute contractions last 1 to 3 minutes and do not migrate from the proximal gut to the distal gut (1). With increasing gestational age, motor contractions become more organized, the duration of a single cluster become longer as is the duration of the motor quiescence separating clusters. As a result this dominant pattern still occupy 75% of the recordings of term infants but clusters are longer (3-4 minutes) and their occurrence is lower (6 to 8 times per minute). The migrating motor complexes (MMC) appears between 32 to 35 weeks postconception, as the overall occurrence of clusters decreases (19). Some of these MMC are poorly organized with slower propagation velocities.

In spite of an apparent immaturity of fasting activity, intestinal motor activity pattern in preterm and term infants change in response to feeding. However the appearance of fed pattern is different at different gestational ages. Term neonates shown a fed pattern similar to that seen in adults. In contrast to term infants, only 25% of preterm infants display a mature type of fed pattern while about 75% display a prompt cessation of motor contraction after feeding. This pattern, associated with a delay in gastric emptying, is probably due to immaturity of vagal regulation.

Feeding and development of motility

There is convincing evidence that acute response of motor activity and peptide release are present with the first enteral feeding and that the provision of early enteral feedings facilitates functional maturation of the human intestine. Babies can respond to enteral nutrition as early as 25 weeks of gestational age (20). These evidences suggest that the small intestinal fed response is a more primitive form of motor activity than is the fasting motor activity. For this reason the practice of delaying the use of enteral nutrition in the very low birth weight infant may not coincide with preterm intestinal physiology of motor function.

Several studies have shown that gut function and subsequent milk tolerance is improved by trophic feeding. Trophic feeding (minimal enteral feeding, gut priming, early hypocaloric feeding) is a practice that involves feeding small volumes of milk, nutritionally insignificant but beneficial to the developing gut. Recent studies have reported that this practice accelerates the whole gut transit probably by enhancing the MMC. The mechanism by which trophic feeding exerts its influence is unknown. It is responsible for surges in the plasma concentration of several enteric hormones and peptides which alter gut motility (motilin, gastrin, neurotensin and peptide YY) and may cause stimulation of the ENS (21).

The manner in which babies are fed may also trigger differences in motor responses. Maturation of motor function requires that nutrient be fed to the neonates because feeding sterile water does not produce this effect (22). Preterm infants fed by a 2 hour infusion display a brisk increase in motor contraction that is associated with a faster gastric emptying compared with infants fed by 15-minutes bolus. Feedings volumes that provide as little as 10% of daily fluid intake significantly induce the premature appearance of MMC as those that provide 30% or 100% (23).

In conclusion minimal feeding volumes can be used to trigger maturation of motor function avoiding at the same time the risk of enterocolitis that larger feeding volumes incute. However,

since cluster represents 60-75% of the motor activity in the term infants who have complete interdigestive cycle, the motor activity in these neonates is still very dissimilar from that seen in the adult, suggesting that further changes occur throughout infancy.

Colonic motility

The role of trigger that the enteral nutrition occupies in the development of gastrointestinal function represents a major factor in the ontogeny of colonic motility, too. It seems that colonic motility matures late in gestation and has different characteristics in the infants compared to the older children and adults.

Meconium can be found in the fetal rectum after the 21 week of gestation and as much as 10 to 20% of total amniotic fluid proteins derive from the fetal gut. These data suggest that defecation in utero occurs physiologically during the late stages of pregnancy and it is now believed that the detection of meconium in the amniotic fluid might reflect impaired clearance of meconium rather than excessive or inappropriate elimination in the amniotic fluid.

The correlation among early enteral feeding, passage of the first stooling, stool frequency and consistency had been largely discussed in pediatric literature.

Coordinated sucking and swallowing, required for the independent utilisation of milk feeds, is not achieved until 32-34 weeks' gestation, after which time most preterm infants are capable of taking feeds by mouth. This gestational age coincides with a significant increase in defecation rate and a surge in circulating concentrations of intestinal regulatory polypeptides (gastrin, motilin and neurotensin) in response to milk feeds.

In newborn infants, who do not have voluntary control, evacuation probably occurs in response to an increasing volume of stool in the rectum. In a large study observing bowel habits in 844 preterm infants, a direct relation between the volume of milk ingested and stool frequency throughout the first eight weeks after birth was reported (24). Infants who received no milk had a modal frequency of one stool each day whereas those receiving greater than 150 ml/Kg/day passed

between three and four stools each day. Infants receiving human milk had consistently higher defecation rate, and passed softer stools, than those receiving formula milk, irrespective of gestational age and feed volume. The finding of a modal frequency of one stool each day in the unfed neonate suggests that there is an intrinsic pattern of large bowel motor activity present as early as 25 weeks' gestation. This daily passage of stool may perform the "housekeeping" function of clearing the colon of intestinal secretions and other unwanted material. Probably, milk feeds override the intrinsic fasting motor activity of the colon and induce regular defecation at a frequency determined directly by the volume of the products of digestion that reach the rectum: the more feeds, the more stools.

In full term and preterm infants, the peak stool frequency occurs during the first week after birth, after which there is a decrease, in spite of increasing milk intake, indicating a maturation of the water conserving ability of the gut. It is not known, however, whether this is due to the increasing efficiency of small intestinal absorption or colonic water retention.

Term newborn infants average four bowel movements/day for the first week of life. The frequency of defecation decreases with age, so that 85% of children 1-4 years old defecate once or twice daily. High amplitude (> 60 mmHg) propagating contractions (HAPCs) are the manometric correlate of the radiologic "mass movements" and are responsible for the rapid movement of feces in the aboral direction. The presence of HAPCs together with an increase in colonic motility after a meal, are markers for neuromuscular integrity of the colon in toddlers and children (17). HAPCs decrease in frequency from several per hour after a meal in awake toddlers to just a few per day in adults (25). The gastrocolonic response seems also more prominent in younger compared to older children. Nevertheless the colon in toddlers seems to have fewer tonic and phasic non-HAPCs contractions compared to the colon of older subjects. Information about age related changes in colonic tone are absent.

The ongoing developmental maturation of bowel function results in intestinal hypomotility with consequent postponement of meconium passage. The first studies to measure intestinal transit

in humans used amniography; aboral transport of contrast did not occur in the intestinal tract of fetuses younger than 30 weeks gestation. Using amniography, Mc Lain observed that gastrointestinal motility increased with advancement of gestational age; progression of contrast material from the oral cavity to the colon took as long as 9 hours at 32 weeks of gestational age, but only half of that time by the time of labour (16). Intestinal transit is approximately three times slower in preterm infants compared with that seen in the adults.

It has been noted previously that more than 90% of full term infants and 100% of post-term infants passed their meconium within the 24 hours. There has been agreement on the general principle that defecation should be avoided in utero and that lack of defecation after birth is a sign of disease. In fact it is generally believed that the passage of meconium into the amniotic fluid is an indicator of fetal distress. Nevertheless meconium-stained amniotic fluid is found up to 30% of all deliveries, and no cause of fetal distress is found in up to 25% of all occurrences of meconium stained amniotic fluid (26).

In premature infants with a birth weight of 1000 g or less the first stool is passed at a median age of 3 days and 90% have their first stool by 12 days after birth (27). Meetze et al (28) found a median age of 43 hours for passage of the first stool in 47 patients with birth weights 1259 g or less. One fourth of these infants had not passed stool by 10 days of age. Weaver and Lucas (24) reported 32% delay in passing meconium greater than 48 hours with an inverse relation between gestational age and the time of first bowel action. Extreme prematurity and delayed enteral feeding were significantly associated with delayed passage of the first stool in more than one study (29-30).

Therefore a delayed passage of meconium and constipation could be induced by a delayed intestinal transit in particular evident at the level of the colonic segments. Naturally, a normal development of the upper gastrointestinal tract (stomach; small intestine) is essential to warrant a correct maturation of the colonic motility, too.

In conclusion, we have underlined as the ontogenesis of gastrointestinal motor activity is influenced by several factors such as the smooth muscle activity, the CNS, the ENS and the neurohumoral system.

We have also seen that early enteral feeding occupies a main role in the promotion of development of small intestinal functions and colonic motility.

Further understanding about the timing of specific motor patterns in humans and their control mechanisms may allow neonatologists to rich the optimal feeding strategies to induce the better gastrointestinal function and to obtain the optimal feeding tolerance.

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Normal Motility and Development of the Intestinal Neuroenteric System

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NORMAL ASPECTS OF SMALL INTESTINAL MOTILITY

Gastrointestinal motility is a complex process deriving from the integration of several mechanisms including myoelectrical activity and contractile activities tone compliance and transit. Initiation and coordination of muscle contraction is regulated by neural and hormonal input. Under physiologic conditions, in the postprandial and fasting period, two basic organized motor patterns characterize the small intestine contractility.

In the fasted state, motor activity is highly organized into distinct and cyclically recurrent events known as the *interdigestive cycle*.^{1,2} The interdigestive cycle consists of at least three distinct phases that occur in sequence, with a combined total average duration of about 60 to 90 minutes (Figure 1). At first, the gut is relatively quiet and exhibits very few contractions. This absence of motor contraction lasts for approximately 60% of the total cycle and is called *motor quiescence*, or phase I. Phase I is gradually replaced by a pattern characterized by increasing but irregular contractions, phase II. Phase III represents the hallmark of the fasting condition and reflects the neuromuscular function. This final pattern is called the *migrating motor complex* (MMC) and consists of a series of intense phasic contractions that are sustained for approximately 5 to 10 minutes and sweep distally throughout the intestine from the distal stomach to the ileum. The propagation velocity also varies, with about 10 cm/min in the duodenum, 7 cm/min in the proximal jejunum, and about 1 cm/min in the distal ileum.

Most phase III complexes originate in the gastroduodenal region, but about one-third of them begin distal to the ligament of Treitz. It has been reported that the terminal pressure waves of phase III in the proximal duodenum are mainly retroperistaltic.³ The MMC develops before birth and persists in a stable fashion throughout life; it is responsible for the aboral movement of intraluminal contents and has such been termed the gut "housekeeper."

An additional brief period of transitional motor activity from the intense phase III to the quiescence of phase I (the phase IV) has been observed.

When nutrients are ingested, the cycling activity of the interdigestive cycle is interrupted by a second motor pattern. The postprandial pattern is induced 5 to 10 minutes after ingestion of a meal, peaks after 10 to 20 minutes and persists as long as food remains in the stomach. The myoelectric pattern of a meal consists of random bursts of spike potentials with the motor findings of continuous sustained contraction of variable amplitude superimposed on small changes of tone (Figure 2). This pattern of muscle contractions results in the mixing and churning of nutrients so that they may be mixed with gastrointestinal secretions and peptides and then exposed to the mucosal surface for absorption. The length of the fed motor period is dependent upon the type of nutrients ingested and the

number of calories consumed, with fats inducing a more prolonged fed pattern than proteins or carbohydrates.⁴

The type of nutrients ingested together with the manner in which babies are fed and the timings of enteral feeding have been shown to be crucial factors in the development of intestinal motor activity in children. Recent advances in biomedical engineering have allowed the study of gastrointestinal motility even in very premature infants. By using miniaturized feeding catheters with an outer diameter of less than 2 mm, multiple recording sites and sleeve sensors, and with rates of water infusion ranging between 0.005 and 0.04 mL/min, we have learned a great deal about the functional ontogeny of esophageal and antroduodenal motility in humans.

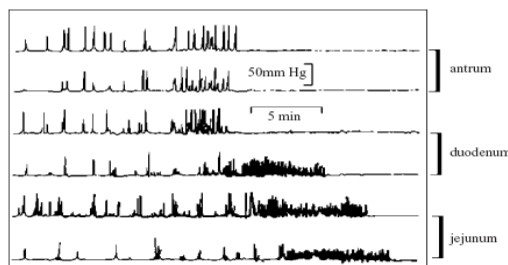


Figure 1 Fasting antroduodenal motor activity. The manometric tracing shows the sequence of phase I, phase II, and the migrating motor complex (MMC).

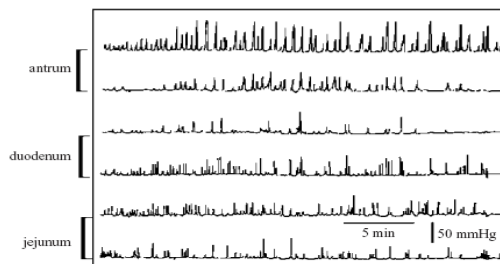


Figure 2 Postprandial small intestinal motor activity. The manometric tracing shows random bursts of contractions.

Development of Small Intestinal Motility in Children

There is convincing evidence that acute response of motor activity and peptide release are present with the first enteral feeding and that the provision of early enteral feedings facilitates functional maturation of the human intestine. Babies can respond to enteral nutrition as early as 25 weeks of gestational age.^{5,6} The evidence also suggests that the small intestinal fed response is a more primitive form of motor activity than is the fasting motor activity. For this reason the practice of delaying the use of enteral nutrition in the very low birth weight infant may not coincide with preterm intestinal motor physiology. Several studies have shown that gut function and subsequent milk tolerance is improved by trophic feeding.⁷⁻¹²

Trophic feeding (minimal enteral feeding, gut priming, early hypocaloric feeding) is a practice that involves feeding small volumes of milk, nutritionally insignificant but beneficial to the developing gut. Typical volumes are from 1 to 24 mL daily/kg/body weight.^{7,8} Several studies have reported that this practice accelerates the whole gut transit probably by enhancing the MMC. The mechanism by which trophic feeding exerts its influence is unknown. It is responsible for surges in the plasma concentration of several enteric hormones, neurotransmitters and other peptides that alter gut motility (motilin, gastrin, neurotensin, and peptide YY). For example, infants who are given small enteral feedings have more mature small intestinal pattern and higher plasma gastrin and motilin concentration than do infants who have been given no feeding.⁹ Furthermore, trophic feeding may cause stimulation of the enteric nervous system (ENS) directly via nociceptors or indirectly via hormone release.¹⁰ In fact, in the preterm intestine it has been reported that minimal enteral feeding can stimulate motor activity also independently from hormonal response.

The manner in which babies are fed may also trigger differences in motor responses. Maturation of motor function requires that nutrient be fed to the neonates because feeding sterile water does not produce this effect.¹¹ Preterm infants fed by a 2-hour infusion display a brisk increase in motor contraction that is associated with a faster gastric emptying compared with infants fed by 15-minute bolus. Feedings volumes that provide as little as 10% of daily fluid intake induce the premature appearance of MMC as well those that provide 30 or 100%.¹²

In conclusion minimal feeding volumes can be used to trigger maturation of motor function avoiding at the same time the risk of enterocolitis that larger feeding volumes incite. However, since clusters represent 60 to 75% of the motor activity in term infants who have complete interdigestive cycle, the motor activity in these neonates is still very dissimilar from that seen in the adult, suggesting that further changes occur throughout infancy.

Although complete interdigestive cycles can be observed occasionally in term infants, they are

very rarely seen in preterm infants. Approximately 75% of the neonatal intestinal motor activity is occupied by a motor pattern that is not typically seen in adults: the nonpropagating cluster of contractions. This pattern consists of bursts of 11 to 13/min contractions last 1 to 3 minutes, which do not migrate distally from the stomach to the distal gut and is prominent in both term and preterm infants.^{13,14} With increasing gestational age, motor contractions become more organized, the duration of a single cluster becomes longer as does the duration of the motor quiescence separating clusters.

Preterm neonates of 27 to 28 weeks' gestational age have short clusters of activity, with a duration of less than 1.5 minutes, that are separated by brief periods (0.25 to 0.5 minutes) of motor quiescence. Compared with older children and adults, the clusters occur more frequently, with a rate of 12 to 14 times per minute. With increasing gestational age, the duration of a single cluster and motor quiescence periods become longer, with period of activity of 2 to 3 minutes occurring 8 to 10 times per minute at 32 weeks of gestation.¹⁵ As a result, in term neonates this dominant pattern still occupies 75% of the recordings, but clusters last 3 to 4 minutes and their occurrence is lower with a frequency of 6 to 8 times per minute.

The migrating motor complexes (MMCs) appear between 32 and 35 weeks' postconception, as the overall occurrence of clusters decreases.¹⁶ Some of these MMCs are poorly organized with slower propagation velocities.

In spite of an apparent immaturity of fasting activity, the intestinal motor activity pattern in preterm and term infants changes in response to feeding. However, as with the fasting pattern, the fed pattern shows a different activity at different gestational ages.¹⁷ Term neonates report a fed pattern similar to that seen in adults. After feeding of at least 15 minutes, the fasting pattern is immediately interrupted by sustained bursts of

motor contractions. In contrast to term infants, only 25% of preterms display a mature type of fed pattern. About 75% of preterm neonates display a prompt cessation of motor contraction after feeding that lasts for approximately 15 to 20 minutes. This pattern of sustained motor quiescence, associated with a delay in gastric emptying, is probably due to immaturity of vagal regulation.

COLON MOTILITY: NORMAL FEATURES AND DEVELOPMENT

The physiology of the human colon requires motor activities that are different from those of the upper gut, to propel intraluminal contents distally, to mix them in a continuous manner and to store and eventually expel the residuals.

Colonic motility may be divided into two main patterns of contraction: the segmental activity and the propagated activity.¹⁸ The segmental activity is represented by single contractions or bursts that appear usually arrhythmic and constitute most of the overall colonic motility (Figure 3). Only in a small percentage of time (<6% of the overall contractile daily activity) will these contractions assume a rhythmic frequency with ranges of 3 cycles/min. This motor pattern has the function of moving the fecal matter distally toward the rectum, allowing absorption of water, electrolytes, short-chain fatty acids, and bacterial metabolites.¹⁹

The propagated activity could be classified, on the basis of the contraction wave amplitude, as low-amplitude propagated contractions (LAPCs) and high-amplitude propagated contractions (HAPCs) (Figures 4 and 5). LAPCs appear with an amplitude of less than 50 mmHg and a high frequency (more than 100 event/die). They allow the transport of fluid contents within the colon, the passage of flatus and the distention of lumen. HAPCs are powerful contractions with an amplitude of more than 100

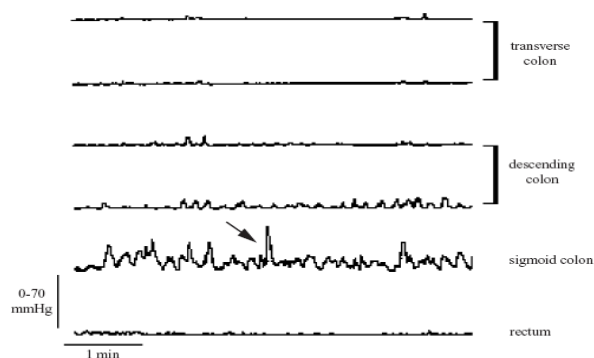


Figure 3 Normal colonic segmental activity: manometric tracing. The arrow points out sporadic contractions exceeding 50 mmHg.

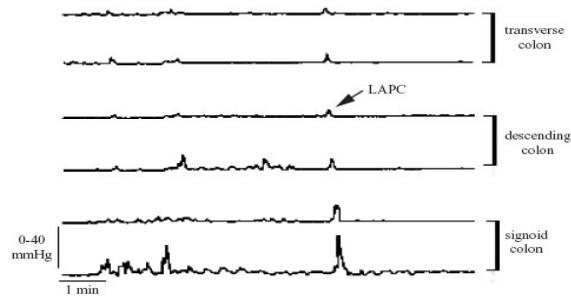


Figure 4 Manometric tracing of colonic propagated activity. The arrow points out a low-amplitude propagated contraction (LAPC).



Figure 5 Normal colonic propagated activity. The manometric tracing shows high-amplitude propagated contractions (asterisk). Note the background arrhythmic segmental activity (arrow).

mmHg that occur about 6 times/d. These contractions play a key role in the defecatory mechanism, as they precede the expulsion of stools.²⁰

Periodic motor phenomena, the “rectal motor complex” and the “periodic colonic motor activity” have been documented in the rectum and in the more distal colonic segments, respectively.²¹ No cyclic or periodic motor activity has been documented in the anal canal. Furthermore, a circadian trend is recognizable with a peak of activity after awaking in the morning and after meals and a deep inhibition during sleep. However, the major stimulus for colonic motor activity is represented by ingestion of food with an increase in smooth muscle tone and in segmental contractions. The type of nutrients influences the motor response with fatty meals eliciting a more sustained motor response than carbohydrates.²²

In contrast to the upper gastrointestinal tract, very little is known about the development of colonic motility in infants and children. Placement of manometric or barostat catheters in the colon requires endoscopy and cannot be justified in healthy infants while noninvasive techniques such as scintigraphic transit studies or ultrasonographic evaluations have not been standardized yet in children.

Enteral nutrition represents a major factor in the ontogeny of colon motility. It seems that colon motility matures late in gestation and has different characteristics in infants compared to older children and adults. Meconium can be found in the fetal rectum after the 21 weeks of gestation and as much as 10 to 20% of total amniotic fluid proteins derive from the fetal gut. These data suggest that defecation in utero occurs physiologically during the late stages of pregnancy and it is now believed that the detection of meconium in the amniotic fluid might reflect impaired clearance of meconium rather than excessive or inappropriate elimination in the amniotic fluid. Term newborn infants average 4 bowel movements/day for the first week of life. The frequency of defecation decreases with age, so that 85% of children 1 to 4 years old defecate once or twice daily.

As described previously, HAPCs (> 60 mmHg) are the manometric correlate of the radiologic “mass movements” and are responsible for the rapid movement of feces in the aboral direction. The presence of HAPCs together with an increase in colonic motility after a meal, are markers for neuromuscular integrity of the colon in toddlers and children.²³ HAPCs decrease in frequency from several per hour after a meal in

awake toddlers to just a few per day in adults.²⁴ The gastrocolonic response seems also more prominent in younger compared to older children. Nevertheless the colon in toddlers seems to have fewer tonic and phasic non-HAPCs contractions compared to the colon of older subjects. Information about age related changes in colonic tone is absent.

The ongoing developmental maturation of bowel function results in intestinal hypomotility with consequent postponement of meconium passage. The first studies to measure intestinal transit in humans used amniography and aboral transport of contrast did not occur in the intestinal tract of fetuses younger than 30 weeks' gestation. Using amniography, McLain observed that gastrointestinal motility increased with advancement of gestational age. Progression of contrast material from the oral cavity to the colon took as long as 9 hours at 32 weeks of gestational age, but only half as long by the time of labor.²⁵ Intestinal transit is approximately three times slower in preterm infants compared with that seen in the adults.

It has been noted that more than 90% of full-term infants and 100% of post-term infants passed their meconium within the 24 hours. It is generally believed that the passage of meconium into the amniotic fluid is an indicator of fetal distress. Nevertheless, meconium-stained amniotic fluid is found up to 30% of all deliveries, and no cause of fetal distress is found in up to 25% of all occurrences of meconium stained amniotic fluid.²⁶

In premature infants with a birth weight of 1000 g or less the first stool is passed at a median age of 3 days and 90% have their first stool by 12 days after birth.²⁷ Meetze and colleagues²⁸ found a median age of 43 hours for passage of the first stool in 47 patients with birth weights 1259 grams or less. One-fourth of these infants had not passed stool by 10 days of age. Weaver and Lucas²⁹ reported 32% delay in passing meconium greater than 48 hours with an inverse relation between gestational age and the time of first bowel movement. Extreme prematurity and delayed enteral feeding were significantly associated with delayed passage of the first stool in several studies.^{30,31}

There is a well demonstrated correlation between early enteral feeding, passage of the first stool and stool frequency and consistency. Coordinated sucking and swallowing, required for the independent utilization of milk feeds, is not achieved until 32 to 34 weeks' gestation, after which time most preterm infants are capable of taking feeds by mouth. This gestational age coincides with a significant increase in defecation rate and a surge in circulating concentrations of intestinal regulatory polypeptides (gastrin, motilin, and neurotensin) in response to milk feeds.

In newborn infants, who do not have voluntary control, evacuation probably occurs in response to an increasing volume of stool in the rectum. In a large study of bowel habits in 844 preterm infants, a direct relation between the volume of milk ingested and stool frequency throughout the first eight weeks after birth was reported.²⁹ Infants

who received no milk had a modal frequency of one stool each day whereas those receiving greater than 150 mL/kg/d passed between three and four stools each day. Infants receiving human milk had consistently higher defecation rate, and passed softer stools, than those receiving formula milk, irrespective of gestational age and feed volume.

The finding of a modal frequency of one stool each day in the unfed neonate suggests that there is an intrinsic pattern of large bowel motor activity present as early as 25 weeks of gestation. This daily passage of stool may perform the "house-keeping" function of clearing the colon of intestinal secretions and other unwanted material. Probably milk feeds override the intrinsic fasting.

Motor activity of the colon and induce regular defecation at a frequency determined directly by the volume of the products of digestion that reach the rectum: the more feeds, the more stools.

In full term and preterm infants, the peak stool frequency occurs during the first week after birth, after which there is a decrease, in spite of increasing milk intake, indicating a maturation of the water conserving ability of the gut. It is not known, however, whether this is due to the increasing efficiency of small intestinal absorption or colonic water retention.

REGULATION OF GUT MOTILITY

The fetal development of the structure and function of the gastrointestinal tract is a complex process. Normal intestinal motility requires the coordinated development of the smooth muscle layers, nerve plexi, and interstitial cells of Cajal (ICC) in the gut wall. These structures allow all the integrated intestinal functions, such as myoelectrical activity, contractile activity, tone, compliance, and transit by generation and modulation of local and circulating neurohumoral substances.

Throughout the intestine, three layers of muscle contract in a coordinated fashion: the muscularis mucosa, a thin layer that lies beneath the villi; the circular muscle, which lies outside of the muscularis mucosa and serves as the pacemaker for gut muscle contraction; and the longitudinal muscle, the outer most layer of the three muscles. These muscles have oscillatory membrane potentials and their contraction rate is reflective of the electrical slow waves. The slow wave has a different frequency at each level of the gut (ie, 9 to 11 time per minute in the duodenum, 8 to 10 times per minute in the jejunum, and so forth). Thus, at each level of the gut, there is an intrinsic phasic contraction rate.

The muscular layers differentiate from the mesenchymal tissue in the gut by the 4th to the 6th week of gestation in a rostral to caudal fashion. The circular muscle layer appears first, and is presenting the small intestine and colon by week 8, followed, after 2 to 3 weeks, by the longitudinal muscle coat, while the muscularis mucosa is formed later, by 22 to 23 weeks' gestation.^{32,33} Similarly, the contractile proteins of smooth muscle cells in animal models appear in a hierarchic

manner; however, no such information is available in humans.

Just as the developmental changes of the contractile proteins occur, the frequency of the slow waves or electric control activity (ECA) of the smooth muscle cells also changes. The frequency of ECA increases with increasing postconceptional age, reflecting developmental changes in the activity of membrane iron pumps or in their modulation. The precise mechanisms that trigger and set the pace of slow waves is unknown. Only a few neurohumoral inputs are capable of influencing the amplitude of plateau potential and the frequency of spike potential determining the magnitude and occurrence of slow waves and phasic contractions in the intestinal cells.³⁴

The evidence for the origin of rhythmicity in intestinal contraction suggests that groups of mesenchymal progenitor cells differentiate to form the ICC, specialized cells capable of multiple processes that project in an ascending and descending manner throughout the length of the circular muscle and to the longitudinal muscle. These cells act as pacemakers by driving the slow wave frequency and coordinate neural input to gut smooth muscle.³² The ICC are distinct from neurons and smooth muscle cells, and they play important roles in the regulation of gastrointestinal motility. By regulating ionic conductance in ICC, neurohumoral substances can influence the resting potential and the excitability of smooth muscle cells.³⁵ ICC have in fact receptors for both the inhibitory transmitter NO and excitatory tachykinin; muscarinic and VIP receptors.^{36,37}

ICC are present from an early stage in human gut development and Wallace and colleagues³³ identified Kit-positive ICC in the human intestine by week 9, after the colonization of the gut by neural crest cells and following the differentiation of the circular muscle layer. The finding that c-Kit positive ICC are present when neural crest colonization of the gut is approaching completion, is consistent with a modulating effect of the fetal ENS on ICC development.

Anatomic studies characterizing the distribution of ICC have measured the immunoreactivity to c-Kit, a proto-oncogene coding for a receptor tyrosine kinase. Six distinct ICC populations were identified in the gut, including intra-muscular ICC, ICC within the myenteric plexus, submucosal ICC in the colon and ICC in the deep muscular plexus of the small intestine. A regional variability has been reported in colonic ICC with the highest density observed in the transverse colon.³⁸

Intrauterine maturation of ICC correlates with the initiation of electrical rhythmicity. In fact, in mutant mice lacking ICC, no spontaneous pacemaker activity is seen.³⁹ Such loss of pacemaker function leads to disruption of organized luminal propagation. Recent studies have reported that a delayed maturation of ICC could be involved in the pathophysiology of gastrointestinal dysmotility seen in some neonates and children.^{40,41} However, since ICC development continues well into postnatal life, interpretation of apparent

abnormalities in their distribution as being of pathological significance should be tempered.

In conclusion it appears that there is an intimate cross talk in the gut between the developing muscles, ICC and extrinsic and intrinsic neural interconnections involved in the control of intestinal motility. Extrinsic neural regulation refers to all nerves that have cell bodies located outside the intestinal tract. Extrinsic neural input to the gastrointestinal tract comes from the central nervous system (CNS), the sympathetic and the parasympathetic systems. Intrinsic neural regulation refers to all nerves whose cell bodies reside in the intestine. The ENS provides most of this regulation. It is capable of functioning independently of the extrinsic nervous system in animals when connections to the extrinsic nerves have been severed.

DEVELOPMENT OF THE INTESTINAL NEUROENTERIC SYSTEM

The ENS comprises a large number of phenotypically different neurons and glial cells, arranged in enteric ganglia interconnected in complex plexiplexi. These plexiplexi are situated between the smooth muscle layers to form the outer myenteric and the inner submucosal plexiplexi. The main functions of the ENS are: to control gut propulsive motor activity (such as peristalsis and MMC) by regulating the contractility of the intrinsic smooth muscle; to modulate the activity of secretory glands present within or associated with the gastrointestinal tract and to regulate the blood flow and the mechanisms of secretion/absorption.

In many aspects, the ENS of vertebrates is similar to the CNS, which has led to its characterization as the "second brain." In fact, unlike the innervation of other organs, the ENS is capable of mediating reflex activity in the absence of input from the brain or spinal cord, due to the presence of motor circuits including sensory neurons, intrinsic primary afferent neurons, interneurons, and excitatory and inhibitory motor neurons.

During the development of the gastrointestinal tract, neuroectoderm-derived neuronal precursors colonize the lengthening gut and become distributed in concentric plexi within the gut wall to form the ENS. All neurons and glial cells of the ENS are derived from the neural crest. Neural crest cell (NCC) ablation studies,⁴² quail-chicks interspecies grafting,⁴³ and other cell-tracing experiments⁴⁴ have allowed the origin of ENS cells to be assigned to the vagal region of the neural crest, adjacent to somites 1–7, which populates the entire length of the gut. A second region of the neuraxis, the sacral neural crest, posterior to somite 28, was also shown to provide cells to the hindgut.⁴⁵

Vagal derived ENS progenitors, which give rise to the majority of neurons and glia of the enteric ganglia, enter the foregut mesenchyme and migrate in an anteroposterior direction colonising the entire length of the gut (primary

migration wave). Lineage and genetic studies in mouse embryos have suggested that the enteric component of the vagal neural crest generates two distinct lineages: the sympathoenteric lineage which is derived from neural tube at the level of somites 1–5 and contributes to the formation of the enteric ganglia and the superior cervical ganglia of the sympathetic chain, and the second, the sympathoadrenal lineage, which generates progeny that colonizes primarily the enteric ganglia of the foregut (esophagus and stomach).⁴⁶ A secondary migration wave of NCCs takes place across the radius of the developing gut in mice and chicks.^{45,47} In the small and large intestine of mice and small intestine of chicks, neurons and glia initially coalesce into ganglion plexoplexi in the myenteric region, while the submucosal plexus develops later.^{48,49} However, the submucosal plexus develops before the myenteric plexus in the large intestine of chicks. In contrast to the vagal derived NCCs, the vast majority of the sacral-derived neural crest progeny is restricted to the colorectum, whereas, far fewer cells are also present in the ceca and in the postumbilical intestine.⁵⁰

Numerous studies have investigated the phases of NCCs spatio-temporal migration within the gut, the possible prespecification of NCCs as ENS precursors and the possible factors implicated in the coordinated migration, proliferation, differentiation, and survival of NCCs within the developing gut. The combination of quail-chicks interspecies grafting to selectively label subpopulations of NCCs, together with antibody double-labeling to identify quail cells and neuronal and glial phenotypes within chick enteric ganglia, has allowed the identification of the subsequent phenotypes of crest-derived cells within the gut and consequently the vagal and sacral NCCs spatiotemporal migration pathways.^{43,45,50} Vagal NCCs initially accumulate in the caudal branchial arches, then enter the foregut mesenchyme at E3 and migrate in a single rostrocaudal wave reaching the level of umbilicus in the chicken at E5, the cecal region at E6 and the colorectum at E7.5. The entire length of the chick gut is colonized by E8.5. Sacral NCCs colonize the gut in an opposing caudorostral direction. They were found to initially congregate in the dorsal wall of the hindgut where they form the nerve of Remak until E7, when nerve fibres project into the hindgut, then migrate into the gut along these nerve fibres colonizing the hindgut in large numbers from E10. From these findings, the sacral NCCs appear to colonize the hindgut 2 to 3 days after it had been colonized by vagal NCCs, supporting the idea that sacral crest derived cells require the presence of signaling molecules released by vagal-derived cells in order to colonize the hindgut.

The same authors demonstrated that unlike the colorectum in avians and mammals, where NCCs initially colonize the myenteric plexus while the submucosal plexus arises from the secondary migration of cells throughout the myenteric region, in chicks vagal NCCs colonize the submucosal region first, before migrating outwards

through the circular muscle layer to populate the myenteric plexus region in a second movement.⁴⁵ Furthermore, these experiments revealed differences in the migration pathways of vagal NCCs within different regions of the chick gut. In fact, migrating crest cells appear randomly distributed within the mesenchyme in the colonization of preumbilical intestine, being muscle layers undeveloped. In the postumbilical intestine the vagal NCCs migration front is initially located in the outermost layers of the mesenchyme, adjacent to the serosal epithelium. As the circular muscle layer begins to develop, while the crest cells progress along the gut to reach the cecum, they become orientated on either side of the circular muscle layers to form the presumptive myenteric and submucosal ganglia.⁴⁵

The topographical pattern of differentiation of human NCCs and gut mesenchyme have been recently studied by Fu and colleagues 2004.³² Previous studies reported that vagal NCCs migrate from the foregut to the hindgut of human embryos between gestational age week 4 and week 7 and that the NCCs at level of the colon differentiate into neurons and glia by week 7 without coalescing into ganglion plexus.⁵¹

Fu and colleagues³² reported that rostral to caudal colonization of the entire gut by the NCC is completed by week 7 of gestation. The formation of the myenteric plexus follows a rostral to caudal pattern. Coalescence of neurons and glia into the myenteric plexus coincides with the differentiation of the longitudinal and circular muscles between gestational weeks 7 and 14. The submucosal plexus develops after the appearance of the myenteric plexus and before the differentiation of the muscularis mucosae. A discernable myenteric plexus first appears at the foregut in week 7 when the mesenchyme surrounding the ganglion has started to differentiate into muscle. At week 7, neurons and glia are localized at the hindgut mesenchyme but are randomly distributed and have not coalesced into recognizable ganglion plexus. Neurons and glia coalesce into plexi in the myenteric region at the hindgut by week 9, 2 weeks after the appearance of myenteric plexus at the foregut. The myenteric plexus is small and consists of a few, closely packed neurons and glia at week 7. From week 12 to week 20 plexus increases in size and neurons and glia become less packed. Intraplexus nerve fascicles become clearly visible from week 14 to week 20. The submucosal plexus develops after the appearance of the myenteric plexus and before the differentiation of muscularis mucosae. Scattered NNCs and glia are first seen at the presumptive submucosa of the foregut and mid gut at week 9 and coalesce into small ganglion plexi in the submucosa inner to the nascent circular muscle layer in these sites. By week 12 the submucosal plexus at the foregut increases in size, remaining relatively small in the midgut. By week 14 inter-cellular spaces and intraplexus nerve fascicles are visible at the foregut and midgut. At the same gestational age the submucosal plexus is seen at the hindgut, firstly localized at the submucosa inner to the

circular muscle. By week 20 it is also localized in the inner submucosa further away from the circular muscle layer at the foregut and the midgut and intraplexus nerve fascicles are evident in the hindgut submucosal plexus. The observation that in the human fetal gut neurons and glia initially coalesce into ganglion plexi at the myenteric region, and that the submucosal plexus develops later, argues for a secondary migration of NCCs from the myenteric region to the submucosa.

The temporal development of ganglion plexi in the human fetal gut is in line with that reported in mice and the small intestine of chicks, but differs from the large intestine of chicks.^{45,47} The molecular mechanisms that control the spatio-temporal development of the ENS in the gut are multiple and represent a field of research in continuous evolution.

Signals and Molecules that Control the Development of ENS

Crest derived cells probably do not migrate as a uniform array of committed and uncommitted precursors, but appear to constitute a heterogeneous population that changes progressively as a function of developmental stage, both as the cells migrate and after they arrive in the target bowel.

Several studies, performed over the past several decades, have clarified that since vagal and sacral NCCs differentiate into specific neuronal phenotypes when transplanted elsewhere along the neuraxis, it is possible that NCC precursors may have some level of prespecification or some special migratory properties that, together with a particular favorable and permissive environment, allow and regulate their spatiotemporal migration within the gut.^{52–54} Another important concept is that vagal and sacral populations seem to present differences in the migratory properties, the rostral vagal NCCs being endowed with a higher invasive and proliferative capacity than sacral cells.^{54,55} In fact by transplanting sacral crest to the trunk region and vice versa, Erickson and colleagues⁵⁵ found that crest cells behaved according to their new position, rather than their site of origin, concluding that sacral NCCs have no cell autonomous properties that allow them to colonize the gut and that at the sacral level the environment is sufficient to allow crest cells from other axial levels to enter the gut mesenchyme.

Along their route of travel the crest derived precursors have ample opportunity to interact with microenvironmental signaling factors, which include growth factors and elements of extracellular matrix (ECM) that irreversibly influence the precursors and contribute to the determination of their fate. The migration, proliferation, survival, and differentiation of enteric NCCs are primarily regulated by interactions between diffusible chemoattractive molecules that originate in the gut mesenchyme and their specific receptors expressed on the enteric NCCs. These essential factors include: glial cell line-derived neurotrophic factor (GDNF) and its receptor tyrosine kinase RET and Gfr α 1^{56,57} neurotrophin-3 (NT-3)

and TrkC receptor,⁵⁸ endothelin-3 (EDN-3) and endothelin receptor-B (EDNRB),⁵⁹ netrin (NTN) and deleted in colorectal cancer (DCC),⁶⁰ bone morphogenetic proteins 2 and 4 (BMP2, BMP4) and the BMP receptors,⁶¹ sonic hedgehog.⁶²

Two intercellular signaling pathways are absolutely necessary for complete colonization of the gut by neural precursors: those mediated by RET and EDNRB.

THE GDNF GFR α -RET MEDIATED PATHWAY

RET is a transmembrane tyrosine kinase and represents the signaling component of multi-subunit receptor complexes for the GDNF family ligands (GFLs). There are four distant members of the transforming growth factors TGF- β superfamily: GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN).⁶³ RET activation requires a glycosylphosphatidylinositol (GPI)-linked coreceptor (GFR α 1-4) that determines RET ligand specificity. GFR α 1 interacts preferentially with GDNF; GFR α 2 with NRTN; GFR α 3 with ARTN, and GFR α 4 with PSPN.⁶⁴

The role of RET in ENS development is well documented and several studies have demonstrated the role of RET-positive NCCs as multipotential ENS progenitors. Taraviras and colleagues⁴⁶ studied the response to GDNF and NTN of primary neuronal cultures (peripheral sensory and central dopaminergic neurons) derived from wild-type and RET-deficient mice and showed that the absence of functional RET receptors abrogates the biological responses of neuronal cells to both GDNF and NTN. Furthermore, cultures of Ret⁺-NCCs, isolated from the gut of rat embryos failed to survive or differentiate in the absence of neurotrophic factors. These findings suggest that GDNF and NRTN promote the survival, proliferation, and differentiation of multipotential ENS progenitors present in the gut rat embryos at relatively early stage of embryogenesis (E12.5 to 13.5). These effects seem to be stage specific, since similar ENS cultures established from later stage embryos (E14.5 to 15.5) had a drastically reduced response to both neurotrophic factors. On the contrary another neurotrophic factor, NT-3, has no effect on early Ret⁺-NCCs progenitors while it promotes the generation of neurons and glia in late NCC derived cultures (E14.5).^{46,65-66}

Despite the comparable ability of GDNF and NRTN to promote ENS precursors proliferation and axonal extension in vitro, the phenotype of Gdnf^{-/-} and Ntn^{-/-} mice is very different. Gdnf^{-/-} mice have hypoganglionosis in the stomach and ganglionosis of the small bowel and colon.⁶⁷ Ntn^{-/-} mice have a normal number of myenteric neurons but a reduced neuronal fiber density and abnormal intestinal contractility.⁶⁸ Gianino and colleagues⁶⁴ have examined the ENS in mice deficient in both GDNF and NRTN (Gdnf^{-/-}/Ntn^{-/-}) and in mice heterozygous for GDNF (Gdnf^{+/-}) as well as Gdnf^{+/-}/Ntn^{-/-}, Ret^{+/-} and Gfr α 1^{+/-}. They confirmed that Gdnf^{+/-} mice have enteric hypoganglionosis that

occurs because GDNF availability determines the rate of ENS precursor proliferation.

Unlike most other parts of the nervous system, neuron number in the wild-type ENS appears to be largely determined by controlling ENS precursor proliferation rather than by programmed cell death. By contrast, NRTN availability determines acetylcholinesterase-stained neuronal fiber density in the mature ENS, but does not influence myenteric neuron number; it may play a minor role in determining submucosal neuron number. Heterozygous mutation produces motility defects, but no major neuron-anatomical changes within the ENS. The hypoganglionosis in Gdnf^{+/-} mice and the reduction in neural cell size and acetylcholinesterase-stained myenteric fiber density in Ntn^{-/-} mice provides strong evidence that mature enteric neurons and their precursors are trophic factor dependent. This dependence appears to switch during development. In the early stages of cell migration and proliferation, GDNF activation of Ret via GFR α 1 is absolutely required for both survival and proliferation of all ENS precursors in the small bowel and colon. As development proceeds, many enteric neurons become dependent on NRTN and GFR α 2 for trophic support. However, because of the complexity of the ENS, it seems likely that subpopulations of enteric neurons will be supported by distinct neurotrophic factors and neurotrophic cytokines.

Yang and colleagues⁶⁹ demonstrated that mesenchymally derived GDNF in addition to its role in survival, proliferation, and neuronal differentiation, plays a role in retaining vagal NCCs within the gut mesenchyme so that they do not migrate into the neighboring tissues via mesentery and promote the migration of NCCs along the gut. The authors postulated that GDNF protein levels could be higher in more caudal uncolonized areas that could act as GDNF "sinks." So the net direction of neuronal precursor cell migration, and the direction of axon projection pattern of the first enteric neurons, could be attracted towards these areas with higher levels of untapped GDNF protein in a gradient-dependent manner. Further supporting this evidence is the study from Natarajan and colleagues.⁵⁷ The authors found that NCCs present within fetal small intestine explants migrate towards an exogenous source of GDNF in a RET-dependent fashion. The chemoattractant role of GDNF is likely to depend on the spatial and temporal regulation of GDNF expression along the developing bowel. When vagal cells colonized the foregut, GDNF expression was high in the stomach and when present in the midgut, GDNF expression was upregulated in a more posterior region, the cecum.

The progressive wave front of the NCCs following the areas with GDNF upregulation has not been described in the hindgut. It appears that during hindgut colonization by ENS progenitors, the cecal domain of GDNF expression extends posteriorly alongside the front of migration cells. This suggests a permissive, rather than an instructive role for GDNF in the postcecal region with distinct mechanisms directing hindgut colonization.

The role of GDNF in promoting proliferation and survival of ENS precursors seem to be modulated by EDN3 which had been shown to inhibit the differentiation of migrating NCCs ensuring that sufficient ENS precursors are available to colonize the entire gut. In fact, in the absence of EDN3, enteric precursors differentiate prematurely and fail to colonize the entire length of the gut.

The EDN3/EDNRB-Mediated Pathway

Endothelins -1,-2 and -3 (EDN1-3) constitute a small family of 21 amino acid peptides which activate heptahelical G-protein-coupled receptors. Only EDN3 is known to be required for normal enteric neurodevelopment. EDN3 is produced by mesenchymal cells adjacent to NCCs as they colonize the gut and the skin, and is expressed at particularly high levels in the ileocecum.⁷⁰ Mature endothelins are produced from large precursor molecules, called pre-proendothelins that are cleaved enzymatically to produce 38-41 amino acid long biologically inactive intermediates, the "big endothelins." The big endothelins are then cleaved by endothelin converting enzyme 1 (ECE-1) to the biologically active 21 amino acid long endothelins.

Mice deficient in ECE-1 exhibit craniofacial and cardiac abnormalities and fail to generate enteric neurons and melanocytes, reproducing the phenotype observed in EDN3 and EDNRB-deficient animals.⁷¹ These findings confirm that ECE-1 is the protease responsible for the in vivo formation of active EDN-1 and EDN3. In vitro studies of cell cultures of ENS precursors suggest that EDNRB activation inhibits differentiation.⁷² A similar phenomenon is believed to occur in vivo to maintain a critical mass of mitotically active crest cell precursors, which are required to colonize the entire length of the large intestine. In the absence of EDNRB or EDN3, colonization of the small intestine is slightly retarded and spread of neuronal precursors from the distal ileum to the cecum is severely impaired. The most commonly held hypothesis is that EDN3 activity maintains the pool of enteric NCC precursors by promoting their proliferation and inhibiting their differentiation into neurons. While several studies have confirmed the capacity of EDN3 to inhibit neurogenesis in cultured NCCs, it has been recently reported that the absence of EDNRB in enteric neural stem cells, does not increase neurogenesis.⁷³

Recently Nagy and colleagues⁶² have studied the role of EDN3 during formation of the avian hindgut ENS. They created chick-quail intestinal chimeras by transplanting preganglionic quail hindguts into the coelomic cavity of chick embryos. The quail grafts develop two ganglionated plexi of differentiated neurons and glial cells originating entirely from the host neural crest. The presence of excess of EDN3 in the graft results in a significant increase in ganglion cell number, while inhibition of EDNRB signaling in the hindgut leads to severe hypoganglionosis. This hypoganglionosis does not derive by increased apoptotic cell death. The EDN3-induced hyperganglionosis can result from

effects on NCCs proliferation, survival, and/or differentiation. This result is consistent with previous *in vitro* works where EDN3 promoted proliferation of cultured NCC and of undifferentiated enteric crest derived cells.⁷⁴ In the absence of EDN3 signaling there are only enough NCCs precursor to populate the intestine to the level of the cecum.

The cecum seems to occupy a very central role in EDN3 function. The cecal expression of EDN3 and GDNF in avians appears just prior to the arrival of crest-derived cells at E5.5, suggesting that these factors may locally influence, directly or indirectly, enteric NCCs and the migratory wavefront. Inhibition of EDNRB activity in the avian gut leads to arrested migration precisely at the level of the cecum,⁶² while EDN3 signaling is only required between E10.5 and E12.5 in mice, just when migrating crest cells are crossing the ileocecal junction. Furthermore, NCC migration appears normal in EDN3 and EDNRB mutant mice until those cells reach the cecum, at which time there is a transient migratory arrest.⁷⁵ These observations suggest that EDN3 may have a specific effect on migrating crest-derived cells as they reach the cecum, serving to expand the population precursors at that point so that they can populate the remaining intestine.

While several studies have supported the capacity of EDN3 to promote NCCs proliferation, it has been reported that the absence of EDNRB in enteric neural stem cells does not increase neurogenesis and that EDN3 also inhibits neuronal differentiation in the ENS, as demonstrated by the absence of nNos-expressing cells in EDN3-treated hindgut.^{62,73} In contrast, terminally differentiated crest-derived cells are present in the cecum reflecting their premature differentiation and inability to migrate further along the gut and confirming the role of EDN3 in expanding the population of precursors in the cecum to allow migration in the remaining intestine where they mature. The role of EDN3-EDNRB signaling is relevant not only in the cecum but also in the hindgut, where EDN3 becomes expressed as NCCs are arriving; in this way, deficient EDN3 activity may create a hindgut environment that does not allow normal colonization by enteric crest derived cells.⁶²

One of the most exciting areas of investigation concerns potential genetic and molecular interactions between the GDNF/RET/GFR α 1 and EDN3/EDNRB pathways.

Coexistent alterations in genes from both pathways confer a significantly greater risk of enteric aganglionosis than the same genetic alterations in isolation. In contrast with homozygotes, Ret^{+/-} heterozygotes do not exhibit aganglionosis. Similarly, Ednrb^{del} mice, have ganglion cells throughout their gastrointestinal tracts. On the contrary, aganglionosis is observed in 100% of Ret^{+/-};Ednrb^{del}, indicating that mutations at one locus will modify the phenotype associated with mutations at the other locus.

The restriction of aganglionosis in EDNRB and EDN3 deficient mouse to the distal colon initially suggested that this pathway could play a

role only during late stages of gut colonization such as E11.5 to E12.5, during which time enteric NCCs cross the cecum and colonize the proximal colon.^{76,78} Recent studies in humans and mice have demonstrated that an interaction between the RET and EDNRB loci regulates ENS development not only in the distal colon,^{76,77} but also into prececal segment.⁷⁴ Barlow and colleagues⁷⁴ studying double mutant embryos, reported that EDN3 specifically cooperates with GDNF to promote the proliferation of the uncommitted ENS progenitors; this function in addition to its antagonistic effects on neuronal differentiation, suggests that endothelins represent a class of extracellular signals that regulate the number of undifferentiated neuroectodermal precursors. At the same time, activation of the EDNRB inhibits the chemoattractive effect of GDNF on ENS progenitors. In fact, EDN3 is expressed at highest levels just near and ahead of the front of migration of enteric NCC, probably regulating the orderly colonization of the gut. In this respect, it is interesting that in EDN3 deficient mice, ectopic ganglia have been identified in the pelvic region outside the enteric musculature in the adventitia of the colon⁷⁹ indicating a possible derivation from ENS progenitors that fail to follow the correct migratory pathways established by GDNF. The mechanism by which EDN3 blocks GDNF-induced chemoattraction wavefront seems to be related to the inhibition of a protein Kinase A (PKA).⁷⁴

Finally, EDN3 may also affect the environment through which NCCs migrate, in fact *in vivo* and *in vitro* studies have shown that EDNRB-mRNA is expressed by non-NCCs in the gut wall; these cells produce high levels of laminin when EDN3 is deficient.^{72,80} These findings support the hypothesis that a non-neural crest, ECM mediated, indirect effect of EDN3/EDNRB signaling promotes premature differentiation of neural precursors.

Extracellular matrix molecules could contribute in several ways to ENS development. They provide the matrices for navigation during cell migration, promote neuronal overgrowth, neuronal differentiation, and cell survival. Alterations in ECM molecules including laminin, collagen type IV, tenascin, fibronectin, and nidogen have been documented in patients with Hirschsprung's disease, thus implicating ECM molecules in enteric neurogenesis.⁸⁰⁻⁸³ The production and deposition of the neurotrophic factors and ECM molecules changes as the gut mesenchyme differentiates. The migratory NCCs may recognize the differentiation state of the mesenchyme by the interpretation of the repertoire of neurotrophic factors and ECM molecules as microenvironmental cues.

The Netrin/DCC Mediated Pathway

Netrins are members of a family of laminin-related proteins which include the UNC-6 gene product of nematodes, netrin A and B of *Drosophila*, netrins 1 and 2 of chicks, netrin 3 of mice and NTN2L of humans. They attract or repel sets of axons; the chemoattractant effects

are mediated by the DCC family of plasmalemmal receptors. The adenosine A2b receptor also binds netrins probably acting as a DCC coreceptor.⁸⁴ Whether effects of netrins are attractive or repulsive depends on coreceptors.⁸⁵

Unlike the colorectum in birds, in the intestine of mammals including humans, NCC initially colonize the myenteric plexus. Then cells migrate from the outmost layers of gut mesenchyme taking a critical perpendicular turn and entering the submucosa to give rise to the submucosal plexus.^{45,47} Recent studies have implicated netrins in the guidance of this secondary migration to the submucosa and pancreas respectively.⁶⁰ These authors demonstrated that netrins and netrins receptor DCC, neogenin and the adenosine A2b receptor, are expressed in the fetal mucosa and pancreas at early stages in development. DCC expression quantified in the murine gut, was detectable as early as E11 when crest-derived cells are known to be present in the small intestine.⁴⁷ By E13 DCC expression reaches a peak; this is the period in which the hindgut is colonized (E12.3 to E14.5) and coincides with the secondary migration of the NCCs in the small intestine from the outer gut mesenchyme to the submucosa.

A subset of enteric crest derived cells thus expresses DCC. These cells migrate towards endogenous sources of netrin within the gut, inwardly through the developing circular muscle and give rise to the submucosal plexus. Expression of DCC by enteric NCCs is maximal when they make their turn to colonize the submucosal plexus and when others enter the pancreas. Transgenic mice that lack DCC lack both the submucosal plexus in the bowel and ganglia in the pancreas, confirming that netrins interact with DCC expressed by crest-derived precursors to guide these cells to the submucosa and pancreas.

A repulsive signal, which prevents crest-derived cells from migrating any further than the submucosa is laminin. Laminin, secreted in abundance by the developing enteric mucosa, converts the attractive effect of netrin to repulsion,⁸⁶ preventing NCC from entering the mucosa and encouraging these precursors to differentiate only in the submucosa.

The presence of netrin in the outer gut mesenchyme may be important also in preventing premature apoptosis among the migrating crest derived cells; in fact, in the absence of ligands, DCC mediates apoptosis.^{87,88}

It can be concluded that the netrin/DCC signaling pathway play a critical role in the development of the ENS for the formation of the submucosal and pancreatic plexi. However, this pathway may also play a role in the establishment of the extrinsic vagal innervation if the intestinal ganglia, the development of intraenteric connections and the regulation of cellular apoptosis.

The Role of Sonic Hedgehog (Shh) and the Bone Morphogenetic Proteins (BMP)

The molecular mechanism that controls the secondary migration of NCCs together with the

spatiotemporal development of the annular structures of the gut wall are poorly understood. The development of the annular structure of the gut wall seems to be regulated by the mucosal secretion of the Sonic hedgehog (Shh) into the underlying mesenchyme. In response to Shh induction, mesenchyme produces and secretes BMPs into the ECM.^{89,90}

In developing gut it has been shown that Shh is expressed in the endodermal epithelium and induces patched and BMP4 expression in the mesenchyme of gut.^{90,91} Sukegawa and colleagues⁸⁹ have investigated the expression and function of the genes that regulate the molecular mechanisms involved in concentric differentiation of chicken gut mesenchyme. They reported that all gut mesenchymal cells have the potency of differentiating into smooth muscle cells and that epithelium inhibits this differentiation. Epithelium seems to inhibit also proliferation of enteric neurons and controls their distribution within the gut mesenchyme. The authors demonstrated that Shh could mimic the effect of epithelium on the topographical differentiation of the gut.

Shh secretion is first detected in the endodermal epithelium just after the establishment of the digestive tube and continue throughout development.⁹¹ Later in development Shh is involved in the region-specific differentiation of both epithelium and mesenchyme. Shh-mediated signaling inhibits differentiation of smooth muscle resulting in differentiation of nonmuscle layers such as the lamina propria and submucosa. The influence of this molecule on the mesenchyme can be monitored by the expression of Shh-responsive genes such as patched and BMP4. These genes are expressed in cells in close proximity of epithelium that are the main sources of Shh.

Shh also inhibits the differentiation of enteric neuronal cells which appear restricted to regions distant from the sources of Shh. It has been reported that Shh-Knockout mouse shows an alteration in smooth muscle differentiation and in the increase of the number of enteric neuronal cells that appear abnormally distributed in the mesenchyme. The gradient of Shh concentration across the radius of the gut may regulate the second migration and differentiation of NCC by the modulation of competence of NCCs towards special microenvironmental "cues" such as GDNF and EDN3 from the differentiated mesenchyme.

Shh has been shown to inhibit neuron differentiation of NCC in the gut by inducing BMP4,^{89,90} while it controls smooth muscle differentiation directly or via induction of factors other than BMP4. However, studies in the rat gut in vivo have demonstrated that BMP2 induces neuron differentiation of NCC due to the addition of mesenchyme derived factors.⁹² In response to Shh induction, mesenchyme produces and secretes BMPs.

Bone morphogenetic proteins (BMP) comprise a subgroup of the TGF- β family of secreted signaling molecules. BMP2, 4, and 7 transduce their signal by binding to a heterodimer consisting of a type I receptor, BMPRIA, or BMPRIB

or, in case of BMP7, activin receptor like kinase (Alk-2) and type II (BMPRII) receptor. Receptor activation leads to phosphorylation and activation of the Smad-signaling cascade.^{90,91} BMP regulates multiple critical functions during organogenesis including the epithelial-mesenchymal interactions that underlie specification, regionalization, and differentiation within the developing gut. In the early embryo BMP directs the formation of NCCs in the dorsal neural tube. During the neural crest migration they continue to act as local environmental cues to promote the development of autonomic neurons. Later in development, exposure to BMP promotes dendrite specific process outgrowth, promoting differentiation to an autonomic neuronal fate and modulating trophic factor responsiveness in primary sympathetic cultures in cell lines.⁹³⁻⁹⁵

The role of BMP signaling in enteric neuronal differentiation is controversial. BMP4 has been shown to either promote or inhibit enteric neuronal differentiation while BMP2 promotes neuronal differentiation of post-migratory enteric crest cells.⁹¹

Pisano and colleagues⁹² reported that BMP2 influences the number of neurones that develop from sympathetic and enteric precursors cells, acting through multiple mechanisms that include regulation of differentiation, proliferation, and survival. The acquisition of trophic factor responsiveness and the increase in expression of neurofilament proteins, in conjunction with studies showing BMP-dependent regulation of neuropeptide expression and dendritic development, argue that BMP promotes neuronal maturation in neural crest derived lineages. The final phenotype of these neurones is determined by BMP signal in the context of other signals expressed in the local environment. This model of multiple interacting signals is supported by studies demonstrating that BMP act in conjunction with retinoic acid to induce expression of a GDNF receptor and to enhance the acquisition of GDNF and NT-3 responsiveness.^{96,97}

Recently Goldstein and colleagues⁵⁹ reported that targeted inhibition of intestinal BMP activity using the BMP inhibitor *noggin*, leads to impaired migration of enteric crest cells by interfering with their responsiveness to GDNF. Enteric crest cells underwent a significant delay in their migration and failed to reach the distal hindgut until well beyond the stage at which this normally occurs. Multiple factors can contribute to delay in migration: increased cell death, premature neuronal differentiation or direct effect on migration. The authors found that there was no change in the rate of cell death or in the timing of neuronal and glial differentiation. Since BMP4 alone is not sufficient to promote migration of crest-derived cells from the gut, they demonstrated that BMP4 can modulate the effect of a known migratory factor: the GDNF.

This observation extend the previously reported data that an interaction between GDNF and BMP4 synergistically enhances enteric neuronal development.^{59,98} The migratory response

of ENS cells to GDNF is known to be mediated by several factors, the activation of RET signaling pathways and the interaction with neuronal cell adhesion molecule (NCAM). In addition, both the EDN3 and Shh pathways appear to be involved, inhibiting the migration of crest-derived cells in response to GDNF.^{57,61,63,64,99} Since BMP4 is expressed both in crest and noncrest derived cells within the gut, its influence on migration may be either a direct effect on the crest cells itself or an indirect one through other molecules produced by the intestinal microenvironment.

Secondly, in the absence of BMP activity, fewer numbers of NCC reach the hindgut and subsequently fail to aggregate normally into clusters to form ganglia. Studies in vitro and in vivo show that BMP2 and BMP4 are required to promote aggregation of isolated enteric neurons to form clusters of cells simulating the appearance of enteric ganglia. This mechanism also requires activation of GDNF which increases the number of neurones per clusters and the ganglion size.^{59,65}

In conclusion, development of the ENS is a complex process during all of embryogenesis and continues in fetal and early postnatal life. Its spatiotemporal determination is dictated by innumerable and complex interactions between different intercellular signaling systems. Alterations in this subtle equilibrium could induce anatomic and physiologic defects in the gut wall, some of which are recognized as specific forms of intestinal dysmotility patterns in humans. Further studies of ENS development and all of the molecules involved in its complex mechanisms of control will improve the understanding of these conditions, leading to better diagnosis and treatment of affected individuals.

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Development of Esophageal Peristalsis in Preterm and Term Neonates

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Background & Aims: High-resolution manometry demonstrates a chain of 3 sequential pressure segments that represent esophageal peristalsis in children and adults. We performed high-resolution manometry in preterm and term neonates to determine the ontogenesis of esophageal motility with regard to this segmental architecture. **Methods:** Sixteen preterm (gestational age 32.9 ± 2.6 weeks at examination) and 14 term neonates (38.9 ± 1.6 weeks) underwent manometry with a 9-lumen perfused catheter having recording side holes spaced at 1-cm intervals. Pressure responses to swallows were evaluated for the presence of peristaltic segments on isobaric contour maps by an investigator who was blinded to gestational age. **Results:** The second segment was well developed in $\geq 50\%$ of swallows in all preterm and term neonates. In contrast, the first segment was present in $\geq 50\%$ of swallows in only 2 preterm neonates (12.5%) and 8 term neonates (57.1%; $P < .05$ for each compared with second segment) with identical findings for the third segment (12.5% preterm and 57.1% term neonates; $P < .05$ for each). Completed peristalses with intact segmental contraction sequences throughout the esophageal body were present in $26\% \pm 6\%$ of swallows in preterm neonates vs $55\% \pm 9\%$ in term neonates ($P = .01$). **Conclusions:** The second pressure segment in the midesophagus (proximal smooth-muscle region) is well developed before term. Presence of other segments significantly improves at term, but peristalsis remains incomplete in nearly half of swallows. Control mechanisms for both striated- and smooth-muscle esophageal regions are incompletely developed in neonates, the outcome of which could participate in infant reflux disease.

Gastroesophageal reflux disease (GERD) remains a source of significant morbidity in neonates and infants, a disorder attributed primarily to esophageal motor dysfunction.^{1–4} Using conventional perfusion manometry, Omari et al showed in 1995 that esophageal peristalsis was poorly formed in many swallows by preterm infants aged 33–38 weeks postconception at time of study.⁵ These and earlier findings suggested that poor development of esophageal clearance mechanisms may

be in part responsible for GERD and its complications.^{5,6} However, later works by Omari et al indicated that the dominant defect in preterm infants was increased acid reflux from transient lower esophageal sphincter (LES) relaxations.^{3,7} Swallow-induced peristalses typically were thought to be complete, shifting the emphasis away from clearance to sphincter-related processes.⁸ The ability to determine the pathophysiology of GERD in a very young population was attributed by these authors largely to the development of microluminal extruded catheters allowing low water burdens from their pneumohydraulic perfusion devices.⁹

These same technical advances have contributed to the development of high-resolution manometry, wherein recording sites are increased in number and spaced closely in the axial direction, and interpolation of pressure data across sites is accomplished to visualize better the space-time relationships using 3-dimensional isobaric contour maps.^{10,11} These techniques have revealed in adults that esophageal peristalsis is composed of a chain of 3 contraction segments—1 in the striated muscle and 2 in the smooth-muscle region—that culminate in LES contraction.^{12–14} Although interswallow and intersubject variation occurs in the location of the defining intersegmental pressure troughs along the esophageal length, all 3 segments can be identified in $>85\%$ of subjects.^{13,14}

High-resolution manometry has been reported in preliminary fashion in a small number of pediatric patients across the age range within this specialty.¹⁵ Although the 3 contraction segments could be identified in each age subgroup (neonates, infants/toddlers, children), the percentage of complete peristaltic chains appeared reduced in the very small number of neonates studied. We presumed that high-resolution manometry could better determine the development of peristalsis in the neonatal period and thereby demonstrate whether faulty esophageal clearance mechanisms potentially contribute to GERD in infancy. Preterm and term neonates were studied to see whether all segments of the peristaltic pressure wave are present late in the gestational period.

Abbreviations used in this paper: LES, lower esophageal sphincter; UES, upper esophageal sphincter.

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0016-5085/07/\$32.00
doi:10.1053/j.gastro.2007.03.042

Table 1. Clinical Characteristics of the Study Population

Characteristic	Preterm neonates ^a n = 16	Term neonates ^a n = 14
Female sex, n (%)	8 (50.0)	5 (35.7)
Gestational age at birth, wk	31.8 ± 3.2	38.0 ± 2.0
Gestational age at examination, wk	32.9 ± 2.6	38.9 ± 1.6
Age since birth, days	9.6 ± 9.3	6.1 ± 4.9
Weight at examination, kg	1.8 ± 0.5	2.8 ± 0.8

^aGrouped data reported as mean ± SD.

Patients and Methods

Subjects

Investigations were performed on 16 preterm neonates (8 male and 8 female) and 14 full-term neonates (9 male and 5 female). Clinical characteristics of the infants at birth and at examination are shown in Table 1. Gestational age at birth was determined using a combination of maternal history, physical examination (calculation of the Dubowitz score), and early ultrasonography.^{16,17} Gestational age at examination was calculated as the sum of gestational age at birth and time following birth until manometry was performed.

The neonates chosen for study were healthy and had no evidence of neurologic dysfunction. They were receiving expressed breast milk (20–24 calorie/ounce) and/or similarly concentrated infant formula at a mean volume of 100 mL/kg/day (range, 75–120 mL/kg/day). The preterm neonates were gavage fed per nasogastric tube until gestational age of 34 weeks. Following this, the neonates were bottle fed every 2–3 hours. None of these infants were receiving prokinetic drugs or any other agent that would affect esophageal motility. The study protocol was approved by the Ethical Research Committee of the Medical School, University Federico II, Naples, Italy, and informed consent was obtained from the parents in writing before the studies were performed.

Manometric Methods

The manometry catheter used in this study permitted measurement of esophageal pressures at a very low rate of water perfusion with an assembly that also could be used for gavage feeding of preterm neonates. An extruded silicone catheter (outside diameter, 2.25 mm) was employed in which 9 microlumina with recording side holes spaced at 1-cm intervals were arranged around a larger feeding channel (Dentsleeve, Wayville, South Australia). The catheter was designed such that it could be autoclaved between studies. Perfusion of the catheter with sterile distilled water for pressure recording was accomplished using a pneumohydraulic device created for this purpose (Mui Scientific, Mississauga, Ontario, Canada). The modifiable device characteristics and rate of water perfusion (0.05 mL/min/lumen) were selected to record pressure upstrokes of at least 100 mm Hg/s based

on previously published data⁵ and on technical information from the manufacturer. Bubble formation and entrapment were minimized by carbon dioxide flushing. Pressure data were acquired with a system designed specifically for high-resolution manometry (MMS, Enschede, Holland).

All infants were studied after at least 2–3 hours of fasting, and esophageal motility recording time did not exceed 20 minutes. The catheter was introduced transnasally and positioned initially so that at least 1 distal recording side hole was in the stomach below the LES. Nares-to-LES distance of the infants studied ranged from 14 to 17.5 cm. Because the focus of this investigation was on esophageal body motility and not on LES characteristics, a specific maneuver for measuring LES resting pressure was not performed. Small volume (0.25–0.5 mL) ambient temperature boluses of 5% glucose solution were offered at least 15 seconds after any motor activity was recorded⁸ until responses to at least 8–10 swallows were obtained. In the case of the preterm infants, spontaneous swallows were considered if they met the same timing criterion. If needed to record from the upper esophageal sphincter (UES) and proximal esophageal body, depending on esophageal length, the catheter was repositioned such that the most proximal recording location rested in the UES region. Pressures from this most proximal side hole were recorded from a water-filled microlumen that was not perfused. In the proximal catheter position, 8–10 swallows were again recorded, total recording time was noted, and catheter was removed or used subsequently for gavage feeding.

Swallowing was determined by the characteristic opening and movement of the UES seen with high-resolution manometry.^{14,18,19} Because the UES was not visualized when the catheter was in the distal position, a surface submental electromyogram also was recorded to determine the presence of a pharyngeal swallowing phase. Stereotypical deflections of the electromyogram that corresponded with UES opening when the UES was visualized were determined for each infant on post hoc review. These same stereotypical deflections were then used to confirm the presence and time the onset of a swallow when the catheter was positioned distally. The number of evaluable swallows in the distal position was therefore reduced during analysis if presence of a swallow could not be verified by a clear electromyographic signal.

Data Analysis and Statistical Methods

The pressure data were reviewed on the system hardware to determine consecutive swallows that met criteria for inclusion (ie, adequate demonstration that a swallow had occurred and no motor activity for at least 20 seconds preceding the swallow). Data from the selected swallows were then exported to a plotting program (Golden Software, Golden, Colorado) for creation of isobaric contour maps, as has been described previously for

the analysis of high-resolution manometric data.^{10–14} Lowest contour lines were drawn 5 mm Hg above gastric baseline²⁰ and at 5- to 10-mm Hg increments, the increment determined by the best demonstration of the segmental pressure architecture of peristalsis for that infant. The selection of swallows, creation of plots, and subsequent analyses were performed by an investigator who was unaware of the gestational age of the infant.

Presence of the major pressure segments and intersegmental troughs that have been described previously in children and adults were determined for each swallow.^{10–15} In children and adults, respectively, a first pressure trough is found at 26.5% and 25.4% of esophageal length separating the first and second pressure segments, presumptively striated and proximal smooth-muscle segments.²¹ The second trough dividing the proximal and distal smooth-muscle segments (second and third segments) roughly in half is found at 66.0% and 63.4% of esophageal length in children and adults, respectively, and the third trough separating the third segment from LES after-contraction is found at 96.0% and 99.8% esophageal length, respectively.^{14,15} Landmarks resembling these reported in previous studies were sought on the pressure maps for each swallow from the neonates. Presence of a segment required the finding of a pressure region with concentric contour lines focused on that region (at least 20 mm Hg at peak) and separated by pressure troughs from neighboring areas. When the major pressure troughs could not be identified on an isobaric contour map, the average location determined from the other swallows for that infant was applied to see whether a pressure segment could be identified between troughs. The studies were evaluated subjectively for additional segments and pressure troughs.

The location of pressure troughs was reported in centimeters above the LES and as a percentage of esophageal length from distal margin of the UES to the proximal margin of the LES. For comparison across subjects and incorporating the varying number of evaluable swallows on which the segment might have been observed (taking into consideration the 2 catheter positions), each study was graded for consistency of segment presence on a scale from 0 to 3 (0, absent; 1, present, but in <50% of swallows; 2, present in ≥50% but <80% of swallows; 3, present in ≥80% of swallows). Swallows also were examined for the propagation direction in the 2 smooth-muscle segments (peristaltic or nonperistaltic, including retrograde contraction) based on prior observations of nonperistaltic responses in neonates.⁵ Intergroup comparisons were performed using Fisher exact test; *P* value <.05 was required for 2-tailed testing. Grouped data are presented as mean ± SD.

Results

The entire esophageal body and both sphincters could be visualized in 1 catheter position in 14 of the

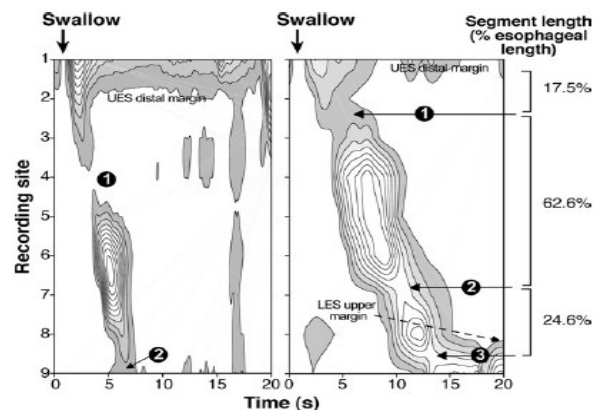


Figure 1. Demonstration of the typical peristaltic pressure segments in a term infant (*left panel*) and a preterm infant (30 weeks gestational age at examination; *right panel*). Isobaric contour lines begin 5 mm Hg above gastric baseline pressure in this and other Figures. In the *left panel*, the catheter is in the proximal position, and only the upper esophageal sphincter and part of the esophageal body are visualized. The first pressure trough (1) separating the first segment (striated muscle) from the second segment (upper portion of the smooth muscle esophagus) and the second pressure trough (2) separating the smooth muscle segments are seen. Locations of these troughs were verified when the catheter was moved to the distal position. In the *right panel*, the esophagus was sufficiently short to visualize all pressure troughs (1–3) and segments in one catheter position. (Sphincter locations were verified by catheter movement.) Lengths of the individual segments relative to total esophageal body length are shown for the *right panel* as calculated for this swallow.

infants, whereas 2 catheter positions were required in the remainder. Ten of the studies in which only 1 catheter position was required were in the preterm neonate group. An average of 8 ± 3 swallows were evaluated in the preterm neonates and 15 ± 5 swallows in the term neonates, the difference reflecting the catheter repositioning more commonly required in the latter group. There were no complications related to manometric evaluation in these infants.

The same segmental architecture to peristalsis was observed in both infant groups as had been reported previously in children and adults (Figure 1), and no additional pressure segments or troughs were identified by subjective review of the maps. On average, the first pressure trough was found 21.0%–23.4% esophageal length, and there was no significant difference in location between preterm and term neonates (Table 2). Likewise, the second pressure trough was located 63.9%–64.5% esophageal length, roughly dividing the smooth-muscle region in half (Table 2). The segmental architecture was distinctive for each infant such that identifying peristaltic sequences was simple using high-resolution manometric techniques (Figure 1).

Table 2. Location of the Peristaltic Segmental Pressure Landmarks

Subject group	Esophageal length (cm)	Intersegmental pressure trough location as percentage esophageal length ^a			Intersegmental pressure trough location in centimeters above LES		
		Trough 1	Trough 2	Trough 3	Trough 1	Trough 2	Trough 3
Preterm neonates (n = 16)	6.1 ± 0.8	21.0 ± 8.6	64.5 ± 11.8	94.3 ± 4.5	4.7 ± 0.6	2.1 ± 0.6	0.4 ± 0.3
Term neonates (n = 14)	7.1 ± 1.6 ^b	23.4 ± 4.5	63.9 ± 6.4	90.5 ± 7.3	5.4 ± 0.9 ^b	2.6 ± 0.9	0.7 ± 0.6 ^b

NOTE. All results reported as mean ± SD.

^aFrom proximal to distal esophagus.^b*P* < .05 compared with preterm neonates.

Each of the individual segments was intermittently present on the swallow maps (Figures 2–4). The segment typically was completely formed when present and easily differentiated from the adjacent segments. The second and third segments overlapped in the distal esophagus as they do in adults¹⁴ such that part of the segment would extend into the neighboring region, yet a set of concentric isobaric contour lines focused on the region (the defining characteristics of a segment) was absent (Figures 3 and 4). The first and third segments were present

in ≥50% of swallows in very few of the preterm neonates and in a significantly larger proportion of full-term neonates—but still only approximately half of the infants (Figure 5). In contrast, the second segment was well developed in ≥50% of swallows in all preterm and full-term neonates, even in the youngest of studied subjects (Figure 5). As would be expected from these segmental findings, the percentage of completed peristalses increased with development, nearly doubling from preterm to full-term evaluation (26% ± 6% vs 55% ± 9%, respectively, *P* = .01). Antegrade propagation was observed in both complete and incomplete peristalses for swallows in

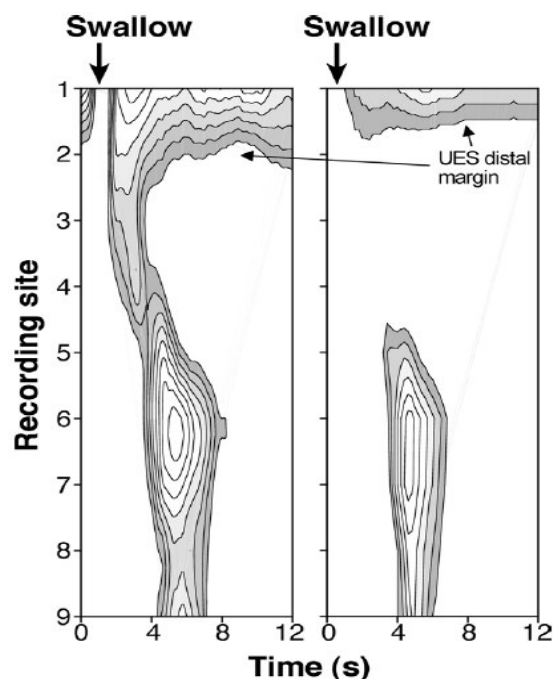


Figure 2. Two swallows from a term neonate demonstrating presence of the first segment in one swallow and absence in another. The second segment is well seen on both swallows with the catheter in a proximal recording position.

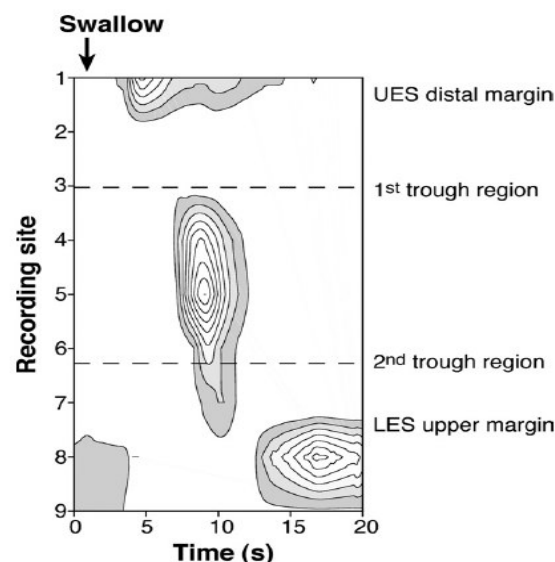


Figure 3. This swallow from a preterm neonate (35 weeks gestational age at examination) shows the entire esophageal body with the catheter in one position. The first segment is not observed at its usual location extending distal to the UES, the second segment is well developed, and the third segment is missing. Location of the second trough was identified from other swallows that contained the second and third segments.

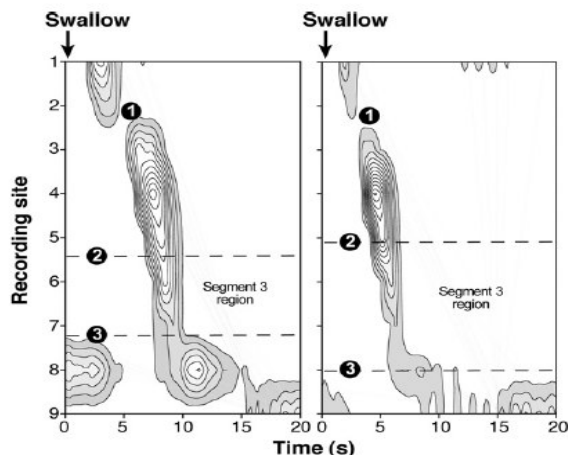


Figure 4. Presence of the first and second segments in a preterm (left panel) and term neonate (right panel) with absence of the third segment above the LES. The second segment overlaps into the region of the absent third segment in each case. The location of the second pressure trough (2) is derived from its average location on remaining swallows in which the trough could be identified. Swallow onset was determined by distinctive electromyographic deflections when the UES was not present on the map.

which at least 1 of the smooth-muscle segments was present; retrograde sequences were not observed.

Discussion

In this report, we found that, as in older children and adults, 3 sequential pressure segments compose esophageal peristalsis in preterm and term neonates. The segmental architecture very closely resembles that described in previous reports of high-resolution manometry in older subjects, with intersegmental trough locations being at similar locations proportional to esophageal length across all age groups.¹³⁻¹⁵ Although all the segments can be identified in infants as young as 27 weeks of gestational age at the time of examination, the consistency of their presence continues to increase through the normal gestational period. At full term, only 55% of swallows have a complete segmental chain, indicating that further development occurs in early infancy. These results support a potential role of inadequate esophageal body motor function in the presentation or manifestations of GERD in infants.

Findings from prior reports can be reconciled with these new observations,^{5,7,22,23} the principal explanation for discrepancies being that the pressure segments identified by high-resolution manometry are not detected easily by conventional manometric techniques. Individual sensors positioned in the distal esophagus cannot interpret the appropriate axial associations of the pres-

sure measurement, such as in regions in which overlapping segments occur (see Figures 3 and 4). Omari et al initially thought that incomplete peristalses were present in more than one third of swallows from preterm infants aged 33-38 weeks (postconceptual age).⁵ The sensors were positioned 1.5, 3, and 4.5 cm above the LES and an amplitude of <10 mm Hg at a site would qualify the response as being incomplete. Our new findings indicate that all 3 sensors would have been located in the smooth-muscle region (second and third segments), and the studies could have been detecting the low prevalence of the third segment. In a subsequent study, Omari et al attributed most of the earlier findings to poor identification of swallows and inclusion of swallow-unrelated motor activity in the analyses.⁷ Only 14% of swallow-related responses were incomplete or failed in the later study, a number considerably lower than the number of incomplete peristaltic chains detected in our present investigation. Similar side hole positioning had been used in the esophageal body with the addition of pharyngeal-located side holes for monitoring swallows. The first segment would not have been detected in most of the studied neonates, and the most distal recording site easily could have been detecting "overflow" pressure from the neighboring second segment in many swallows.

Jadcherla and Shaker also initially concluded that primary peristalsis was well developed at term when they found that 82% of swallows induced peristaltic responses

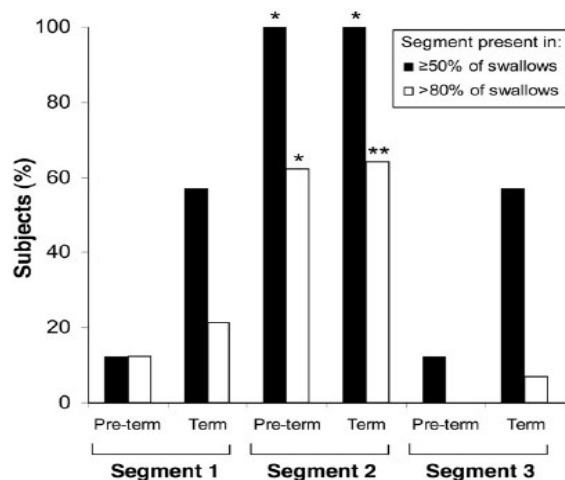


Figure 5. Percentage of subjects in each group demonstrating pressure segments following at least 50% of swallows (grade 2 or 3; solid bars) or following at least 80% of swallows (grade 3 only; open bars). The second segment was more commonly observed than either the first or third segment in preterm and term neonates. * $P < .01$ compared with the first and third segments in the same subject group. ** $P < .05$ compared with the third segment in the same subject group.

in a group of full-term, healthy neonates.²² Three side holes were positioned in the esophageal body at unstated locations. In a recent report from Jadcherla et al, the incomplete development of primary peristalsis in the neonate was established.²³ Eighteen preterm neonates were examined 2 times at a mean of 33 and 35.7 weeks gestational age at examination. The findings were compared with those of 4 full-term neonates and 9 healthy adults. In the neonates, the most proximal esophageal recording side hole was positioned approximately 2 cm below the UES with more distal side holes spaced at 1.75- to 2.0-cm intervals distally. If a propagated swallow was detected in 2 esophageal sites, the peristalsis was considered complete. Peristalsis in 28% of swallows from the youngest group was incomplete or failed; this number reduced to 20%–23% at repeat study and in the full-term subjects. Only 1% of peristalses were incomplete or failed in the adult subjects. Like our findings, these data confirm peristaltic maturation from 33 weeks to term that continues into adulthood. The catheter assembly used by Jadcherla et al²³ would have overlooked the first (striated) segment, and conclusions about the integrity of distal peristalsis would have been limited by the lack of high-resolution data to understand fully the presence or absence of the segments required for completely intact responses. Thus, the percentage of incomplete responses is lower than what we have detected. Taken together, our findings in conjunction with prior studies demonstrate that peristalsis can be complete in preterm neonates, but inconsistent presence of the segments results in incomplete or failed responses that were only partially detected in prior studies; maturation of the process remains incomplete at term.

The first segment likely represents the striated esophageal region, although a direct anatomico-physiological relationship has not been established. The segment is seen in the esophagus of both the human and the opossum, which share similar muscular anatomy of the organ.²⁴ The first pressure trough occurs at a location that corresponds well to the midpoint of conversion from predominantly striated to predominantly smooth muscle,²⁵ and the changes in propagation characteristics across this trough are consistent with a change in muscle type or control.²¹ The mechanisms responsible for the 2 smooth-muscle segments (second and third segments) are less secure. However, the second trough dividing these segments occurs at a location consistent with the transition from predominantly cholinergic to predominantly nonadrenergic, noncholinergic intramural control.²⁶ Other characteristics, including the selective response of the second segment to cholinergic stimulation,²⁷ favor this explanation. Nitric oxide-containing inhibitory nerves play a more significant role in timing and contraction of the most distal region.

Our findings indicate that the second segment develops early and is most consistently present, even in pre-

term neonates. This segment may have particular value in esophageal clearance,²⁸ its early development, thus, being of teleologic importance. Pressures in the second segment augment with cisapride, potentially explaining its benefits on reducing esophageal acid exposure time.²⁷ The segment accentuates in response to increased intrabolus pressures accompanying increased resistance at the esophagogastric junction following fundoplication.²⁹ Control mechanisms for this segment are best developed in the preterm and term neonate. In contrast, sporadic representation of the third segment could reflect immature central or intramural control.³⁰ Defects of nitrergic innervation are found in congenital gut anomalies such as pyloric stenosis and Hirschsprung's disease, suggesting that a lack of nitric oxide-mediated, nonadrenergic, noncholinergic inhibitory control may be responsible for the delay of maturation.

Sporadic presence of the first segment was an unexpected finding. We speculate that the central control mechanisms for this striated muscle region are not completely developed at term because intramural mechanisms play a limited role in this segment. Jadcherla et al also noted differences in UES characteristics as maturation toward term occurred.²³ The authors presumed that brainstem immaturity was in part responsible. Separate development of neural control for the UES and first segment is plausible because the motor events in these regions are distinct and have been differentiated using high-resolution manometry in adult subjects.^{14,19} A slight pressure trough between the UES and first segment was present but not specifically identified in early studies of esophageal peristalsis using high-resolution methods.¹⁴ Williams et al clearly demonstrated this separation in high-resolution evaluation of the pharyngo-esophageal segment,¹⁹ and the initiation of what appears to be a discrete peristaltic sequence timed with but separate from UES contraction is seen on review of the propagating axial waveform generated from high-resolution manometry in this region (refer to http://gastro.wustl.edu/axial_trans.htm for a movie generated from high-resolution manometry data recorded from the UES and proximal esophagus of a normal adult volunteer).³⁰ The central program required for successful initiation and completion of this segmental contraction apparently still is developing at term.

Limitations of this investigation include the spacing of side holes on the catheter considering the subject ages and the method of measuring swallows when the catheter was in a distal position. One could argue that a 1-cm interval between side holes is inadequate to formulate accurate isobaric contour maps when the esophageal length only averaged 6–7 cm in these infants. A greater density of recording sites in the axial direction would have been optimal, but at least 2 recording sites were positioned in each segment, and identification of the pressure troughs was simple in each subject. Addition-

ally, the locations were reproducible when the catheter was moved from the distal to proximal position. The electromyographic method for identifying swallows when the catheter was in a distal position and the pharyngeal-UES region was not represented can be suboptimal, but we had the advantage of comparing the signal with UES opening in the same study. Identification of distinctive electromyographic deflections that corresponded to a swallow was not difficult because the subjects were cooperative with the study. Differing bolus sizes from swallow to swallow can influence peristaltic characteristics such as amplitude,³¹ but it is unlikely that this factor had a major effect on the presence or absence of segments, as measured in this study.

The focus of this investigation was on peristalsis and not LES development because previous reports in neonates have used methods better suited to studying transient LES relaxations as a precursor to reflux events.^{3-5,7,8} Nevertheless, high-resolution manometry could provide information on swallow-induced relaxation and anatomic interactions at the esophagogastric junction that is not available from conventional techniques.³² Recent work in adults demonstrates that high-resolution methods can help dissect the key events leading to junction opening during a transient LES relaxation, eg, crural diaphragm inhibition, esophageal shortening, and favorable pressure gradient between the stomach and esophageal lumen.³³ The axial density of sensors on the catheter used in the present study (1 per cm) was not considered sufficient in neonates to examine the junction adequately, and the radially oriented side holes on a water-perfused high-resolution system are inappropriate considering regional radial asymmetry. We await the further miniaturization of circumferentially sensing, solid-state catheters now being used for high-resolution manometry in adults and older children so that development of motor behavior at the LES level can be investigated more fully.

In summary, this first application of high-resolution manometry to preterm and term neonates demonstrates the development in esophageal peristalsis that occurs through late gestation into term. The 3 segments responsible for intact peristalsis in older children and adults are found in all infants and can be identified in neonates as young as 27 weeks gestational age at examination. Differential development occurs, such that the second segment in the midesophagus (proximal smooth-muscle segment) is present in the majority of swallows in all preterm and term neonates while the other 2 segments at proximal and distal ends of the esophageal body lag behind, developing in tandem despite very different underlying control mechanisms. These findings suggest a teleologic role for the second segment, possibly in enhancing clearance and preventing GERD and its complications. The fact that only half of swallows show completely intact segmental architecture at term, however,

indicates that development of esophageal peristalsis continues into infancy.

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Received December 5, 2006. Accepted February 15, 2007.

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The authors have no conflict of interest to disclose.

CHAPTER 2

EPIDEMIOLOGY AND PATHOPHYSIOLOGY OF THE MAIN FUNCTIONAL GASTROINTESTINAL DISORDERS IN CHILDREN

- Campanozzi A, Boccia G, Pensabene L, Panetta F, Marseglia A, Strisciuglio P, Barbera C, Magazzu' G, Pettoello-Mantovani M, and Staiano A. **Prevalence And Natural History Of Gastroesophageal Reflux: Pediatric Prospective Survey.** Pediatrics (submitted in October 2007)

- Boccia G, Manguso F, Sarno MA, Masi P, Pensabene L, Staiano A **Functional defecation disorders in children: Rome II criteria vs Rome III and PACCT criteria.** J Pediatr 2007;151:394-8

- Boccia G, Buonavolontà R, Coccorullo P, Manguso F, Masi P., L Fuiano, Staiano A. **Dyspeptic symptoms in children: the result of a constipation- induced “cologastric brake”?** Clinical Gastroenterol Hepatol (submitted with revisions, October 2007)

**PREVALENCE AND NATURAL HISTORY OF GASTROESOPHAGEAL REFLUX:
PEDIATRIC PROSPECTIVE SURVEY**

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ACKNOWLEDGMENTS :

The authors would like to thank the following pediatricians, who made this study possible:

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ABSTRACT

The prevalence and natural history of gastro-esophageal reflux (GER) in infants have been poorly documented. **Aim:** To evaluate the prevalence and natural history of infant regurgitation in Italian children during the first two years of life. **Methods:** A detailed questionnaire prepared on the basis of the Rome II criteria was completed by fifty-nine primary care pediatricians to assess infant regurgitation on consecutive patients seen in their office over a 3-month period. A total of 2642 patients aged zero to 12 months were prospectively enrolled during this 3-month period. Follow-up was performed at 6, 12, 18 and 24 months of age. **Results:** A total of 313 children (12 %; 147 girls) received the diagnosis of infant regurgitation. Vomiting was also present in 34/313 patients (10.9%). Their score for the Infant Gastro-esophageal Reflux Questionnaire (I-GERQ) was 8.51 ± 4.75 (mean \pm SD). Follow-up visits were carried out to the end in 210/313 subjects. Regurgitation had disappeared in 56/210 infants (27%) by the first 6 months of age, in 128 (61 %) by the first 12 months, in 23 (11 %) at 18 months and in 3 patients (1 %) by the first 24 months. At follow-up 1/210 (0.5 %) patients had developed a GER-disease with esophagitis endoscopically and histologically proven; another patient received a diagnosis of cow milk protein intolerance. The I-GERQ score reached 0 after 8.2 ± 3.9 months in breast fed infants (89/210), and after 9.6 ± 4.1 months in artificially fed infants ($p = 0.03$).

Conclusion: We have found that 12% of Italian infants satisfied the Rome II criteria for infant regurgitation. 88% of 210/313 who had completed a 24 month follow-up period had improved at the age of 12 months. Only one patient later turned out to have GER disease. Breast milk was associated to a shorter time necessary to reach a complete normalization of the I-GERQ.

INTRODUCTION

Gastroesophageal reflux (GER) is a common reason for pediatric visits and referrals to pediatric gastroenterologists. Regurgitation occurs more than once a day in 67% of healthy 4-month-old infants. Many parents believe regurgitation is abnormal; 24% bring this symptom to their clinician's attention during their infant's sixth month (1). Although the symptoms of GER are common and often treated in a primary care setting, the natural history of GER in childhood has been poorly documented. Most studies have been cross-sectional, retrospective, or biased toward hospital-based populations. Only two previous studies have prospectively examined infants in the community to determine the outcome of infant spilling (2-4). Nelson et al. (1) reported that at least one episode of regurgitation a day was present in half of zero-to-three-month-old infants. Peak reported regurgitation was 67% at 4 months, and the prevalence of symptoms decreased dramatically from 61% to 21% between 6 and 7 months of age. By the age of 10 -12 months, 5% of study subjects were still spilling. This study was limited because in its selection process, the authors cross-sectionally chose children who were younger than 13 months, and prospectively followed only children who were 6 months or older at enrolment. Furthermore, they followed patients only once, 1 year later. Martin et al.(5) longitudinally evaluated the natural history of infant spilling during the first 2 years of life and determined the relationship between infant spilling and GER symptoms at 9 years of age. A total of 41% of infants were spilling most feed between 3 and 4 months of age and the percentage gradually declined, according to age, falling to <5% between 13 - 14 months; symptoms disappeared by 19 months of age. However, all these studies lack of a standard definition of GER.

The Rome II criteria for functional gastrointestinal disorder in children provided a standard definition for the diagnosis of infant GER for the first time (6). These criteria represent a valid instrument to assist the physician in a positive diagnosis of GER – thus allowing them to avoid

many invasive batteries of tests - and to assist the researcher in order to use definitions which are as standardized as possible.

Our objective was to perform a prospective survey in the general Italian pediatric population during the first two years of life in order to determine the prevalence and natural history of GER, as defined by the Rome II criteria.

METHODS

In Italy all children from birth to 14 years of age are enrolled in the National Health Service (NHS). NHS pediatricians have under their care approximately 800 children each, who are distributed evenly throughout the country to cover the health needs of the entire Italian pediatric population. Initially seventy-five primary care pediatricians, from north-central and southern Italy, were selected. Those pediatricians were chosen from communities of all sizes, throughout the territory, by random selection of evenly numbered members provided from the membership list of the regional pediatric society. Subsequently, regional coordinators of the study within each territory presented the aim, outline and questionnaires of the study to the selected pediatricians. The survey was conducted by 59 pediatricians who agreed to participate in the study.

From April 1 to June 30, 2004, each participating pediatrician was asked to record the number of infants examined per day in their office for acute, chronic care or routine follow-up evaluation, and to complete for each consecutive patient a detailed questionnaire to assess infant regurgitation according to the Rome II Criteria (6) (Table 1). Evidence of metabolic, gastrointestinal or central nervous system diseases, chronic debilitating diseases, neurological abnormalities, previous surgery of the gastrointestinal tract, use of acid-suppressive therapy (H₂-antagonists, proton pump inhibitors) were considered exclusion criteria. In addition, infants with hematemesis, anemia, aspiration, apnea, failure to thrive, abnormal posturing and feeding or swallowing difficulties, were excluded from the study.

Each child with a diagnosis of infant regurgitation according to the Rome II criteria was then reexamined by the same pediatrician with an interval of 2 months, until the age of 24 months, to determine whether there had been a resolution/worsening of symptoms or a change in the diagnosis (i.e. GERD, cow milk protein intolerance). After the diagnosis, additional investigation and treatment were left to the discretion of the primary care pediatrician. However, meetings were held

before, during, and at the end of the study period to standardize the protocol and the diagnostic/therapeutic management of patients, according to the current practice (7). Treatment options included reassurance, formula thickening with dry rice cereal and postural manipulations. The use of prokinetics (domperidon), alginate and/or aluminium hydroxide was allowed on demand as rescue medication.

A validated score (2) (I-GERQ GERD, modified) was completed at enrolment and during the follow-up visits. The I-GERQ GERD modified, consisted of 14 items regarding characteristics of regurgitation (frequency and volume), feeding refusal, weight gain, irritability and crying (daily frequency and correlation with meal), hiccups, arching back, respiratory symptoms and posture, and assigned points for positive response, for a maximum possible score of 31. All patients received a standard history and a physical examination; in particular the information collected were demographics, symptoms (e.g. regurgitation, vomiting, weight deficit, pain suggestive of esophagitis, respiratory symptoms), possible provocative factors (e.g. smoke exposure, prematurity, history of atopy), feeding (breast milk or formula), familiarity with gastrointestinal symptoms.

The study protocol was presented and approved by the Italian Society for Pediatric Gastroenterology, Hepatology and Nutrition (SIGENP). Informed consent for participating in the study was obtained from parents of all patients, and the experimental design was approved by the Independent Ethics Committee of University of Naples, Federico II.

Statistical analysis

Continuous data (age, weight, I-GERQ score) were compared, between initial and final values, using t-tests, unpaired or paired as appropriate. Cross-tabulations were evaluated by using the χ^2 method. Statistical significance was predetermined as $p < 0.05$. Percentages were rounded to the nearest whole numbers

RESULTS

Fifty-nine out of 75 randomly selected pediatricians agreed to collaborate, with a participation rate of 78%. A total of 2642 infants were prospectively enrolled during the 3-month recruitment period. Their age range was from 1 to 12 months, with a mean \pm SD of 5.6 ± 3.6 months. The socioeconomic status of all patients ranged from lower-middle class to upper-middle class. A total of 313 children (12 %) received the diagnosis of infant regurgitation according to the Rome II criteria. The mean age of affected infants was 3.8 ± 2.7 months, and the male:female ratio was 166:147. The mean value of the I-GERQ score at the study entry was 8.51 ± 4.75 ; it was > 7 in 151/313 (48%). Children with I-GERQ score < 7 did not show significant differences from children with a score ≥ 7 in terms of nutritional status, being their BMI z-scores 0.3 ± 1.23 and 0.21 ± 1.19 ($p = 0.2$) respectively. Vomiting was also present in 34/313 patients (10.9%), whose initial score was significantly different from the score of children without vomiting (I-GERQ score mean \pm SD: 11.6 ± 3.7 vs 7.6 ± 4.5 , respectively; $p = 0.0003$). These scores continued to be significantly different at the moment of the first follow-up visit (2 months later), being 6.8 ± 4.3 in children with vomiting and 3.8 ± 3.4 in children without vomiting ($p = 0.0003$). Such a difference was lost later on. No significant difference was found, during the first follow-up visit, between children with or without vomiting in terms of body-weight gain (mean \pm SD: gr 1174 ± 437 and 1287 ± 614 , respectively; $p = 0.3$). As illustrated in Fig. 1, most children with GER are in their first 150 days of life. Two hundred and thirty-three (74 %) out of 313 children were 1 - 5 months old and their score at inclusion in the study protocol (8.8 ± 4.7) was significantly higher compared to children older than 5 months (No: 80; age: 6 - 12 months; I-GERQ: 7.5 ± 4.8 ; $p = 0.04$). Such a difference remained persistently significant also after 4 and 6 months (Fig. 2). Considering the potential risk factors for GERD, no significant difference was found at enrolment between the I-GERQ score of children who were preterm at birth and that of children who were at term ($p = 0.08$) (Tab. 2). A significant difference ($p = 0.005$) was present between scores of children born from atopic parents and children born from non-atopic parents (Tab. 2). Such a difference was lost after a 6-months

period (I-GERQ score: 5.3 ± 4.5 and 5.0 ± 3.8 respectively; $p = 0.2$) and it had no impact on the time necessary to reach a complete normalization of the I-GERQ. No significant difference was found between scores of children born from smoking mothers and children born from non-smoking mothers ($p = 0.09$) (Tab. 2). Since 103 patients were lost at follow-up visits, a complete data collection of the follow-up evaluation was only available for 210 children out of the original 313 patients (67%). Regurgitation had disappeared in 56/210 infants (27%) by the first 6 months of age, in 128 (61%) by the first 12 months, in 23 (11%) by the first 18 months and in 3 patients (1 %) at 24 months (Fig. 3). They had been treated with reassurance in 72 % of cases (I-GERQ score: 8.1 ± 4.7), formula thickening in 6 % (I-GERQ score: 8.5 ± 4.2), antacids in 9 % (I-GERQ score: 10.8 ± 4.8), prokinetics in 3 % (I-GERQ score: 6.3 ± 2.9). At follow-up visits 1/210 (0.5 %) patients had developed GER disease with esophagitis endoscopically and histologically proven. Another patient received a diagnosis of cow milk protein intolerance.

The I-GERQ score reached 0 after 8.2 ± 3.9 months in breast fed infants (112/265), and after 9.6 ± 4.1 months in artificially fed ones ($p = 0.03$).

DISCUSSION

Regurgitation in infants is among the most common causes for physician consultation worldwide, but the heterogeneity of its diagnostic criteria and the lack of a reliable and valid non-invasive instrument to evaluate progression and remission of GER over time has restricted any possibility to evaluate its exact prevalence and natural history. In other words, “infant regurgitation” has not been a standardized diagnostic definition before the Rome criteria for functional disorders in children had been developed and published. That is why epidemiologic studies regarding regurgitation in infancy might not have been appropriate in the past - in fact data from literature reported a wide variety of percentages (8), sometimes accounting for serious manifestations of complicated GER, sometimes limiting the observations to children with one regurgitation episode per day (1). In the present study, for the first time, the Rome II criteria (7) have been used to evaluate the prevalence of regurgitation in children who had healthy baby check-up during their first year of life. Moreover, since evaluating an infant with regurgitation is often misleading without an objective instrument able to differentiate clinically meaningful change from clinically insignificant change, the I-GERQ score was chosen as an objective evaluative tool to obtain prospective longitudinal information regarding the natural history of infant regurgitation during the first two years of life. Infant regurgitation was diagnosed in 12% of the 2642 infants recruited for the study protocol, which is a prevalence lower than previously observed in other studies. Nelson et al reported that at least one episode of regurgitation a day was found in a half of 0- to 3-month-olds and peak reported regurgitation was 67% at 4 months (1). In a recent prospective longitudinal study of the natural history of spilling in children followed from birth, it was shown that spilling reaches a peak of 41% among infants between 3 and 4 months old (5). Since we doubt we have overlooked many infants with regurgitation in our study – due to the fact that Italian parents are encouraged by the NHS to see pediatricians for the smallest problem – a possible explanation accounting for our lower prevalence could be that we have made use of a stricter definition of infant regurgitation

based on both frequency (2 or more episodes per day) and duration (3 or more weeks), according to the Rome II criteria. None of our infants had any GER-related complications, such as aspiration, apnea, abnormal posturing, and there was no significant difference in BMI z-scores between children with a score higher or lower than 7, which is the value usually indicated as the threshold limit over which the presence of GERD is a possible risk. Considering that a value higher than 7 was observed in almost a half of our infants with regurgitation, these data might also suggest that an I-GERQ score higher than 7 is consistent with functional GER, with no reduction in growth velocity and any other associated alarm symptoms for GERD. In addition to regurgitation, vomiting was also observed in 10.9% of cases and did not seem to have any effects on the patient's nutrition, thus suggesting that – again – functional GER might be accompanied by symptoms currently considered indicative of GER-related complications. Children with or without vomiting showed significantly different I-GERQ score values, which were obviously higher in the former group; this difference was still present at their first follow-up visit (2 months later) and was lost later on. The data show that infant regurgitation is mainly prevalent in the first 5 months of life and the younger the infant is, the higher the I-GERQ score becomes. Infants who used to regurgitate between 6 and 12 months of age were no longer doing so a year later. On the basis of these data, and according to Nelson et al.(1), pediatricians can predict that when regurgitation lasts for over 6 months of age it usually resolves over the following year. Considering the possible risk factors for GERD, we did not find any differences, in terms of I-GERQ score, between preterm- and term-infants at enrolment in the study protocol, but these data are limited by the exiguous number of preterm-infants (only 27) in our patient population. Infants born from atopic parents showed significantly higher I-GERQ score values, which did not seem to have an impact on the time necessary to reach a complete normalization of the scores. This overt discrepancy might be related to the consciousness of their parents to be atopic, which indicates a higher sensitivity towards any atopy-related symptom. In other words, atopic parents could view infant regurgitation as a problem more often than non-atopic parents. ETS (= exposure to environmental tobacco smoke) has been

reported to induce lower esophageal sphincter relaxation (9, 10) and it has been shown a strong correlation between esophageal pH and ETS exposure in infants exposed to apparent life-threatening events (11). Passive smoking has also been proposed as a risk factor for esophagitis in children (12). However, our study, in accordance with Martin et al.(5), does not indicate a clear relationship between maternal smoking and ETS in general, or infant regurgitation, neither in terms of prevalence, nor in terms of duration of the symptoms. Furthermore, we compared the frequency of regurgitation in infants receiving breast milk - seen as a possible protective factor from GERD - and in those receiving infant formula and, despite data from literature (5, 2, 13), we observed no significant difference in the prevalence of regurgitation, but a significant difference in terms of the time needed to reach a complete normalization of I-GERQ. These data indicate that breastfed infants stop regurgitating earlier than formula-fed ones. Although a complete data collection regarding the 24-month longitudinal follow-up was only available in 210 out of the original 313 patients, we observed that the prevalence of infant regurgitation decreased markedly at 12 months of age, by which time the symptoms had disappeared in 88% of cases, and no children displayed regurgitation at 24 months. Treatment was left to the discretion of the primary care pediatrician and consisted in reassuring about the physiologic nature of the symptoms and educating parents (frequent small feeds, postprandial burping, correct positioning), which were sufficient measures in 72% of cases. The remaining infants were treated with formula thickening in 6% of cases, antacids in 9% and prokinetics in 3%. It is noteworthy that infants receiving antacid medication have been observed to show the highest I-GERQ score at inclusion. Only one infant was finally diagnosed as having cow milk protein intolerance – thus suggesting this is a rare condition - even though it is considered prudent to recommend a trial of hypoallergenic formula in the medical treatment of an infant with reflux (7). Only one patient received the diagnosis of GERD, with esophagitis endoscopically and histologically proven.

To conclude, infant regurgitation according to the Rome II diagnostic criteria is a common disorder, but less frequent than one might think in the past. In most cases its natural history is resolution within the first 18 months of life, and its treatment should be decided on the basis of the facts exposed in this study. Children older than 18 months who continue to regurgitate regularly should prudently receive additional evaluation. Cow milk protein intolerance and esophagitis should be considered infrequent conditions among children with infant regurgitation. Since there have been no changes from the Rome II to the Rome III criteria for the diagnosis of infant regurgitation (14), these data should be considered also in the light of the more recent diagnostic criteria for functional disorders.

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FIGURE LEGENDS

Fig. 1: I-GERQ score vs Age. Children with GER are mostly gathered in the first 150 days of age.

Fig. 2: Age at recruitment vs I-GERQ score. I-GERQ score at inclusion of children 1 - 5 months old was significantly higher compared to children older than 5 months. Such a difference remained persistently significant also after 4 and 6 months.

Fig. 3: 24 months follow up in 210 children with regurgitation. Regurgitation disappeared in 56/210 infants (27%) by the first 6 months of age, in 128 (61%) by the first 12 months, in 23 (11%) by the first 18 months and in 3 patients (1 %) at 24 months.

Table 1. Diagnostic criteria of Infant Regurgitation according to the Rome II classification (Gut 1999;45(SII):II60-II68).

- Regurgitation 2 or more times per day for 3 or more weeks;
- There is no retching, hematemesis, aspiration, apnea, failure to thrive, or abnormal posturing;
- The infant must be 1-12 months of age and otherwise healthy;
- There is no evidence of metabolic, gastrointestinal, or central nervous system disease to explain the symptom.

Tab. 2 – Risk factors for GERD

Risk Factors	Number of children	I-GERQ score	p
Preterm children	27	9.51 ± 4.8	0.08
At term children	286	8.7 ± 7.9	
Atopic parents	77	9.8 ± 5.6	0.005
Non atopic parents	227	8.0 ± 4.4	
Smoking mothers	38	9.0 ± 4.9	0.09
Non-smoking mothers	275	8.5 ± 4.7	

Data are expressed in mean ± SD

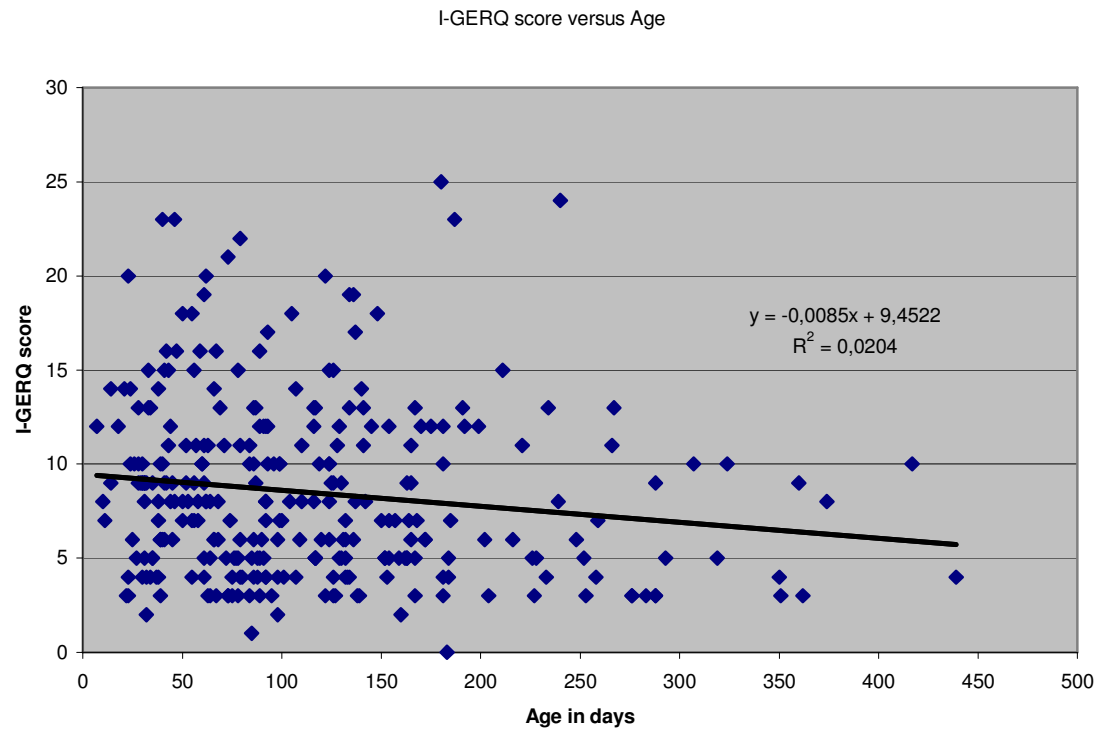


Fig.1

Age at recruitment vs I-GERQ score

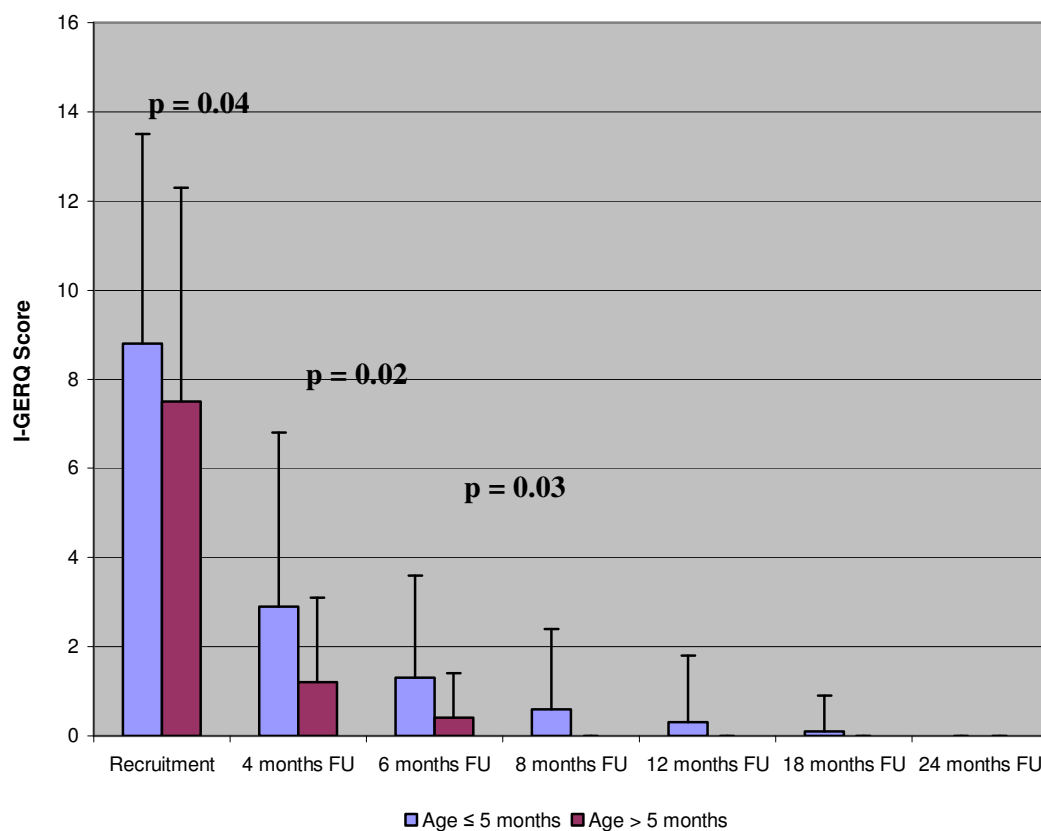


Fig.2

24 months follow up in 210 children with regurgitation

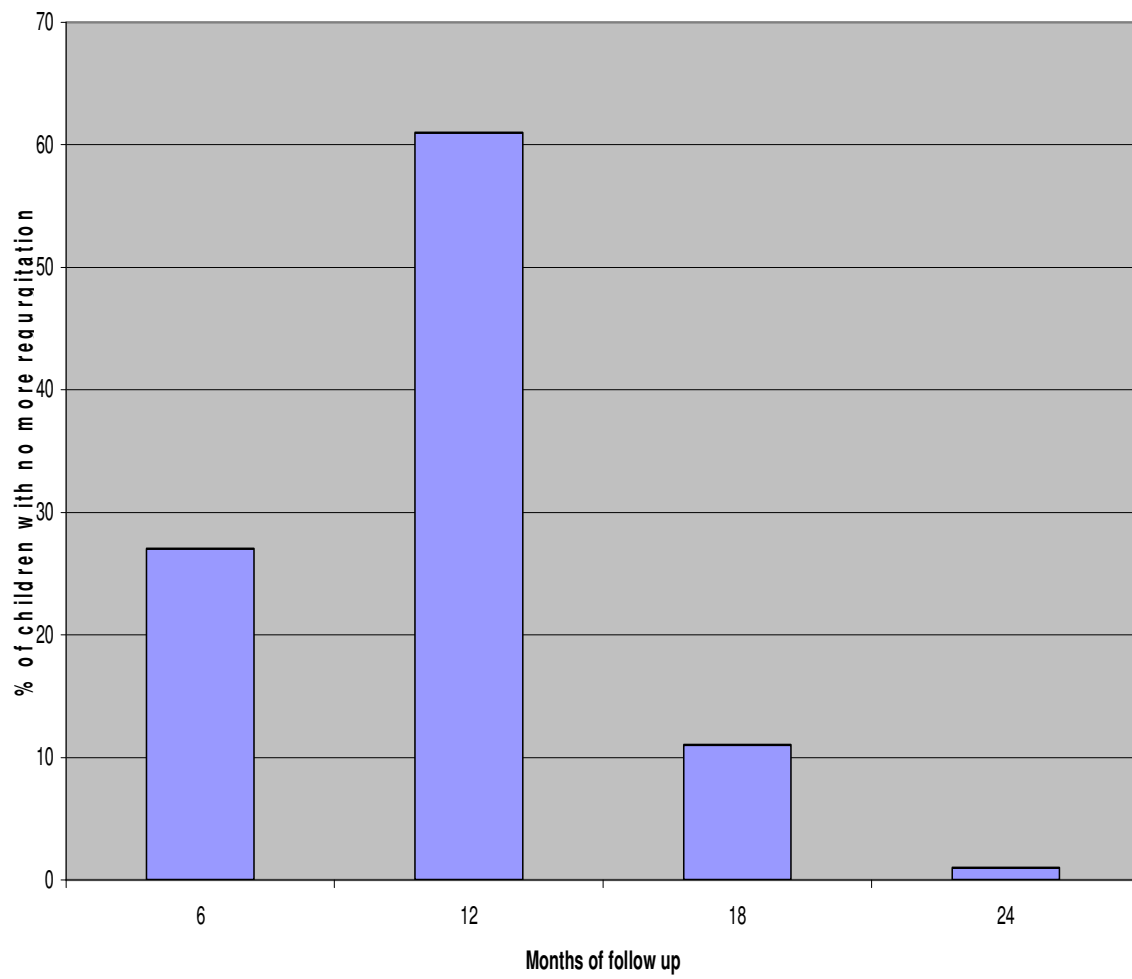


Fig. 3

Functional Defecation Disorders in Children: PACCT Criteria Versus Rome II Criteria

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Objectives To evaluate the clinical validity and applicability of the Paris Consensus on Childhood Constipation Terminology (PACCT) versus the Rome II criteria for pediatric functional defecation disorders (FDDs).

Study design Children from infancy to 17 years who had been referred to a tertiary center for chronic constipation were recruited for the study. A prospective longitudinal design was used. The Questionnaire on Pediatric Gastrointestinal Symptoms (QPGS) for parents of children age 0 to 4 and 4 to 17 years and for children age 10 to 17 years was used for diagnosis of FDDs.

Results Children ($n = 128$; mean age, 67.2 months; 62 males) were screened consecutively. FDDs were diagnosed significantly more often by PACCT than by the Rome II criteria (112 [88.9%] vs 60 [47.6%]; $P = .001$). The agreement Cohen's kappa test showed $\kappa = .173$. A statistically significant difference was reported between Rome II and PACCT in the 4- to 17-year-old group ($P = .001$). Scybalous, pebble-like stools and defecation with straining were the main symptoms reported (80%), followed by painful defecation (66%).

Conclusions The PACCT criteria show greater applicability than the Rome II criteria for FDDs. The poor agreement implies that they do not identify the same types of patients. Because such a high percentage of constipated children reported the symptoms of defecation with straining, scybalous pebble-like stools, and painful defecation, including these symptoms in any revised criteria should be taken into consideration. (*J Pediatr* 2007;151:394-8)

One of the most common functional gastrointestinal disorders (FGIDs) in childhood is chronic constipation.¹⁻³ According to the Rome II criteria, functional defecation disorders (FDDs) are divided into functional constipation (FC), functional fecal retention (FFR), and functional nonretentive fecal soiling (FNRFS).⁴ However, recent studies have suggested that the Rome II criteria are too restrictive and have pointed out the lack of validated questionnaires for the diagnosis of FGIDs in children.⁵⁻¹¹

Loening-Baucke⁷ reported that the Rome II criteria for FFR identify few children with encopresis who have symptoms of FFR, suggesting that the following features be taken into consideration when making the diagnosis: a history of bowel movements that obstruct the toilet, chronic abdominal pain relieved by enema or laxatives, and abdominal or rectal fecal mass. Another study showed that 16% of children fulfilling the classic Iowa criteria for constipation were not recognized by the Rome II criteria.⁸ These studies clearly demonstrate that the development of new and more complete diagnostic criteria for pediatric FGIDs, particularly FDDs, is necessary.

The Paris Consensus on Childhood Constipation Terminology (PACCT) better defines the terms used in childhood constipation, and the same working definitions were also adopted for the revised pediatric Rome III criteria (Table I; available at www.jpeds.com).¹²⁻¹⁴ In addition, the pediatric Rome II criteria were recently validated for FGIDs using appropriate questionnaires on pediatric gastrointestinal symptoms.^{15,16} These questionnaires consisted of 3 different forms, allowing them to be applied to children of all ages and allowing separate reporting by parents and older children.

The aims of the present study were to evaluate the prevalence of FDDs defined according to PACCT and Rome II criteria and to compare their clinical validity and applicability.

CC	Chronic constipation	NRF	Nonretentive functional incontinence
FC	Functional constipation	PACCT	Paris Consensus on Childhood Constipation Terminology
FDD	Functional defecation disorder	QPGS	Questionnaire on Pediatric Gastrointestinal Symptoms
FFR	Functional fecal retention		
FGID	Functional gastrointestinal disorder		
FNRFS	Functional nonretentive fecal soiling		

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Submitted for publication Sep 6, 2006; last revision received Mar 7, 2007; accepted Apr 9, 2007.

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0022-3476/\$ - see front matter

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10.1016/j.jpeds.2007.04.011

METHODS

Subjects

Study subjects were consecutively recruited among otherwise healthy children between infancy and under age 18 years with complaints of chronic constipation of at least 2 months duration. They were referred to the tertiary academic gastroenterology ambulatory center of Federico II University of Naples. Recruitment occurred between November 2005 and May 2006. Children with organic causes of defecation disorders (including Hirschsprung's disease, spina bifida occulta, hypothyroidism, chronic intestinal pseudo-obstruction, previous surgery of the gastrointestinal tract, mental retardation, and chronic severe illness) were excluded from the study.

To allow an objective study of symptoms, laxative treatments were discontinued for at least 2 weeks before entrance into the study.

Measures and Procedures

The Questionnaire on Pediatric Gastrointestinal Symptoms (QPGS) is a validated and scored research instrument designed to classify gastrointestinal symptoms associated with FGIDs according to the Rome II criteria.^{15,16} Because diagnostic criteria are distinct for children and adolescents (from 4 to under 18 years) and for infants and toddlers (from 0 to under 4 years), each questionnaire is presented in 3 forms: Form A, for parents of children and adolescents age 4 to 17 years; Form B, for parents of infants and toddlers up to age 4 years; and Form C, for children and adolescents age 10 to 17 years. All 3 forms include sociodemographic and medical/developmental information. Our Rome II-QPGS comprised 19 items for Form A, 13 items for Form B, and 17 items for Form C. For the PACCT criteria, we used the modified-QPGS (QPGS-PACCT) comprising 48 items for Form A, 17 items for Form B, and 47 items for Form C.

Parents of all children and children and adolescents age 10 to 17 years completed the QPGS and QPGS-PACCT in the waiting room. A research assistant introduced the questionnaires and was present to assist if needed. Completion of both questionnaires took about 20 minutes.

Questions were posed concerning the main characteristics of children's bowel habits: frequency of bowel movements, consistency of stools, onset of constipation symptoms, family history of constipation, hard or painful bowel movements, urgency to have a bowel movement, straining during bowel movements, feeling of an unfinished bowel movement, mucus in stool, history of large-diameter stools that may block up the toilet, withholding of bowel movements, fear of pain during bowel movements, squeezing the legs or buttocks together (retentive posturing), fecal incontinence (staining or soiling) during the day and/or night, a large fecal mass in the rectum, and the presence of associated symptoms. For the PACCT questionnaire, a section concerning abdominal pain was proposed to exclude a diagnosis of irritable bowel syndrome, according to the new criteria.

All patients underwent a standard history and physical examination, including abdominal and digital rectal examination to detect any large fecal mass in the abdomen or rectum.¹⁷ The rectal examination could not be done in 11 patients (8.7%) due to high levels of fear and anxiety. All data were analyzed to establish how many patients met the Rome II, the PACCT, and both the Rome II and PACCT criteria.

Informed consent to participate in the study was obtained from the parents of all patients. The experimental design was approved by our institutional review board.

Statistical Analysis

Continuous data are expressed as mean \pm standard deviation (SD). For categorical variables, either the χ^2 test with or without exact correction or Fisher's exact test was applied, as appropriate. With regard to constipation, to test the agreement of the PACCT and Rome II criteria, Cohen's κ measure was applied. A 2-sided P value $< .05$ was considered to indicate statistical significance. Statistical tests were performed using SPSS software, version 14.0.2 (SPSS Inc, Chicago, IL).

RESULTS

In a 6-month period, 128 children (62 males, 66 females; mean age, 67.2 ± 45.6 months; age range, 6 to 210 months) were consecutively screened. Two of these patients were excluded because their parents were not able to answer the QPGS questionnaires. Among the 126 children included in the study, 49 (38.9%) were under age 4 years and 77 (61.1%) were age 4 to 17 years; in particular, 16 children (12.7%) were age 10 to 17 years. QPGS Forms A and B were completed by 61 parents of children age 4 to 17 years and 49 parents of children under age 4 years, respectively; all 16 patients age 10 to 17 years were able to complete Form C by themselves.

Functional defecation disorders were diagnosed significantly more often by PACCT criteria (112 [88.9%]) than by Rome II criteria (60 [47.6%]) and the agreement Cohen's κ test showed $\kappa = 0.173$. Fifty-nine patients (46.8%) met both criteria, 53 (42.1%) children who fulfilled the PACCT criteria were not recognized by the Rome II criteria. Only 1 constipated patient according to Rome II, did not fit the PACCT criteria because he responded affirmatively to only 1 of 6 items. Thirteen patients (10.3%) did not fulfill any criteria and so could not be considered affected by FDDs. These patients were all age 4 to 17 years.

In the under-4 group, 31 patients (63.3%) were positive under Rome II criteria and 48 (98%) were positive under PACCT criteria. In the 4 to 17 group, 29 (37.7%) were positive under Rome II and 64 (83.1%) were positive under PACCT. The distribution of FDDs in the 126 patients according to the Rome II and PACCT criteria, stratified for the 2 age classes, is shown in Table II. A statistically significant difference was reported between the Rome II and PACCT criteria for FDDs in the 4 to 17 group ($P = .001$) but not in the under-4 group.

Table II. Distribution of the 126 patients according to the Rome II and PACCT criteria, stratified for the 2 age classes

Age group	Rome II-/PACCT-	Rome II-/PACCT+	Rome II+/PACCT-	Rome II+/PACCT+	P
Under 4 years (n = 49)	0 (0)	18 (36.7)	1 (2.0)	30 (61.2)	1.000
4 to 17 years (n = 77)	13 (16.9)	35 (45.5)	0 (0)	29 (37.7)	.001
Total (n = 126)	13 (10.3)	53 (42.1)	1 (0.8)	59 (46.8)	.001

Data are number (%).
+, positive; -, negative.

Table III. Distribution of Rome II FFR, FC, and NRFS and of PACCT CC and NRFI for all study patients according to age group

	All patients	4 to 17 years	<4 years	P
Rome II (n = 60)				
FC	21 (35)	6 (10)	15 (25)	.027
FFR	37 (61.7)	21 (35)	16 (26.7)	
NRFS	2 (3.3)	2 (3.3)	—	
PACCT (n = 112)				
CC	102 (91.1)	54 (48.2)	48 (42.9)	.005
NRFI	10 (8.9)	10 (8.9)	—	

Data are n (%).

The prevalence in all patients of Rome II FDDs (FFR, FC, and NRFS), together with PACCT chronic constipation (CC) and nonretentive fecal incontinence (NRFI), is shown in Table III. Among the 60 children positive for Rome II constipation, FFR was the most frequently observed disorder, followed by FC and NRFS. According to the PACCT criteria, the majority of the 112 positive children were affected by CC. FC was diagnosed significantly more often in the under-4 group, although CC was equally distributed between the 2 age groups. FFR was observed more frequently in the age 4 to 17 group of constipated children.

Clinical characteristics of all patients and of the "PACCT criteria positive only" group according to the different age ranges are given in Tables IV and V. A history of defecation with straining was the most prevalent symptom (83.3%), followed by scybalous, pebble-like stools (80.2%). These 2 symptoms, together with a history of large-diameter stools, were the most frequent in the "PACCT criteria positive only" group. In this group, fecal incontinence was the main reported symptom by children age 4 to 17. In a minority of cases, parents of children under age 4 responded "I don't know" in regard to some clinical aspects, such as retentive posturing, painful defecation, large fecal mass, and defecation with straining (Tables IV and V).

DISCUSSION

One of the key problems in managing childhood constipation is the lack of a generally accepted definition. After the Rome II FGID classification,⁴ the PACCT Group reached a consensus on terminology to describe childhood defecation disorders.¹² The 2 most important results of this

consensus were (1) the unification of the previous classification of Rome II FC and FFR under the umbrella of just 1 category—CC—and (2) the recommendation that the terms "encopresis" and "soiling" be replaced by the term "incontinence." These definitions were accepted and published as the pediatric Rome III criteria for FDDs.^{13,14} One of the main changes in the Rome III criteria was that now a child must meet 2 of 6 criteria to be diagnosed with FC (without differentiation in FC and FFR). The 6 constipation characteristics are the same as those obtained by the consensus on childhood constipation terminology reached by the PACCT group (Table I). On this basis, because our aim was to compare the applicability of PACCT criteria versus the Rome II criteria, we assume that all of our results can be applied to the Rome III criteria as well.

The prevalence of FDDs in our study population was significantly higher when using the PACCT criteria than when using the Rome II criteria (88.9% vs 47.6%; $P = .001$); 53 of 126 (42.1%) constipated children were not recognized by the Rome II criteria. The prevalence of Rome II constipation in patients recruited from tertiary care settings varied from 34.2% to 69%.^{7-8,16} Voskuil et al⁸ reported that 58% of patients were positive for both classic and Rome II criteria, whereas 16% were not recognized by the Rome II criteria and 11% were not recognized by the classic criteria.

Among signs and symptoms of constipation in our study population, defecation with straining and scybalous, pebble-like stools were reported in about 80% of the patients, a history of painful defecation was reported in 66%, and large-diameter stools were found in 63%. These symptoms, along with the presence of retentive posturing, were reported primarily in the "PACCT criteria positive only" group, and the prevalence of fecal incontinence was greatest in the children age 4 to 17 years (Table IV).

These data support the importance of the revised Rome III diagnostic criteria for FC, in which 2 of 6 criteria must be satisfied. In the previous diagnostic criteria, the presence of retentive posturing together with fewer than 2 bowel movements per week were needed for a diagnosis.⁴ It should be noted that the Rome III criteria define retentive posturing as merely a feature of FC. Based on our results, we can argue that a numerous constipated children were considered "false negatives" by the Rome II criteria, confirming the wider validity and clinical applicability of the PACCT and Rome III criteria.

Such symptoms as defecation with straining, painful and/or hard bowel movements, large-diameter stools, and

Table IV. Clinical characteristics of all study patients and according to age

Patient characteristics	All patients	4 to 17 years	<4 years	P
Number of patients	126	77	49	—
Mean age (months) \pm SD	67 \pm 46	95 \pm 38	25 \pm 12	—
Males, n (%)	62 (49)	42 (55)	20 (41)	—
Positive family history, n (%)	50 (39)	30 (39)	20 (40.8)	.836
Symptoms, n (%)				
Defecation frequency <3/week	76 (60.3)	45 (58.4)	31 (63.3)	.590
Scybalous, pebble-like, hard stools	101 (80.2)	55 (71.4)	46 (93.9)	.002
Painful defecation				<.0001
No	37 (29.4)	32 (41.6)	5 (10.2)	
Yes	83 (65.9)	45 (58.4)	38 (77.6)	
I don't know	6 (4.8)	—	6 (12.2)	
Defecation with straining				.004
No	18 (14.3)	16 (20.8)	2 (4.1)	
Yes	105 (83.3)	61 (79.2)	44 (89.8)	
I don't know	3 (2.4)	—	3 (6.1)	
History of large-diameter stools	79 (62.7)	50 (64.9)	29 (59.2)	.515
Retentive posturing				<.0001
No	45 (35.7)	34 (44.2)	11 (22.4)	
Yes	74 (58.7)	43 (55.8)	31 (63.3)	
I don't know	7 (5.6)	—	7 (14.3)	
Large fecal mass (by rectal examination*)				<.0001
No	38 (33.0)	27 (38.6)	11 (24.4)	
Yes	67 (58.3)	43 (61.4)	24 (53.3)	
I don't know	10 (8.7)	—	10 (22.2)	
Fecal incontinence	50 (39.7)	50 (64.9)	—	—

SD, standard deviation.

*Not performed in 11 patients.

Table V. Clinical characteristics of the “PACCT criteria positive only” group in all patients and according to age

Patient characteristics	All patients	4 to 17 years	<4 years	P
Number of patients	53	35	18	—
Mean age (months) \pm SD	83 \pm 52	111 \pm 40	27 \pm 10	—
Males, n (%)	24 (45.3)	17 (48.6)	7 (38.9)	—
Positive family history, n (%)	22 (41.5)	17 (48.6)	5 (27.8)	.146
Symptoms, n (%)				
Defecation frequency <3/week	18 (34)	16 (45.7)	2 (11.1)	.012
Scybalous, pebble-like, hard stools	36 (67.9)	20 (57.1)	16 (88.9)	.019
Painful defecation				.002
No	20 (37.7)	18 (51.4)	2 (11.1)	
Yes	30 (56.6)	17 (48.6)	13 (72.2)	
I don't know	3 (5.7)	—	3 (16.7)	
Defecation with straining				.025
No	16 (30.2)	14 (40)	2 (11.1)	
Yes	36 (67.9)	21 (60)	15 (83.3)	
I don't know	1 (1.9)	—	1 (5.6)	
History of large-diameter stools	33 (62.3)	22 (62.9)	11 (61.1)	.901
Retentive posturing				.007
No	19 (35.8)	16 (45.7)	3 (16.7)	
Yes	31 (58.5)	19 (54.3)	12 (66.7)	
I don't know	3 (5.7)	—	3 (16.7)	
Large fecal mass (by rectal examination)	22 (41.5)	13 (37.1)	9 (50)	.368
Fecal incontinence	24 (45.3)	24 (68.6)	—	—

SD, standard deviation.

fecal incontinence could be considered different clinical aspects of fecal retention. Often, for rectal accommodation, children who have been constipated for years do not need to withhold to delay stool passage; furthermore, adolescents may find it difficult to explain the concept of excessive withholding,¹⁶ and parents may misinterpret withholding as an attempt to defecate and may not understand that incontinence can be a consequence of retentive behavior.^{7,8} In some cases, the presence of large fecal mass causing a painful evacuation may induce retentive behavior.²

We suggest that the following items be added to the PACCT and Rome III criteria for FC: painful defecation, defecation with straining, and scybalous, pebble-like stools. Painful defecation was included in the Rome III criteria, but it was combined with "a history of hard bowel movements," thus forming a single item. Scybalous, pebble-like stools was a Rome II criterion for FC but is excluded from the Rome III revised diagnostic criteria. These constipation characteristics were included in the questionnaires used in this study as independent items. In our patients, scybalous, pebble-like stools and defecation with straining were the most commonly reported symptoms (80%), followed by painful defecation (66%). Because such a high percentage of constipated children reported these symptoms, we believe their inclusion should be taken into consideration in any revised criteria.

According to previous data, the Rome II FC was the most common disorder of defecation.⁸ We found that among the 60 children who were positive for FDDs according to the Rome II criteria, FFR was the most common disorder (61.7%). This finding may result from the careful use of appropriate scored questionnaires for parents and for children age 10 to 17 years.

Our results demonstrate that FDDs can be diagnosed more often by the PACCT criteria; however, a statistically significant difference was found between the Rome II and PACCT criteria in the children age 4 to 17 but not in those under age 4. Up to 60% of our patients could not meet the Rome II criteria for FC only because they were older than preschool age, even if they reported a defecation frequency of less than 3 times per week and scybalous, pebble-like stools. Of the children fulfilling the criteria for FC, 24.6% could not be diagnosed as constipated according to the Rome II criteria solely because they were over 6 years old.⁸ The PACCT criteria's unification of FC and FFR under the category of FC and its exclusion of age limits may have increased the likelihood of diagnosis in older children while possibly decreasing the power of discrimination in infants and toddlers.

Our finding of poor agreement between the PACCT and Rome II criteria implies that the 2 criteria do not identify the same types of patients. On the basis of the PACCT consensus, the Rome III criteria were revised to make diag-

nostic criteria more applicable to clinical practice. A wider spectrum of clinical features to explain constipation in all of its different aspects were introduced. Ideally, diagnostic criteria must meet the needs of both practitioners, who may prefer sufficiently broad criteria to include atypical cases, and researchers, who may prefer sufficiently strict criteria to allow them to properly select their study sample. Are the Rome III criteria too broad, including too many false positives? Our findings are based on patients who present with a history of chronic constipation and cannot be generalized to an unselected population. Future research should address the applicability and validity of Rome III diagnostic criteria for FC and other FGIDs in unselected populations and in both general practice and research settings.

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Table I. Diagnostic criteria according to the Rome II, PACCT, and Rome III criteria

Rome II criteria

Functional constipation: In infants and preschool children (from 1 month to 6 years), at least 2 weeks of

- Scybalous, pebble-like, hard stools in a majority of stools, or
- Firm stools 2 or fewer times/week, and
- No evidence of structural, endocrine, or metabolic disease

Functional fecal retention: From infancy to 16 years old, a history of at least 12 weeks of

- Passage of large-diameter stools at intervals <2 times/week, and
- Retentive posturing, avoiding defecation by purposefully contracting the pelvic floor. As pelvic floor muscles fatigue, the child uses the gluteal muscles, squeezing the buttocks together.

Functional nonretentive fecal soiling: Once a week or more for the preceding 12 weeks, in a child over age 4 years, a history of defecation

- In places and at times inappropriate to the social context,
- In the absence of structural or inflammatory disease, and
- In the absence of signs of fecal retention.

PACCT criteria

Chronic constipation: Occurrence of 2 or more of the following characteristics during the preceding 8 weeks:

- Fewer than 3 bowel movements per week
- More than 1 episode of fecal incontinence/week
- Large stools in the rectum or palpable on abdominal examination
- Passage of large-diameter stools that may obstruct the toilet
- Display of retentive posturing and withholding behaviors
- Painful defecation

Fecal incontinence: Passage of stools in an inappropriate place

- Organic fecal incontinence: fecal incontinence resulting from organic disease
- Functional fecal incontinence: nonorganic disease that can be subdivided into:
 - Constipation-associated fecal incontinence: functional fecal incontinence associated with the presence of constipation
 - Nonretentive (non-constipation-associated) fecal incontinence: passage of stools in an inappropriate place, occurring in children with a mental age of 4 years and older, with no evidence of constipation based on history and/or examination

Rome III Criteria

Functional constipation: Must include 2 or more of the following in a child with a developmental age of at least 4 years with insufficient criteria for diagnosis of irritable bowel syndrome:

- Two or fewer defecations in the toilet per week
- At least 1 episode of fecal incontinence per week
- History of retentive posturing or excessive volitional stool retention
- History of painful or hard bowel movements
- Presence of a large fecal mass in the rectum
- History of large-diameter stools that may obstruct the toilet

Criteria must be fulfilled at least once per week for at least 2 months before diagnosis.

Nonretentive fecal incontinence: Must include all of the following in a child with a developmental age of at least 4 years:

- Defecation into places inappropriate to the social context at least once per month
 - No evidence of an inflammatory, anatomic, metabolic or neoplastic process that explains the subject's symptoms
 - No evidence of fecal retention
-

Dyspeptic symptoms in children: the result of a constipation- induced “cologastric brake”?

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Abbreviations: BMs=bowel movements; FC= functional constipation; FD= functional dyspepsia;
FFR=functional fecal retention; FGIDs=functional gastrointestinal disorders; GI =gastro-intestinal;
IQR=interquartile range; QPGS= Questionnaire on Pediatric Gastrointestinal Symptoms; TGEt=
total gastric emptying time; TSS=total symptom score.

Conflict of interest: There is no conflict of interest

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ABSTRACT

Background & Aims: Patients with constipation frequently complain of dyspeptic symptoms that may be explained by reflex inhibition of upper gastrointestinal motor activity by colonic stimuli. We sought to evaluate: 1) the prevalence of functional constipation (FC) and gastric emptying characteristics in children with functional dyspepsia (FD), and 2) the efficacy of osmotic laxatives on constipation, dyspeptic symptoms and gastric motility. **Methods:** We recruited 42 children (M/F 22/20; mean age 80.5 months) affected by FD (Rome II criteria). All subjects underwent ultrasonographic measurement of the total gastric emptying time (TGEt) at baseline (T0) and after three months (T3). Children's bowel habits and the dyspeptic symptomatic score were evaluated at entry and after one (T1), two (T2) and three months (T3). Constipated patients were treated with osmotic laxatives for three months. Dyspeptic children without constipation represented the comparison group. **Results:** FC was present in 28/42 (66.6%) patients. Constipated dyspeptic children had significantly more prolonged TGEt than subjects without constipation (median value (IQR) 180 (50) vs 150 (28) minutes, respectively; $p=0.004$). Patients on osmotic laxatives had a significant decrease in TGEt at three months ($p<0.001$). The median dyspeptic symptomatic score as well as the number and consistency of evacuations per week significantly improved at T1 in comparison to T0 and even more at T2 and T3 ($p<0.001$, for each). **Conclusion:** In our study group, the majority of children with FD were affected by FC associated with delayed gastric emptying. Normalization of bowel habit may improve gastric emptying as well as dyspeptic symptoms.

INTRODUCTION

Dyspeptic symptoms including fullness, bloating, early satiety, heartburn, nausea, intermittent vomiting, and upper abdominal pain may be commonly seen in patients with constipation (1,2). Borowitz et al (3) reported that 34 children rapidly and completely resolved chronic upper gastrointestinal (GI) symptoms when their constipation was treated.

Some of these upper GI symptoms related to delayed gastric emptying may be explained by reflex inhibition of upper GI motor activity by colonic stimuli (4-8). Studies in experimental animals showed that balloon distension of the colon caused rapid inhibition of gastric and intestinal contractions and tone. When the balloon was deflated, there was an immediate rebound increase in tonus and motility which suggests a nervous component (6,7). It was also shown that in adults, the intermittent distension of the rectum, at a level below that which caused any discomfort, delayed the passage of a solid meal through both the stomach and the small intestine (8). In addition, it has been reported that voluntary suppression of defecation delayed gastric emptying in healthy subjects (1). Therefore, this “cologastric brake” may be involved in the pathogenesis of upper abdominal symptoms in constipated patients.

Although gastric emptying was studied in a variety of childhood diseases (9), it was poorly investigated in constipated children. Ultrasonographic evaluation of gastric emptying was shown to be a reliable method to assess gastric emptying disorders in children (10-13). However, widespread clinical application of ultrasonography has been limited by paucity of data from studies in disease states (14).

The aims of our study were to evaluate: 1) the prevalence of functional constipation (FC) and the gastric emptying characteristics in children affected by functional dyspepsia (FD); 2) the efficacy of osmotic laxatives not only on constipation, but also on dyspeptic symptoms as well as on gastric motility.

SUBJECTS AND METHODS

Ninety-nine patients were consecutively recruited from children with complaints of upper GI symptoms referred to our tertiary academic gastroenterology outpatient clinic. Upper GI endoscopy was performed when indicated. Recruitment took place from June 2005 to July 2006. Forty two of them (22 males; mean age 80.5 months; range 48-199) were affected by FD according to the Rome II criteria (Table 1)(15) and represented our study group. Apart from age and gender, subject demographics were not included because they were not relevant to the evaluation of the study's outcomes. The Questionnaire on Paediatric Gastrointestinal Symptoms (QPGS) was used to make the diagnosis of FD at entry and a diagnosis of FC (Table 1) -section C (bowel movements)- in the group of dyspeptic patients (15,16). This questionnaire is a validated instrument for qualitative and quantitative assessment of constipation according to the paediatric Rome II criteria. We didn't choose Rome III criteria for diagnosis of FC and FD because they had not yet been published when the study protocol started.

Children with organic causes of defecation disorders, including Hirschsprung's disease, spina bifida occulta, hypothyroidism, chronic intestinal pseudo-obstruction, previous surgery of the gastrointestinal tract, or with systemic disease, central nervous system disease and chronic severe illness, were excluded from the study. In addition, children who were taking any form of constipation treatment were excluded and all drugs directly and indirectly affecting GI motility, including acid suppressive therapy, were discontinued at least two weeks prior to the study.

A full medical history together with a complete physical examination were obtained at entry (T0). Constipated patients were treated with oral lactulose at the standard dose of 1 mg/Kg/day, once daily, for a period of three months. Dyspeptic children without constipation did not receive any treatment and were used as comparison group. No patient underwent acid suppressive therapy during the study period. The use of alginate and/or aluminium hydroxide was allowed as rescue medication, on demand.

Dyspeptic symptoms and bowel habit were evaluated with validated questionnaires at enrolment (T0); and after one (T1), two (T2) and three (T3) months of follow-up. All subjects underwent measurement of the total gastric emptying time (TGEt) performed by real time ultrasonography of the antral area at entry (T0) and after three months (T3).

Informed consent for participation in this study was obtained from parents of all patients, and the experimental design was approved by the Independent Ethics Committee of University of Naples, Federico II

ASSESSMENT OF SYMPTOMS

Information concerning dyspepsia and constipation questionnaires and diaries were obtained by the parents of the children aged 0-9 years and by the children themselves aged 10-17 years.

Dyspepsia

A standardized questionnaire was used to assess the presence of epigastric pain, heartburn, nausea, early satiety, vomiting and bloating (17). Caregivers were also asked to keep a weekly diary of symptoms. The dyspepsia questionnaire was always administered by the same investigator during the clinic visits at entry (T0), at one (T1) two (T2) and three months (T3) of follow-up. Symptoms were scored numerically for frequency and severity. Frequency was scored as follows: 0_ absent, 1 _ 1 day per week, 2 _ several times per week. Severity was scored as follows: 0 _ absent, 1 _ present but not interfering with daily activities, 2 _ present and interfering with daily activities. A score for each symptom was calculated by multiplying the severity grade by the frequency grade, with a possible range for each score of 0 to 4. The total symptom score (TSS) (range 0 to 24) was obtained by adding up the scores for each symptom and was calculated for each patient.

Constipation

The defecatory pattern was established for the last week and prospectively for the first 3 consecutive days from the scheduled visits (18). Questions were posed concerning the duration of constipation, frequency of bowel movements (BMs), presence of large diameter stools, presence of

stool withholding, of abdominal pain and/or of blood with BMs, presence of urinary incontinence, fecal incontinence (stains or soils underpants). Stool consistency was assessed as follows: hard like rock, pellets = 0, firm=1, soft= 2, loose =3 and watery = 4 (19). A weekly diary was kept from patients and parents to record the BMs characteristics during the three months of follow-up. At 1-month, 2-months and 3-months visits the interim history was assessed and the stool diaries were collected and evaluated.

Assessment of Gastric Emptying

Measurement of gastric emptying time was performed by real time ultrasonography of the gastric antrum after ingestion of a mixed solid-liquid meal (Table 2) (10). All subjects were examined using a 5-MHz linear probe applied to the epigastrium, with minimal abdominal compression. Baseline scans were performed on an empty stomach, and follow-up measurements were performed at 30 and 60 minutes, and then at 15-minute intervals until emptying was complete. The gastric emptying time was calculated by measuring the cross-section of the gastric antrum at the sagittal plane passing through the superior mesenteric vein. The antral cross-sectional area, elliptical in shape, was calculated by the following formula:

$$\text{Area} = \pi \times \text{longitudinal diameter} \times \text{antero-posterior diameter} / 4 \quad (11)$$

and the stomach was considered empty when the cross-sectional area returned to baseline and persisted unchanged for at least 30 min. The total gastric emptying time (TGEt) was calculated in relation to the start of the meal.

RESULTS

Eighty of 99 consecutively recruited children complaining of upper GI symptoms underwent upper GI endoscopy. Ten patients had no indication for endoscopy because of very mild symptoms, whereas nine caregivers did not give their consent. Forty-two children received a diagnosis of FD according to Rome II criteria. All 42 children with a diagnosis of FD underwent upper GI endoscopy. By endoscopy, thirty-five (83.3%) of them showed normal macroscopic finding; 7 (16.6%) had *H.pylori* gastritis. The following disorders were found in the remaining patients: mild-moderate peptic esophagitis in 21 (26.2%); hiatal hernia in 5 (6.2 %); *H.pylori* gastritis in 7 (8.75%) and not *H.pylori* related gastritis in 5 (6.2%).

Because none of the dyspeptic children infected by *H.pylori* showed evidence of gastric or duodenal ulcer, no eradication therapy was performed.

Functional constipation was present in 28 (66.6%) out of 42 dyspeptic patients. The TGEt at enrollment (T0) was significantly more prolonged in dyspeptic children with constipation than in subjects without constipation (median value, Interquartile range (IQR) 180 (50) vs 150 (28) minutes, respectively; $p=0.004$). Patients on lactulose had a significant decrease in the TGEt after three months (T3) of constipation treatment ($p<0.001$, compared with T0 (entry) (Figure 1). There was no significant change of the TGEt in the group of dyspeptic children without constipation during the follow-up period in comparison with baseline (Figure 1).

The median (IQR) total symptomatic score in dyspeptic constipated children at entry (T0) was 9 (10), with values of 3 (8) at T1, of 1.5 (4) at T2, and of 0 (2) at T3. The total dyspepsia symptomatic score significantly decreased from baseline (T0) at T1 ($p<0.001$ vs baseline). Moreover a significant reduction was observed for this symptomatic score between T1 and T2 ($p=0.01$) and between T2 and T3 ($p=0.001$) (Figure 2). No statistically significant difference was found in the group of dyspeptic children without constipation, regarding the total dyspepsia symptomatic score, during the three months of follow-up (T1, T2, T3) compared with T0 ($p=0.880$) (Figure 2).

The frequency of BMs per week improved significantly during the 3-month treatment period (T1, T2, T3) as compared with the initial evaluation (T0) ($p<0.001$), in the dyspeptic children with constipation; while no significant change was observed in the group of dyspeptic children without constipation (Figure 3). Dyspeptic children with constipation showed a significant reduction of the stool consistency assessed by the constipation score after three months of lactulose treatment (median value (IQR) T0 1(1); T1 2(1); T2 2(1); T3 3(1); T0 vs T3 $p<0.001$). The constipation score remained unchanged during the 3-month follow-up period in the dyspeptic patients without constipation (median value (IQR) T0 2 (1); T1 2 (1); T2 2 (1); T3 2 (1); T0 vs T3 $P=0.821$).

Among the presenting dyspeptic symptoms, epigastric pain was the most frequent (100%) followed by vomiting (82.1%) and bloating (71.4%), in the dyspeptic constipated patients. All these symptoms were improved (epigastric pain 32%; bloating 18%) or disappeared (vomiting), after three months of constipation treatment (T3).

DISCUSSION

Studies in children and adults suggest that constipation is part of a generalized GI motor disorder in which proximal GI motility can also be impaired (1,3-5,8). In our study 66.6% of patients with FD were affected by FC. All of our constipated children with FD showed a significantly prolonged TGEt compared with dyspeptic subjects without constipation.

In healthy, unconstipated adults, intermittent rectal distension significantly retarded the emptying of the meal from the stomach and delayed small intestine transit (8). The delay in gastric emptying was abolished if the subject was pretreated with ranitidine (an acid-suppressant). This suggests that the inhibition of gastric emptying by rectal distension may be mediated by an increase in meal stimulated gastric acid secretion, which may then slow emptying by interaction with duodenal receptors. Coreman et al. (20) determined the effect of continuous isobaric rectal distension on gastric emptying time and oro-cecal transit in young, healthy females and found that while gastric emptying was inhibited, small bowel transit was not. The voluntary suppression of defecation for more than three days significantly slowed gastric emptying in the same manner (1).

The mechanism by which rectal distention inhibits gastric emptying remains speculative but probably involves both neural and humoral components (6-8). Colonic loading with faecal materials may activate a recto-gastric inhibitory reflex altering the function of gastrointestinal tract regions proximal to the colon. This “cologastric brake” may be involved in the pathogenesis of dyspeptic symptoms. In our study dyspeptic patients affected by constipation reported a prolonged gastric emptying time with a more severe dyspepsia symptomatic score. In addition, the improvement of the bowel habit during the 3-month constipation treatment was correlated to a significant decrease of the dyspepsia symptomatic score as well as of the gastric emptying time.

Gastric emptying, gastric accommodation and visceral hypersensitivity abnormalities have been demonstrated in symptomatic adult patients affected by FD (21-23). However, there are conflicting data about an exact relationship between constipation and dyspepsia. Van der sijp et al.(5) reported that patients with severe idiopathic constipation have delayed gastric and small

bowel transit that may be related to upper GI symptoms. A significant correlation was shown between the reduction of postprandial fundus relaxation and the presence of daily upper GI symptoms in slow transit constipated patients (4). On the contrary, in a recent study the authors concluded that although motor abnormalities of both colon and proximal GI tract regions are present in patients with chronic constipation and dyspepsia, they do not appear to play a role in the genesis of different dyspeptic symptoms (2). A number of reports have associated abnormalities in the GI transit in children with upper GI symptoms (24-27,3) but data on gastric motor activity and on the prevalence of specific dyspeptic symptoms among constipated patients are lacking. In previous papers, delayed gastric emptying has been reported in up to 68% of pediatric dyspeptic patients (28-29). Chitkara et al. (27) demonstrated that symptoms such as bloating and abdominal pain in pediatric and adolescent patients affected by FD may be associated with abnormal gastric or small bowel transit measurements. Recently it has been reported that 100% of 34 children complaining of chronic upper GI symptoms such as recurrent vomiting, nausea, gastroesophageal symptoms, abdominal pain, and early satiety suffered from chronic unrecognized constipation. The authors reported a significant improvement of the upper GI symptoms once constipation was adequately managed (3). Another study investigated 28 children with functional fecal retention (FFR) and chronic constipation and found that postprandial symptoms of nausea, poor appetite and early satiety had disappeared after treatment of constipation. In contrast to our results, they showed no significant change in gastric emptying time (30).

Two aspects of our data need explanation: 1) In our study *H.pylori* gastritis was found in 7 (8.75%) children affected by FD. These children were included into the study and did not receive any eradication treatment. 2) *H.pylori* gastritis in these children may have affected gastric motility. The current recommendation from the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) is that *H. pylori* infected children with non ulcer dyspepsia or recurrent abdominal pain, or both, do not require treatment (31). According to these guidelines, eradication treatment is recommended only for children who have a duodenal or gastric

ulcer identified at endoscopy and H.pylori documented by histopathology and in the rare child with lymphoma or proven atrophic gastritis with intestinal metaplasia. About the finding of H.pylori-associated gastritis, in the absence of peptic ulcer disease at endoscopy, the committee concluded that there is insufficient evidence to support either initiating or withholding eradication treatment. We chose not to treat the patients since there was no clear indications in the guidelines in use, and because the use of acid-suppressant (included in the eradication therapy) could interfere indirectly with gastric motility

Although a correlation between slowing of gastric emptying and increased severity of H. Pylori gastritis has been reported in adults (32-34), there are no studies in children. Recently Friesen et al. (35) reported that chronic gastritis in children is not associated with abnormalities of gastric electrical rhythm or solid food gastric emptying.

It is widely recognised that patients suffering from functional gastrointestinal disorders (FGIDs) may have symptoms of multiple disorders concurrently. In a recent study on a large series of adult patients, a significant overlap was found between upper and lower gastrointestinal symptoms and a relationship between different categories of functional gastrointestinal disorders (FGIDs)(31). Caplan et al (16) showed that among children 4-9 years old, the majority of overlaps with other FGIDs diagnosis according to Rome II criteria were observed for FD, FFR and irritable bowel syndrome, (IBS) while in children of 10-18 years, the diagnosis of FD overlapped with that of IBS (2.9%), FFR (2.9%), functional constipation (FC) (0.7%) and cyclic vomiting syndrome (1.4%).

Our data confirm the presence of overlaps among different functional gastrointestinal disorders. However, the limitations of our study include the absence of a control group and the lack of a validated method for measurement of gastric emptying time and dyspeptic symptoms..

In our study group, the majority of dyspeptic patients resulted affected by FC with a delayed gastric emptying time. The resolution of dyspeptic symptoms and gastric abnormalities after constipation treatment and bowel habit normalization suggests that, in a specific subset of patients,

dyspepsia may be induced by constipation. The enterogastric feedback activated by faecal stasis in the rectum should be considered among the possible mechanisms involved in the pathogenesis of dyspepsia.

The demonstration of significant overlap raises the question as to whether the functional gastrointestinal disorders should be considered multiple separate disorders or as a unique clinical entity with a common pathophysiology, common symptoms and also, when possible, a common treatment.

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FIGURE LEGENDS

Figure 1 : Total gastric emptying time evaluated at entry (T0) and after three months of follow-up (T3) in dyspeptic patients with functional constipation (FC yes) who received lactulose and in dyspeptic patients without functional constipation (FC no) who did not receive any treatment.

Figure 2: Dyspepsia symptomatic score evaluated at entry (T0) and after one (T1), Two (T2) and three (T3) months of follow-up in dyspeptic patients with functional constipation (FC yes) who received lactulose and in dyspeptic patients without functional constipation (FC no) who did not receive any treatment.

Figure 3: Number of bowel movements per week evaluated at entry (T0) and after one (T1), two (T2) and three (T3) months of follow-up in dyspeptic patients with functional constipation (FC yes) who received lactulose and in dyspeptic patients without functional constipation (FC no) who did not receive any treatment.

Table 1. Rome II functional dyspepsia and functional constipation diagnostic criteria (*Rasquin-Weber et al. Gut 1999; 45(S2):II60-II68*)

FUNCTIONAL CONSTIPATION

In infant and preschool children, at least two weeks of:

- Scybalous, pebble-like, hard stools for a majority of stools; or
- Firm stools two or less times/week; and
- There is no evidence of structural, endocrine, or metabolic disease.

FUNCTIONAL DYSPEPSIA

In children mature enough to provide an accurate pain history, at least 12 weeks, wich need not to be consecutive, within the preceeding 12 months of:

- Persistent or recurrent pain or discomfort centered in the upper abdomen (above the umbilicus; and
- No evidence (including at upper endoscopy) that organic disease is likely to explain the symptoms; and
- No evidence that dyspepsia is exclusively relieved by defecation or associated with the onset of a change in stool frequency or stool form.

Table 2. Test meals for gastric emptying time administered in different age groups

Age (years)	Bread (g)	Ham (g)	Butter (g)	Fruit juice (ml)
3-6	70	25	4	100
>6-9	80	30	7	125
>9-12	90	30	7	125
>12	100	40	8	125

Bread, 290 kcal/100 g; ham, 370 kcal/100g; butter, 750 kcal/100 g; fruit juice, 56 kcal/100 ml

	T0	T1	T2	T3
Patients who experienced symptoms, n (%)	28 (100)	19 (67.8)	16(57.1)	13(46.4)
Frequency for each reported symptom, n (%)				
Vomiting	18(64.2)	4(21)	4(25)	3 (0)
Heartburn	2(7.1)	2(10.5)	1(6.2)	2 (0)
Epigastric pain	28(100)	10 (52.6)	4(25)	0 (0)
Nausea	16(57.14)	7 (36.8)	5(31.2)	1 (2.2)
Early satiety	20(71.4)	5 (26.3)	3 (18.2)	0 (0)
Bloating	23(82.1)	8 (41.1)	4(25)	0 (0)

Table 3. Frequency of reported dyspeptic symptoms at enrollment (T0), at one-month (T1) two-months (T2) and at three-months (T3) after constipation treatment started in the 28 constipated dyspeptic children.

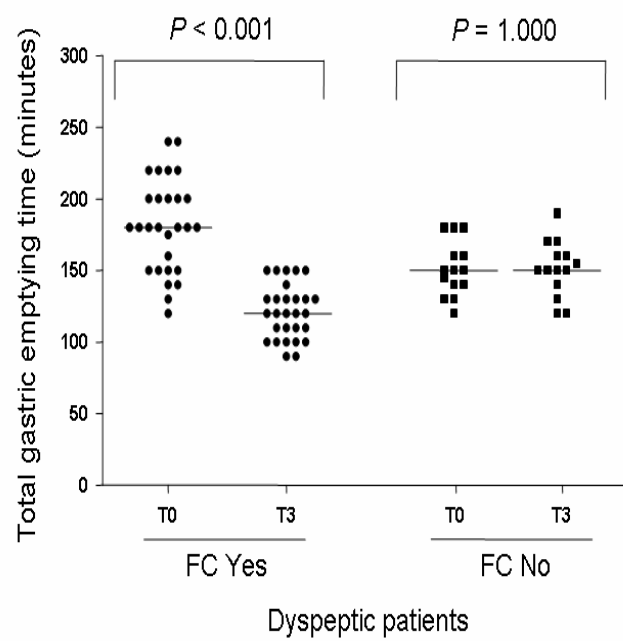


Figure 1

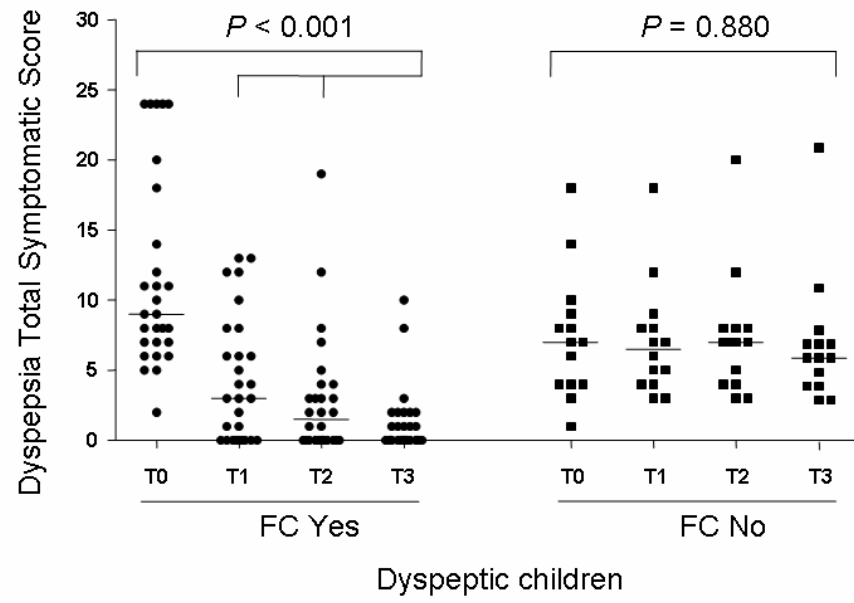


Figure 2

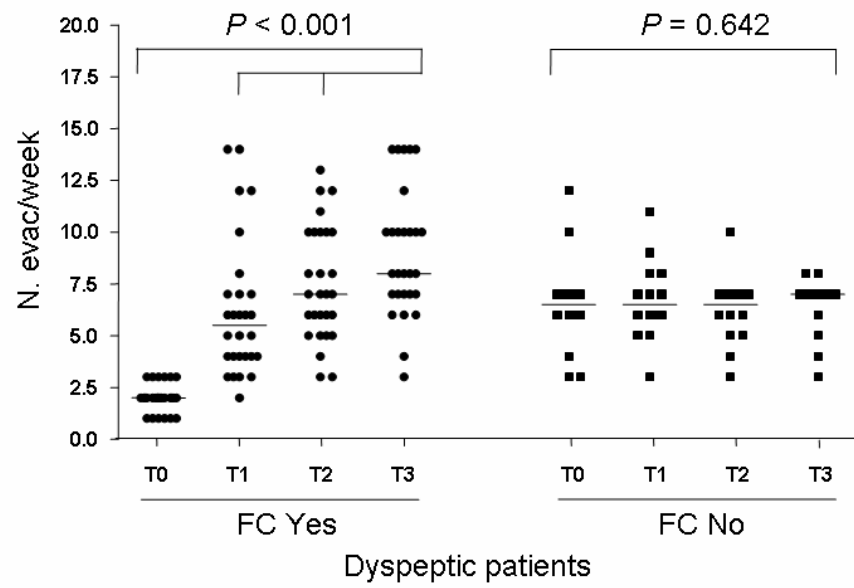


Figure 3

CHAPTER 3

DIAGNOSIS AND TREATMENT OF THE MAIN PEDIATRIC GASTROINTESTINAL MOTILITY DISORDERS.

-Staiano A, Boccia G, Miele E, Clouse RE. **Segmental characteristics of oesophageal peristalsis in pediatric patients.** Neurogastroenterol Motil. 2007 Nov 21; [Epub ahead of print]

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Segmental characteristics of oesophageal peristalsis in paediatric patients

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Abstract High-resolution manometry (HRM) in adults identifies a sequential chain of pressure segments that together form normal oesophageal peristalsis. HRM was performed in 40 neonates, infants/toddlers and children (age 1 day–14 years) to see if a similar segmental pattern could be identified in paediatric subjects. A chain of three pressure segments was found with inter-segmental troughs at $27.4 \pm 1.1\%$, $62.6 \pm 1.3\%$ and $94.9 \pm 0.8\%$ oesophageal length. The first and second pressure troughs were similarly distributed along the oesophagus across age groups; the third was 7.6–8.9% oesophageal length further from the lower oesophageal sphincter in neonates ($P < 0.05$ compared with other age groups). There were no significant differences in trough locations between subjects with or without oesophageal disease, controlling for age. Consistent presence of all three segments was less common in neonates, primarily because of fewer swallows demonstrating the first (proximal) and third (distal) segments compared with children. HRM in paediatric patients demonstrates, from neonates to children, the distinctive chain of pressure events that also characterizes oesophageal peristalsis in adults. The segmental character to oesophageal peristalsis should be taken into consideration in manometric investigation of all age groups – for example, in testing pharmacological responses and evaluating clearance mechanisms.

Keywords children, oesophageal manometry, oesophageal motility.

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Received: xx xxxx 200x

Accepted for publication: xx xxxx 200x

INTRODUCTION

High-resolution manometry (HRM) was developed to increase interpretative consistency and diagnostic accuracy of oesophageal manometry.^{1,2} The spacing of pressure sensors along the catheter is decreased and the number of sensors increased so that pressure data can be interpolated in the axial direction. Three-dimensional pressure maps of peristalsis, plotting pressure against time and sensor location, demonstrate distinctive features of normal and abnormal swallowing responses in adult subjects.^{2–6}

One important observation made in adults that accentuates the value of HRM is that oesophageal peristalsis is comprised a specific chain of sequential pressure segments.^{3–5,7} These segments, one in the striated-muscle region and two in the smooth-muscle region, appear as concentrated pressure loci separated from each other by lower amplitude pressure troughs on the three-dimensional maps.^{3–5,7} We recently demonstrated that this peristaltic chain, although incompletely formed, can be detected in healthy preterm and term neonates using HRM methods adapted for this very young age group.⁸ Whether a similar peristaltic 'architecture' or pattern is present throughout the age range of paediatric patients remains unknown but is highly suspected. Determining the presence of this pattern in all age groups should be valuable in interpreting manometric studies of paediatric patients when few pressure sensors are used, e.g. in studying oesophageal response to pharmacological provocation, as pressure profiles for the individual subject vary markedly in relation to the peaks and troughs.^{5,9} The HRM appearance of peristalsis also has facilitated recognition of motor disorders and lower oesophageal sphincter (LOS) location in adults.¹⁰

In the present study, HRM was performed during clinical evaluation of 40 neonates, infants/toddlers and children whose presentations indicated oesophageal manometry. Our objectives were to determine if a

segmental pattern is present in all paediatric patients and to see if the pattern varies with age group or in the presence of oesophageal disease. Because the segments reflect physiologically distinct regions of the oesophagus, we hypothesized that a segmental pattern would be detected in each subject group.

MATERIALS AND METHODS

Subjects

Each subject in this report underwent oesophageal manometry for clinical indications at either the Department of Pediatrics, Federico II University, Naples, Italy ($n = 27$), or Barnes-Jewish Hospital, Washington University Medical Center, St Louis, MO, USA ($n = 13$). Informed consent was obtained from the parents of each subject for performance of the study. The 40 subjects averaged 7.3 ± 0.8 years of age, and 14 (35.0%) were females. The subjects were further divided into three subgroups by age: neonates (<1 month), infants/toddlers (1 month to <2 years) and children (2–14 years). Further characteristics of the subjects by age group are shown in Table 1. Review of clinical data for this report was approved by the institutional review board (IRB) of each participating institution.

Manometric methods

Oesophageal manometry was performed following a fast appropriate for subject age. Extruded microluminal silicone catheters with recording side holes spaced at 1-cm intervals were used, the number of recording sites and catheter diameter varying by age group: a 2.25-mm-diameter catheter with nine recording sites was used in neonates; a 2.5-mm-diameter catheter with 11 recording sites was used in infants/toddlers; and the 2.5-mm-diameter catheter or a 4.0-mm-diameter catheter with 21 recording sites was used in children. The microlumina were perfused with a pneumohydraulic perfusion

system (Mui Scientific, Mississauga, ON, Canada). The modifiable device characteristics and rates of water perfusion (0.05 – $0.28 \text{ mL min}^{-1} \text{ cm}^{-1}$) were selected depending on catheter diameter to record pressure upstrokes of at least 100 mmHg s^{-1} in neonates and infants/toddlers and $>300 \text{ mmHg s}^{-1}$ in older subjects based on previously published data^{10–12} and on technical information from the manufacturer. Pressure data were acquired and displayed using a system designed for HRM and capable of displaying isobaric contour maps, three-dimensional maps in which a series of lines at specified pressure levels plot pressure against time and side hole location on the catheter (MMS, Enschede, Holland). These maps resemble topographic plots of geographical elevations.

The catheter initially was advanced transnasally until distal recording sites were positioned in the LOS or proximal stomach so that the LOS and majority of the oesophageal body could be sampled. The catheter then was withdrawn so that proximal recording sites were in the upper oesophageal sphincter (UOS) and remaining recording sites sampled the oesophageal body. When possible, the entire oesophageal body and sphincters was studied in one catheter position. Water boluses (0.5 – 4 mL depending on subject age) were offered until at least 10 evaluable swallows were obtained from each catheter position. In children, swallows were spaced by $\geq 20 \text{ s}$,¹³ in younger subjects, dry swallows interspersed between water boluses prohibited the 20-s delay in some instances. Once an adequate number of swallows and a pull-through manoeuvre for measuring LOS resting pressure (children only) were obtained, the catheter was removed.

Swallowing was determined by the characteristic opening and movement of the UOS seen with HRM in adult subjects.^{5,14,15} Because the UOS was not visualized when the catheter was in the distal position, a surface submental electromyogram was also recorded to determine the presence of a pharyngeal swallowing phase in all but older children wherein observed swallows were marked on the maps. Stereotypical

Table 1 Subject characteristics by age group

Subject group (n)	Age [mean \pm SEM (range)]	Sex	Indication for manometry (n)
Neonates (5)	7.0 ± 3.1 (1–16) days	3F/2M	Vomiting/regurgitation (5)
Infants/toddlers (6)	10.0 ± 2.5 (6–24) months	1F/5M	Vomiting/regurgitation (4) Dysphagia/anorexia (2)
Children (29)	9.9 ± 0.6 (3–14) years	12F/17M	Dysphagia/regurgitation (19) Chest pain (7) pH probe placement (3)

F, female; M, male.

deflections of the electromyogram that corresponded with UOS opening when the UOS was visualized were determined on *post hoc* review. These same stereotypical deflections were then used to confirm the presence and time of onset of a swallow when the catheter was positioned distally.

Isobaric contour maps were created by drawing the first line 5 mmHg above gastric baseline pressure; subsequent lines were drawn at 5–20 mmHg increments as required to best demonstrate the peristaltic pattern. Gastric baseline pressure was established as an average across respiratory variation from swallows in which distal recording sites were intragastric in location. Maps were scrutinized for pressure segments and pressure troughs separating the segments using previously described characteristics in adult subjects as the primary template (Fig. 1).^{3–5} A locus of pressure activity with concentric isobaric contour lines having a peak amplitude of ≥ 20 mmHg was required for identifying a pressure segment. Initial map review in the subject groups revealed that a similar pattern was present across all ages, and, thus, additional pressure segments and troughs were not recorded.

Locations of the UOS distal margin and LOS proximal margin were determined, and oesophageal length was measured as the difference between these sites. Location of each pressure trough (trough 1, separating the striated- from proximal smooth-muscle segment; trough 2, separating the two smooth-muscle oesophageal body segments; trough 3, separating the second smooth-muscle segment from LOS after contraction) was reported in centimetre above LOS and per cent oesophageal length from proximal to distal direction. Percentage of swallows demonstrating each contraction segment was calculated for each subject. If a pressure trough was not identified for a specific swallow, the average location of the pressure trough for that subject was superimposed on the map. Segments were considered present only if a distinct pressure locus was detected in the expected region (delineated by evident or superimposed troughs) with concentric isobaric contour lines focused on the region.

Manometric patterns were classified using a scheme described previously.¹⁰ LOS relaxation was assessed from conventional tracings using the recording site located most closely to the centre of the LOS. The transsphincteric gradient also was measured; the gradient was measured for up to 4 s after the swallow using techniques reported previously, a value of >5 mmHg being considered abnormal.¹⁶ LOS relaxation data were available only in subjects with intragastric pressure sensors and primarily were used to assist in classifying aperistaltic disorders. Final diagnosis for

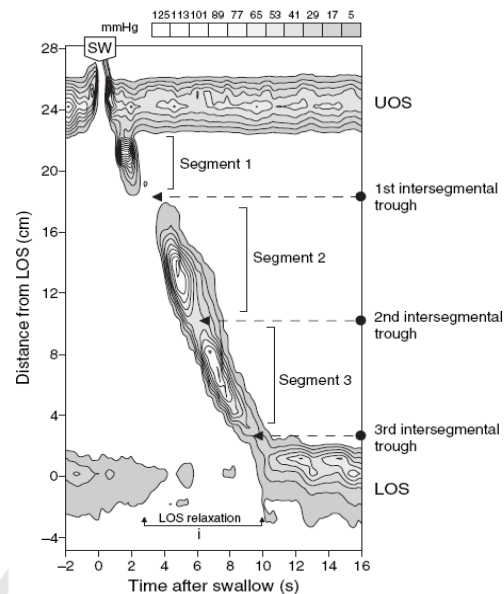


Figure 1 Typical features of the segmental peristaltic architecture using high-resolution manometry (HRM) and isobaric contour mapping in an adult. A solid-state catheter system spanning 35 cm (sensors at 1-cm intervals) was used to visualize the entire oesophagus and its sphincters. A short pressure region (segment 1) extends from the upper oesophageal sphincter (UOS) following the swallow (SW). The first intersegmental pressure trough separates this segment from the remaining smooth-muscle oesophageal body. The third pressure trough then separates this peristaltic chain from the lower oesophageal sphincter (LOS) after contraction. Increasing pressure amplitudes are represented by concentric isobaric contour lines and shading as demonstrated on the grey-scale legend (in mmHg above gastric baseline pressure).

each subject was established from review of all clinical data, and subjects ultimately diagnosed as having a definable non-oesophageal explanation for symptoms were considered together as the control subgroup with regard to the location of peristaltic architectural landmarks.

Statistical methods

Data are reported as mean \pm SEM throughout. Pressure trough locations and percentage of swallows demonstrating each pressure segment were compared across age groups using a one-way ANOVA followed by unpaired Student's *t*-tests if the ANOVA was significant. Linear regression analyses controlling for age were used to

compare pressure trough locations and presence of swallows demonstrating each pressure segment between control and oesophageal-disorders subgroups. Statistical analyses were conducted using SPSS v14.0 (SPSS Inc., Chicago, IL, USA); $P < 0.05$ was required for statistical significance using two-tailed testing. Statistical trends were reported for P -values < 0.1 .

RESULTS

The entire oesophagus and sphincters could be sampled in a single catheter position in four subjects (Fig. 2); proximal and distal positions were necessary to visualize the entire oesophagus and both sphincters in the remainder (Fig. 3). Clinical diagnoses and manometric findings in the subjects are shown in Table 2. The same chain of pressure segments identified previously in adults was seen in each subject with the exception of seven with aperistalsis, six of whom were ultimately diagnosed as having achalasia (Fig. 4). One

of the seven had severe hypocontractility and reflux disease.

Relative locations of the inter-segmental troughs and the proportion of swallows demonstrating the individual pressure segments are shown in Table 3. As expected, oesophageal length was significantly greater in the oldest group when compared with each younger group ($P < 0.05$ for each comparison). The first and second pressure troughs were similarly distributed across oesophageal length in each age group, but the third trough was located proportionately less closely to the upper margin of the resting LOS in the neonates compared with infants/toddlers or children ($P < 0.05$ and $P < 0.001$ respectively).

The first pressure segment was more consistently present in children than in the other two age groups ($P < 0.05$ for each comparison), and the percentage of swallows with the third pressure trough was decreased in neonates compared with children ($P < 0.05$). Consequently, completely formed peristaltic chains were less commonly observed in the neonates, but the number of subjects was too small to confirm that this was a meaningful finding. No differences in trough locations or percentage of swallows with individual segments were found between groups with or without oesophageal disease when the analyses were controlled for age ($P \geq 0.3$ for each comparison). The segments and troughs could be identified in the presence of oesophageal motor disorders other than aperistaltic disorders (Fig. 5).

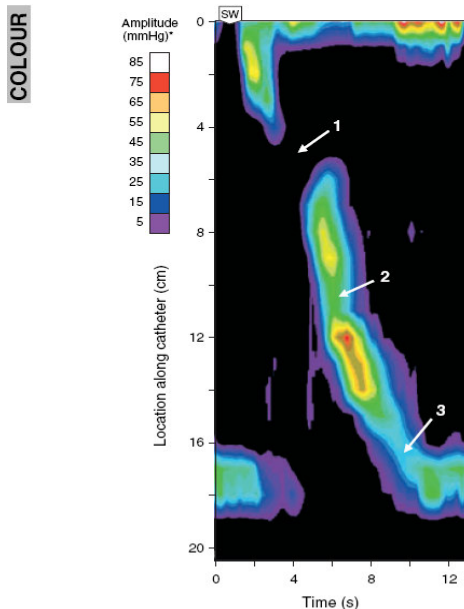


Figure 2 A complete peristaltic chain is seen in this 12-year-old male child studied with a 21-lm catheter. The segmental pressure architecture resembles that seen in the adult. The three inter-segmental troughs are indicated on the figure, and the pressure amplitudes represented by the isobaric contour regions are shown in the colour legend (in mmHg above gastric baseline pressure; pressures below the first isobaric contour are shown in black). SW, swallow.

DISCUSSION

In this report, we successfully applied HRM to a small number of subjects representing the broad age range seen in paediatrics to examine the appearance of peristalsis using these techniques. A segmental oesophageal peristaltic sequence resembling that observed previously in adults was present in all age groups with minimal variation in the relative location of defining landmarks along oesophageal length.³⁻⁵ Although a distinctly normal control group was not employed, we found no difference in the presence or distribution of the pressure segments within the oesophageal body in subjects who had symptoms ultimately attributed to oesophageal disease or who had other explanations for the presenting complaints. Consequently, our hypothesis that these segmental features would be detected with HRM across all paediatric age groups was confirmed. The relative distribution of the segments along the oesophageal length appears fixed at birth unless the chain is obliterated by disorders underlying aperistalsis.

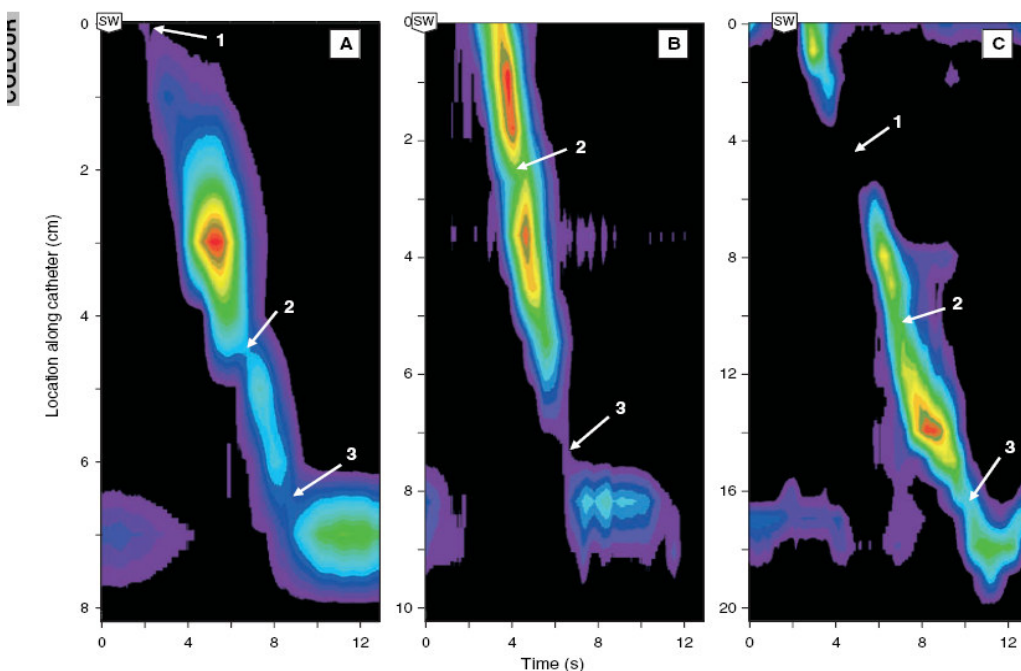


Figure 3 Examples of oesophageal peristalsis in each paediatric age group. The female neonate (age 20 days) in panel A is studied with catheter having nine recording sites, and the distal oesophagus and lower sphincter region are visualized. The third pressure segment is not well developed on this swallow. In panel B, a 6-month-old male infant studied with a catheter having 11 recording sites has typical segmental architecture in the distal oesophagus. The proximal segment and first pressure trough were only seen when the catheter was re-positioned more proximally. In panel C, all but the upper oesophageal sphincter is well visualized in a 6-year-old male child studied with a 21-lm catheter. Again, the typical segmental pressure architecture is identified with three distinctive inter-segmental pressure troughs. In each case, the recording sites are separated by 1 cm. SW, swallow.

Table 2 Clinical diagnoses and manometric findings

Subject group (n)	Final clinical diagnosis (n)	Manometric findings (n)
Neonates (5)	Achalasia (1) GORD (3) Functional regurgitation (1)	Achalasia pattern (1) Normal (3) Normal (1)
Infants/toddlers (6)	GORD (3) Normal (3)	Hypocontractility (2); normal (1) Normal (3)
Children (29)	Achalasia (5) Functional symptoms (5) GORD (11) Normal (8)	Achalasia pattern (5) LOS dysfunction (3); NSSD (2); normal (1) NSSD (5); normal (5); aperistalsis/severe hypocontractility (1) Normal (8)

GORD, gastro-oesophageal reflux disease; NSSD, non-specific spastic disorder; LOS, lower oesophageal sphincter.

The fact that the pressure signature of oesophageal peristalsis is comprised a series of sequential segments had not been appreciated prior to use of HRM in adults.

The first pressure trough had been observed, but detailed analyses from HRM now indicate that this trough separates two distinct propagating wave forms,

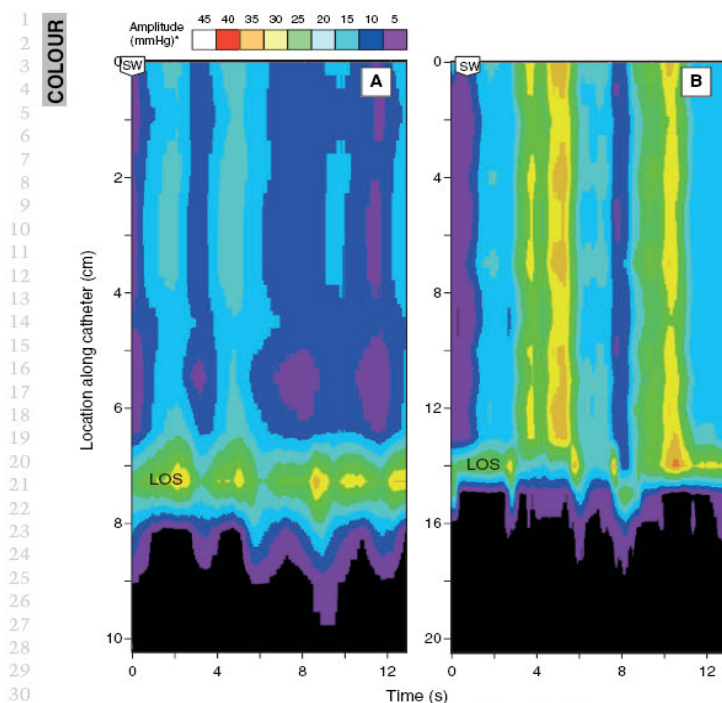


Figure 4 Peristaltic segments are absent in this 7-year-old child (A) and 14-year-old child (B) with achalasia. The peristaltic chain is replaced by isobaric contour stripes spanning the oesophageal body that end in a 'dam' effect at the non-relaxing lower oesophageal sphincter (LOS), features that have been described by high-resolution manometry in adults with achalasia.² In each of these subjects, the upper oesophageal sphincter and most proximal oesophageal body were not seen until the catheter was positioned proximally for additional sampling. SW, swallow.

consistent with transition from striated- to smooth-muscle activity.⁷ The second pressure trough dividing the smooth-muscle oesophagus into two similarly sized regions had been overlooked by conventional manometry. Preliminary data indicate that this pressure trough also is seen in the opossum, an animal sharing some oesophageal neuromuscular characteristics with the human.¹⁷ Based on comparisons to physiological studies in this animal and the outcome from pharmacological manipulations in the human, it is plausible that the smooth-muscle segments correspond to transition of dominant neural control mechanisms (from cholinergic to non-cholinergic).^{9,18}

The present study confirms that all of these features can be detected in even the youngest paediatric subjects requiring manometric evaluation. We recently demonstrated in an HRM study of healthy preterm and term neonates that maturation of the peristaltic chain continues to occur through late gestation and beyond term birth.⁸ Our present findings in symptomatic neonates and infants/toddlers replicate these findings.

They suggest that maturation may continue through the infant/toddler period such that presence of the complete peristaltic chain at rates matching the adult pattern only becomes most evident during childhood years. This conclusion must be tempered, however, by the fact that numbers of subjects in the younger groups were small and that symptomatic subjects might not represent the normal population.

The identification of peristaltic segments has several important implications. From a clinical standpoint, the HRM appearance of peristalsis has facilitated recognition of normal and abnormal motor function in adults. Finding even small fragments of the normal sequence helps differentiate primary hypocontractility from achalasia, the latter having no normally timed peristaltic elements and, most often, no segmental characteristics at all.^{10,19} The distinctive peristaltic pattern also helps identify LOS location. We previously demonstrated that differences between LOS location by conventional pull-through and from isobaric contour maps exceeds ≥ 2 cm in 11.9% of adult patients and

Table 3 Peristaltic landmarks identified on the isobaric contour maps

Subject group (n)	Oesophageal length (cm)	Inter-segmental pressure trough location as % oesophageal length*			Inter-segmental pressure trough location in centimetre above LOS			Percentage of swallows demonstrating the pressure segment		
		Trough 1	Trough 2	Trough 3	Trough 1	Trough 2	Trough 3	Segment 1	Segment 2	Segment 3
All subjects† (33)	12.5 ± 0.7	27.4 ± 1.1	62.6 ± 1.3	94.9 ± 0.8	9.1 ± 0.5	4.6 ± 0.3	0.6 ± 0.1	88 ± 4	88 ± 3	72 ± 5
Neonates† (4)	7.9 ± 0.8	24.7 ± 2.8	59.1 ± 2.1	88.1 ± 2.9	6.1 ± 0.2	3.2 ± 0.5	0.9 ± 0.2	72 ± 11	88 ± 7	40 ± 23
Infants/toddlers (6)	10.0 ± 1.0	30.2 ± 1.8	59.2 ± 2.6	97.0 ± 2.0	7.0 ± 0.7	4.1 ± 0.5	0.3 ± 0.2	78 ± 12	80 ± 5	66 ± 11
Children† (23)	14.0 ± 0.7‡	27.2 ± 1.4	64.1 ± 1.6	95.7 ± 0.6‡	10.2 ± 0.6‡	4.9 ± 0.3‡	0.6 ± 0.1‡	96 ± 2‡	91 ± 3	79 ± 4‡
Non-oesophageal disorders (12)	13.4 ± 0.7	27.9 ± 2.3	66.6 ± 2.1	96.0 ± 0.6	9.7 ± 0.6	4.4 ± 0.3	0.5 ± 0.1	98 ± 2	86 ± 6	79 ± 4
Oesophageal disorders† (21)	12.0 ± 1.1	26.7 ± 1.2	60.7 ± 1.5	94.1 ± 1.3	8.8 ± 0.8	4.6 ± 0.4	0.6 ± 0.2	82 ± 6	91 ± 2	71 ± 8

*Reported as mean ± SEM.

†Exclusive of patients with aperistalsis.

‡ $P < 0.05$ on ANOVA across age groups.

COLOUR

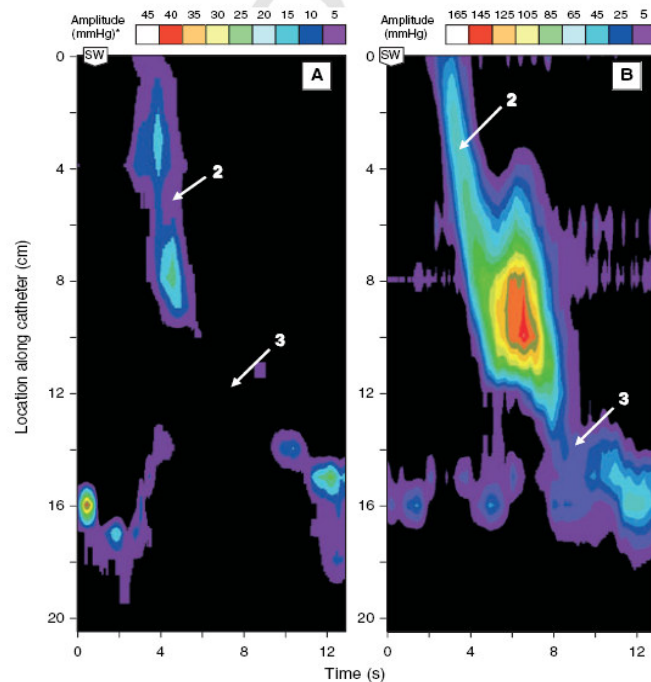


Figure 5 Segmental features in two children with abnormal oesophageal motility. (A) Hypocontractility of the distal oesophagus with evidence of the two distal segments, each of low amplitude. The third segment is foreshortened, extending the region of the third pressure trough proximal to lower sphincter after contraction. (B) The prominent third peristaltic segment is forked with repetitive contraction in its proximal extent in this child with a spastic disorder. Forked regions on high-resolution manometric studies correspond to double-peaking on conventional pressure tracings.²² SW, swallow.

reaches 3 cm or more in a small but important 2.4%.¹⁰ Predictably, discrepancies are more common with low LOS resting pressures when identification of sphincter

location by pull-through is most difficult.¹⁰ From an investigational standpoint, the recognition of peristaltic segments is important in interpreting manometric

data when few intra-oesophageal sensors are used. Inconsistent positioning with regard to segmental peaks and inter-segmental troughs could interfere with detecting treatment effects or understanding clearance mechanisms, as examples.^{9,18}

HRM may prove to have clinical advantages in paediatric patients as it has in adults, but further proof of its effectiveness in these subjects will be required. Current limitations of HRM in paediatrics relate largely to the pneumohydraulic perfusion of a catheter having multiple microlumina and the need for fastidious maintenance of this system to ensure accurate recordings. Recent development of a 36-sensor solid state catheter having circumferential pressure transducers embedded along its length has eliminated water perfusion, allows sampling of the entire oesophagus without catheter repositioning, and has simplified HRM in adults.^{6,14,20,21} The 4.2-mm diameter of the presently available catheter restricts its use in paediatrics to older children, and water-perfused systems will be required for HRM in younger paediatric subsets throughout the foreseeable future. One could argue that the density of sensors (1 per centimetre) on each catheter used in the present study may have been inadequate to detect peristaltic segments accurately, especially in neonates, a group with seemingly less consistently developed peristaltic chains. We believe this is unlikely, as at least two recording sites would have been present in each segmental region, even in the neonate group.⁸ More detailed assessment of oesophageal motility by HRM in the youngest groups may require greater sensor density at the expense of shorter sampled regions (e.g. restricted to the oesophagogastric junction).

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Chronic Cough and Acidic Gastroesophageal Reflux Disease in Children: which Test for Symptomatic Correlation?

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Key words: gastroesophageal reflux disease; respiratory symptoms; esophageal pH-metry

Short running title: Chronic cough and GERD in children.

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ABSTRACT

Objectives: To evaluate in children with chronic cough the prevalence of acidic GERD, and to establish if a temporal correlation exists using the traditional symptom (SI) and symptom sensitivity (SSI) indices and the new symptom association probability (SAP). **Methods:** Distal esophageal 24-hour pH monitoring was performed in 47 children (mean age \pm SD: 34 ± 41 months, range: 1-108 months; M/F: 27/20) affected by chronic unexplained cough. During recording time, caregivers documented each bout of coughing, both in a diary card and by pressing an event button on the digitrapper. Pathological reflux was defined as the percentage of time pH<4 exceeded 4%. SI, SSI, and SAP were calculated for each pH-tracing and considered positive if greater than 50%, 5%, and 95%, respectively. **Results:** GERD was diagnosed in 30 (64%) children with chronic cough, while one or more indices were positive in 16 (34%). In particular, SI was positive in 6 (13%) children (all with GERD), SSI in 12 (26%) (7 with GERD), while SAP was abnormal in 6 (13%) (4 with GERD). The Cohen's kappa test reported a high agreement between SSI and SAP ($k=0.60$; $p<0.0001$) and a poor one, between SI and SSI, and between SI and SAP ($k=0.06$ and $k=0.24$, respectively). **Conclusions:** Children with chronic cough had a high prevalence of acidic GERD. Each of the three used indices identified a possible symptomatic association.

INTRODUCTION

Cough is the most common presenting symptom to general practitioners in western societies (1). Non-specific cough has been defined non-productive cough in the absence of identifiable respiratory disease or known aetiology (2). Gastroesophageal reflux disease (GERD) is one of the three most common causes of chronic cough in children along with postnasal drip syndrome and asthma (3). The cough, without associated heartburn or acid regurgitation, may be the only presenting manifestation of GERD in 10-45% of cases, in both prospective and retrospective studies (4-6). However, few studies have examined whether a temporal correlation actually occurs between documented episodes of reflux and cough.

In the diagnosis of GERD, ambulatory 24-hr esophageal pH monitoring is a valuable tool not only for the quantitative measurement of esophageal acid exposure but also for the assessment of the association in time between symptoms and reflux (7-9). The most frequently used parameter in determining the significance of the symptom correlation on these pH tracings is currently the symptom index (SI), defined as the percentage of reflux related symptoms episodes (8). The symptom-sensitivity index (SSI), defined as the percentage of symptom-associated reflux episodes was introduced as an additional parameter to overcome the drawbacks of the SI (9). This index, however, fails to take into account the total number of symptom episodes, rendering its use of limited value.

In suspected GERD patients, a consistent temporal association may clarify whether cough is induced by or provokes reflux and may help in the establishment of the appropriate treatment. The symptom association probability (SAP) is a single parameter which is able to provide more objective information on the probability that the association in time between reflux and symptoms does not occur by chance (10). The aims of this study are: 1) to evaluate the prevalence of GERD

related to acid in children with chronic cough and 2) to establish if a temporal correlation exists between cough and GERD comparing the SI and SSI and the new SAP.

SUBJECTS AND METHODS

We prospectively investigated, from January 1 through December 30, 2005, 47 consecutive patients (27 males; mean age \pm SD: 34 ± 41 months, range 1-108 months) with chronic unexplained cough, referred for 24-h esophageal pH-monitoring to the Gastrointestinal Endoscopy and Motility Unit of the Department of Pediatrics, University “Federico II” of Naples, Italy.

Chronic cough was defined as a cough which persisted greater than 3 weeks.

Other causes of chronic cough were excluded. All patients were immunocompetent, had a normal chest x-ray and asthma was excluded by negative spirometry and/or allergy testing and/or absence of improvement with antiasthmatic medication. Postnasal drip syndrome was excluded both clinically and by sinus imaging.

At enrollment, all patients underwent clinical evaluation and 24-hr esophageal pH-monitoring. If patients were receiving empirical antireflux therapy, acid suppressive medication was stopped at least two weeks before the 24-hr esophageal pH-monitoring. A validated cough diary, using the verbal category descriptive score for daytime and nocturnal cough, was recorded by children’s caregiver, in order to obtain a measure of symptom severity and frequency (11). Cough was scored for each day as follows: 0, no cough; 1, cough for one or two short periods only; 2, cough for more than two short periods; 3, frequent coughing but does not interfere with school and other activities; 4 frequent coughing that interferes with school and other activities; and 5, cannot perform most activities due to severe coughing. A visual analogue scale from 0 to 10 was used for parental rating of cough severity and a full medical history was obtained for all patients at enrollment.

A one-channel esophageal pH probe (ambulatory system with semidisposable monocrystalline antimony pH electrode, Medtronic) was placed in all patients. Before each study, the pH electrode was calibrated in buffers of pH = 1.07 and pH = 7.01. The intraesophageal pH electrode was positioned 5 cm above the lower esophageal sphincter (LES), using Strobel's equation based on patient length (12). Children's caregivers were instructed to fill in a diary card the times of the meals, the sleeping periods and the onset of children's bouts of coughing, during the whole recording time. In addition, caregivers were given an event button for cough and were instructed on using it to indicate each time that a bout of coughing occurred. The diary data needed as a check of appropriate use of the event button. During the 24 hr examination the children were invited to perform a normal daily routine and eat normally. Patient data were stored in a portable disposable (Digitrapper Mk III, Medtronic), and the results for each day were analyzed subsequently using a specific software (Polygram for Windows, Medtronic).

The following parameters were measured for each 24-hr tracing: 1) total reflux index (RI) = percent of investigation time with pH <4; 2) number of episodes with pH < 4; 3) number of episodes with pH < 4 lasting longer than 5 minutes; 4) duration of the longest episode with pH < 4. Reflux was considered pathological if the total time the pH was less than 4.0 exceeded 4.0 %.

A clinician (GB) experienced in interpreting 24-hr pH tracings, who was blinded to the study, calculated the SI and SSI for each patient to establish whether a significant correlation between cough and reflux occurred. The symptom index (SI) was defined as the number of reflux-related cough episodes divided by the total number of cough episodes multiplied by 100 %. The symptom sensitivity index (SSI) was defined as the number of cough-associated reflux episodes divided by the total number of reflux episodes multiplied by 100 %. SI and SSI were calculated for each pH-tracing and were considered positive if greater than 50 % and 5 %, respectively.

A separate investigator (FM) calculated the SAP for each patient using the following procedure adapted from Weusten et al (10). The 24-hr esophageal pH signal was divided into consecutive 2-min periods. Each period was then evaluated for the presence or absence of reflux.

A drop in pH below 4.0 lasting at least 5 sec at any point during the 2-min window was considered positive for reflux. If cough occurred within a 2-min span, following reflux, the period was considered positive for both cough and reflux. Subsequently, a contingency table was constructed for each patient containing four fields (Table 1). Fisher's exact test was employed to calculate the probability (P value) that the observed association between reflux and symptoms occurred by chance. The SAP was calculated as $(1.0 - P) \times 100\%$ and it was considered positive if greater than 95 %. SAP values were compared with SI and SSI previously calculated.

Informed consent for participation in this study was obtained from parents of all patients, and the experimental design was approved by the Independent Ethics Committee of the University of Naples, Federico II.

STATISTICAL ANALYSIS

Continuous variables are expressed as mean \pm standard deviation (SD). For categorical variable either the unpaired and paired t-test or the Fisher's exact test were used, as appropriate. To test the agreement among SI, SSI and SAP, Cohen's k measure was applied. Statistical tests were performed with SPSS software, version 14.0.2.

RESULTS

Esophageal pH monitoring was performed in all the 47 enrolled patients affected by chronic unexplained cough. Three of them had received acid-suppressive therapy (ranitidine) at the dose of 8 mg/Kg/day, for a period of 4 weeks, until 2 weeks before the esophageal pH-monitoring was performed. No symptomatic remission was observed. GERD related to acid was diagnosed in 30 (64%) children with chronic cough. The duration of esophageal pH-monitoring was not significantly different in children with GERD compared with children without GERD (mean \pm SD: 22 ± 1.23 hrs vs 22.3 ± 1.3 hrs, respectively). Distribution at enrollment of the esophageal pH-monitoring parameters and of the cough scores according to the acidic *GERD positive* (GERD +) and acidic *GERD negative* (GERD -) group of patients are reported in Table 2.

Cough severity and frequency, obtained using the *cough score* and the *visual analogue scale*, were significantly higher in children with GERD than in children without GERD. One or more indices were positive only in 16 (34%) out of 47 patient. In particular, SI was positive in 6 (13%) children (all with GERD), SSI in 12 (26%) (7 with GERD), while SAP was abnormal in 6 (13%) (4 with GERD). Table 3 shows the distribution of the three symptomatic indices in children with chronic cough with and without GERD.

The Cohen's kappa test reported high agreement between SSI and SAP ($k = 0.60$; $p < 0.0001$), and poor agreement between SI and SSI, and between SI and SAP ($k = 0.06$ and $k = 0.24$, respectively).

DISCUSSION

Chronic unexplained cough in children may be the only presenting manifestation of GERD in 45% of cases, in both prospective and retrospective studies (4-6,13). In our study, GERD was diagnosed in 64% of children with chronic unexplained cough. This higher prevalence is probably due to the fact that participants in our study were recruited from tertiary care settings and probably had more severe symptoms than general population.

It has been shown that cough is associated with GERD, but there is conflicting data as to whether or not it is the consecutive factor (14, 15). Cohort studies in adults suggest that GERD related to acid causes 21-41 % of chronic non-specific cough, including many patients with no gastrointestinal symptoms of GERD (16, 1). Several possible mechanisms underlying a relation between reflux and respiratory symptoms have been proposed. Heightened bronchial reactivity, microaspiration, and a vagally mediated reflex mechanism are possible pathways (17). Exposure to small amounts of acid has recently been proposed as a cause of impaired laryngopharyngeal sensitivity and therefore may potentially increase the risk of aspiration (18). However, it has also been suggested that asthma causes or aggravates reflux (13).

While the association between respiratory symptoms and GERD has been well demonstrated in adults, until now it has been unclear in children (19). In pediatric literature, studies on chronic unexplained cough and GERD are scarce and include one prospective study, one retrospective study and various observational studies (20-25). The only pediatric RCT study evaluated whether cough frequency was increased by the thickening of infant milk formula feedings. It demonstrated an increased cough in infants with atypical GER (26). One case report documented a temporal association of gastroesophageal reflux episodes and cough in infants (27). Our study is the first prospective randomized evaluation of temporal correlation between GERD and chronic unexplained cough in a large group of children. In contrast, many pediatric studies focused on the association between asthma and GERD. A high percentage of children with persistent asthma have

gastroesophageal reflux detectable by abnormal esophageal pH monitoring. The reported prevalence ranges from 25 % to 75 %. However, in children there is no consistent evidence that specific asthma symptoms or response to asthma therapy correlates with abnormal esophageal pH monitoring (28, 29).

In children with atypical GERD, defined by the presence of extra-intestinal symptoms such as cough, 24-hr pH recording is the gold standard for correlating the acid reflux with cough, according to the most recent guidelines; however, evidence for this correlation is still lacking (30). We found acidic GERD in 64% of children with chronic unexplained cough and each of the three symptomatic indices identified a possible temporal association. In adults Wunderlich et al.(31) examined how a temporal correlation could often be found between coughing episodes and acid reflux events. They then compared the traditional method of analysis, which involves SI and SSI, and the potentially more precise SAP method revealing a significant number of patients with a temporal correlation between cough and reflux. In fact, in adults, it has been well documented that although SI and SSI are not effective indicators of correlation between respiratory symptoms and gastroesophageal reflux episodes, SAP seems to be a more reliable statistical tool in the diagnosis of atypical GERD. This was why we evaluated the effectiveness of all these indices. In our study SI was found to be positive in 13% of children (all with GERD), SSI in 26% of children (7 with GERD) and SAP was abnormal in just 13% of children (4 with GERD). In particular, we found high agreement between SSI and SAP, and poor agreement between SI and SAP. SI is defined as the number of reflux-related symptom episodes divided by the total number of symptom episodes multiplied by 100%. However, one major drawback of this parameter, is that it does not take into account the total number of reflux episodes. The higher the frequency of reflux episodes, the greater the chance that a symptom will have a temporal correlation with reflux. Thus a patient with frequent reflux and only one episode of cough may have a positive SI by chance alone. The lack of correlation between SI and SAP in our study may indicate that the number of symptom episodes experienced in this subgroup of patients was relatively low, making them more susceptible for

random symptom-reflux association. This finding confirms the limited usefulness of SI, compared to SSI and SAP, in the evaluation of a temporal correlation between gastroesophageal reflux episodes and symptoms. In our study, once again in contrast to what was reported in adults, none of the three indices proved to be accurate in proving a relationship between cough and GERD. This may suggest that chronic unexplained cough in children is due to different pathogenetic mechanisms than in adults. It also suggests that though cough and GERD often co-exist in children, their association does not imply cause and effect.

On the other hand, we know that esophageal 24-hr pH-monitoring only recognizes acid reflux, while in literature it has been reported that respiratory symptoms are mainly due to non-acid reflux. Esophageal pH-impedance, is able to detect acidic and non acidic reflux and could better evaluate the relationship between cough and GER but it is a method not yet validated in children. For this reason in our study we used the esophageal 24-hr pH-monitoring, currently considered the gold standard for the diagnosis of atypical GERD in children (30). Future studies on the correlation between non acidic reflux and respiratory symptoms are of course auspicious, but only after a validation of esophageal impedance in the paediatric population with the availability of clear age-related range of values for the detection of normal and abnormal condition. They should be double blind, randomised controlled, parallel designed with validated subjective and objective cough outcomes and also should ascertain the time needed to respond to therapy as well as assess acid and/or non acid reflux while on therapy.

LIST OF ABBREVIATIONS

GERD: gastroesophageal reflux disease

SI: symptom index

SSI:symptom sensitivity index

SAP: symptom association probability

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Table 1 . Model of the contingency table constructed for each patient

	Cough +(C+)	Cough-(C-)	
Reflux + (R+)	C +R+	C-R+	R+ total
Reflux- (R-)	C+R-	C-R-	R- total
	C+ total	C- total	total

C = cough; R= reflux

Table 2. Values of 24-hr pH monitoring and symptomatic score in the 47 patients studied.

	*GERD positive N° patients(%)	*GERD negative N° patients (%)	P value	(*G ER D= Gast roes opha geal reflu x dise ase) Data
Esophageal pH-measurements				
Investigation time with pH <4 (%)	13.6 ± 12	1.56 ± 1.0	<0.001	
N° of episodes with pH < 4	251.8 ± 170.7	63.8 ± 16.8	<0.05	
N° of reflux longer than 5 minutes	5.1 ± 0.94	0.34 ± 0.12	<0.0001	
Cough score	3.2 ± 1.1	1.1 ± 1.0	<0.01	
Visual analog scale	6 ± 2.08	2.1 ± 1.1	<0.001	

are expressed in mean ± standard deviation

Table 3. Distribution of the 3 symptomatic correlation indices in children with chronic cough with and without gastroesophageal reflux disease (GERD).

Pts. with GERD 30 (100)	Positivity to one or more indices	Pts. without GERD 17 (100)	Positivity to one or more indices
19 (63.3)	-	12 (70.6)	-
7 (23.3)	4 SI, 3 SSI	3 (17.6)	3 SSI
2 (6.7)	2 (SSI + SAP)	2 (11.8)	2 (SSI +SAP)
2 (6.7)	2 (SI +SSI +SAP)	0 (0)	-

Data are expressed in Nrs(%)

Maintenance Therapy for Erosive Esophagitis in Children After Healing by Omeprazole: Is It Advisable?

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- OBJECTIVES:** To evaluate the efficacy of acid-suppressive maintenance therapy for gastroesophageal reflux disease (GERD) in children, after the healing of reflux esophagitis.
- METHODS:** Forty-eight children (median age 105 months, range 32–170) with erosive reflux esophagitis were initially treated with omeprazole 1.4 mg/kg/day for 3 months. Patients in endoscopic remission were assigned in a randomized, blinded manner by means of a computer-generated list to three groups of 6-month maintenance treatment: group A (omeprazole at half the starting dose, once daily before breakfast), group B (ranitidine 10 mg/kg/day, divided in two doses), and group C (no treatment). Endoscopic, histological, and symptomatic scores were evaluated at: T0, enrollment; T1, assessment for remission at 3 months after enrollment (healing phase); T2, assessment for effective maintenance at 12 months after T0 (3 months after the completion of the maintenance phase). Relapse was defined as the recurrence of macroscopic esophageal lesions. After the completion of the maintenance phase, patients without macroscopic esophagitis relapse were followed up for GERD symptoms for a further period of 30 months.
- RESULTS:** Of 48 initially treated patients, 46 (94%) healed and entered the maintenance study. For all patients, in comparison to T0, the histological, endoscopic, and symptomatic scores were significantly reduced both at T1 and T2 ($P < 0.0001$, for each). No significant difference was found in these three scores, comparing group A, B, and C at T1 and T2. A relapse occurred in one patient only, who presented with macroscopic esophageal lesions at T2. Three months after the completion of the maintenance phase, 12 (26%) patients complained of symptoms sufficiently mild to discontinue GERD therapy, excluding the patient who showed macroscopic esophagitis relapse. Three of 44 (6.8%) patients reported very mild GERD symptoms within a period of 30 months after maintenance discontinuation.
- CONCLUSIONS:** Our pediatric population showed a low rate of erosive esophagitis relapse and GERD symptom recurrence long term after healing with omeprazole, irrespective of the maintenance therapy.

(Am J Gastroenterol 2007;102:1–7)

INTRODUCTION

Gastroesophageal reflux disease (GERD) is being recognized at increasing rates in infants, children, and adolescents (1, 2). After the first year of life, GERD becomes a chronic relapsing condition and older children are more likely to have the adult pattern of GERD (3, 4). As such, long-term treatment could be targeted toward prevention of esophageal epithelial injury, management of atypical manifestations, and control of symptoms.

In children, proton pump inhibitors (PPIs) have been shown to be safe and effective for short-term treatment of erosive esophagitis and GERD symptoms that are refractory to other measures. Pediatric studies using omeprazole and lansoprazole have reported endoscopic healing rates ranging from 40 to 100% and symptom relief rates from 70 to 100% (5–9).

The pathophysiology of GERD is complex, and likely includes environmental influences (diet, posture, obesity, smoke exposure, abdominal strain, etc.) as well as intrinsic irreversible pathophysiological factors. However, little is known about GERD natural history and the long-term outcome of treatment in most pediatric subgroups.

The available literature supports the value of sustained PPI treatment (at least 6 months) in preventing symptomatic and endoscopic GERD relapse and treating adults and children refractory to other interventions (4, 7, 10–12). Whether such treatment will have very long sustained effects has not been established. Likewise, it is not known how useful and safe this approach will be in preventing GERD relapses and complications.

The primary aim of our study was to evaluate the efficacy of maintenance therapy for GERD with two different acid

suppressants (omeprazole and ranitidine), compared with a group without treatment, to keep patients in endoscopic remission after the healing of erosive reflux esophagitis. Our secondary end point was to evaluate long-term symptomatic remission in the same groups of patients.

MATERIALS AND METHODS

Patients and Methods

Patients were recruited from May 2001 to July 2002 among children aged 1–16 yr with macroscopic reflux esophagitis, grade 2 or above on the Hetzel and Dent scale, as reported in Table 1 (13).

Criteria for exclusion were: treatment with a PPI within the previous 6 months, esophageal abnormalities caused by general or systemic diseases, previous esophageal and/or gastric surgery, the presence of esophageal stenosis, renal, cardiac, hepatic, or pulmonary disease, organ transplantation, central nervous system disease, food allergy and celiac disease, and expected poor compliance with treatment.

In the first part of the study, 48 consecutive children (M/F 26/22, median age 105 months, range 32–170) fulfilling the inclusion criteria entered a 3-month open-healing phase and were treated with omeprazole at a dose of 1.4 mg/kg (7, 14). The daily drug dose was given in the morning, 15–30 min before the first meal of the day. For children unable to swallow intact capsules, the capsules were opened and the omeprazole granules were given in a weakly acidic (pH < 5) vehicle such as yogurt or apple or orange juice.

After 3 months of treatment, endoscopy was repeated. Healing was defined as macroscopically normal esophageal mucosa (grade 0–1 on the Hetzel and Dent scale). Any patients who had not healed were withdrawn from the study and were treated according to current practice (15). Healed patients were eligible to enter the maintenance part of the study.

Children in remission were assigned, in a randomized, blinded manner by means of a computer-generated list, to one of three groups of 6-month maintenance treatment: group A (omeprazole at half the starting dose, once daily in the morn-

ing before breakfast), group B (ranitidine 10 mg/kg/day, divided in two doses), and group C (no treatment). The clinician was blinded to the patient's grade of esophagitis and histological and symptomatic scores at enrollment, as well as to the kind of drugs administered during the maintenance regimen. Another researcher administered the maintenance treatment before the evaluation by both the clinician and the endoscopist.

Informed consent for participation in this study was obtained from parents of all patients, and the experimental design was approved by the Independent Ethics Committee of University of Naples, Federico II.

The following procedures were performed at: T0, enrollment; T1, assessment for remission at 3 months after enrollment (healing phase); and T2, assessment for effective maintenance at 12 months after T0 (3 months after the completion of the maintenance phase):

1. A full medical history together with a physical examination were obtained.
2. At each clinic visit, children's esophageal symptoms (heartburn, epigastric pain, vomiting and regurgitation, irritability with meals, dysphagia and/or odynophagia, respiratory symptoms, and hematemesis) over the preceding 7 days were recorded as a response to a menu. Caregivers were also asked to keep a diary of symptoms. The severity of symptoms was classified as follows: grade 0, no symptoms; grade 1, mild symptoms with spontaneous remission and no interference with normal activity or sleep; grade 2, moderate symptoms with spontaneous but slow remission and mild interference with normal activity or sleep; grade 3, severe symptoms without spontaneous remission and marked interference with normal activity or sleep.

The frequency of symptoms was classified as follows: grade 0, absent; grade 1, occasional (symptoms present less than 2 days a week); grade 2, frequent (symptoms present 2–4 days a week); and grade 3, very frequent (symptoms present more than 4 days a week). Hematemesis was scored as grade 0, absence; grade 1, 1 or 2 episodes with minimal blood present; grade 2, recurrent minor episodes on a weekly basis; grade 3, a single major episode of bleeding or recurrent episodes daily or several times weekly (7). A score for each symptom and a total symptom score were calculated (16). The score for each symptom was calculated by multiplying the severity grade by the frequency grade, with a possible range for each score of 0–9. The total symptom score (range 0–57) was calculated by adding up the scores for each symptom.

During maintenance follow-up, children's caregivers were instructed to contact the investigator at any time if moderate or severe general symptoms were noted for more than 7 days. In such a case, an unscheduled endoscopy was performed and the patient was withdrawn from the study, regardless of the endoscopic results.

Table 1. Macroscopic Appearance of Esophageal Mucosa Scored by the Hetzel and Dent Classification (13)

Grade 0:	Normal esophageal mucosa, no abnormalities noted
Grade 1:	No macroscopic erosions visible but erythema, hyperemia, and/or friability may be present
Grade 2:	Superficial erosion(s) or ulcer(s) involving <10% of the mucosa of the distal 5 cm of esophageal squamous mucosa
Grade 3:	Superficial erosion(s) or ulcer(s) involving 10–50% of the mucosal surface of the distal 5 cm of esophageal squamous mucosa
Grade 4:	Deep ulcers anywhere in the esophagus or confluent erosion or ulceration of >50% of the mucosal surface of the last 5 cm of esophageal squamous mucosa

Table 2. Scoring System for Histological Features of Reflux Esophagitis (17)

Grade 0:	None
Grade 1:	Basal zone hyperplasia (>20% of the epithelial thickness) and elongation of papillae (if their height is >50% of the total epithelial thickness)
Grade 2:	Grade 1 + ingrowth of vessels in the papillae
Grade 3:	Grade 2 + 1–19 eosinophils and/or neutrophils on the most involved high-power field
Grade 4:	Grade 3 + >20 eosinophils and/or neutrophils on the most involved high-power field
Grade 5:	Mucosal erosions and/or ulcerations

- At least three esophageal biopsy specimens were taken by grasp forceps from each patient, avoiding the distal 2 cm of the esophageal mucosa. Samples were taken from both the endoscopically normal and abnormal areas, excluding frank erosions or ulcerations. Endoscopies were scored by the same endoscopist for each patient at T0, T1, and T2. The endoscopist was blinded to the time point of the study, to the patient's maintenance regimen, and to symptomatic, histological, and endoscopic scores regarding the previous controls. Additional antral biopsy specimens were taken for routine histology and *Helicobacter pylori* identification. Endoscopic and histological scores were obtained as shown in Table 1 and Table 2, respectively (13, 17). Relapse was defined as the recurrence of macroscopic esophageal lesions.
- Serum samples were taken for analysis of routine hematology and biochemistry with additional measurement of vitamin B-12, folic acid, and fasting gastrin serum concentration.
- Children's caregivers were also contacted by telephone every 6 months for a period of 30 months after the final maintenance follow-up (T2), in order to obtain information about the patient's health status and symptoms. If

moderate or severe general symptoms were noted for more than 7 days, an unscheduled endoscopy was performed to determine if there had been a recurrence of erosive reflux esophagitis. In addition, children's caregivers were instructed to contact the investigator at any time if moderate or severe general symptoms were noted for more than 7 days, between the scheduled telephone contacts.

Statistical Analysis

For categorical variables, the χ^2 test with or without the exact correction was applied as appropriate. For continuous variables, the Friedman test was used for the repeated measures, while the Kruskal-Wallis H test was used to compare the three groups of cases. The Dunn test was used for the *post hoc* analysis. Continuous data are presented as median and min–max values (range). Two-sided *P* values of less than 0.05 were considered to indicate statistical significance. Statistical tests were performed with SPSS software, version 14.0.2 (SPSS, Chicago, IL).

RESULTS

In the healing phase of the study, 48 patients were enrolled (M/F 26/22, median age 105 months, range 32–170). Endoscopic healing was obtained after 3 months of treatment with omeprazole 1.4 mg/kg/day in 46 patients (94%). Two patients were not healed and were excluded from entry into the maintenance part of the study.

All 46 healed patients entered the maintenance study. Sixteen were allocated to the treatment with omeprazole at half the starting dose (group A), 16 to ranitidine at the dose of 10 mg/kg/day (group B), and 14 did not receive any treatment (group C). Patient characteristics at enrollment, according to the maintenance treatment groups, are shown in Table 3. No differences regarding these demographic and clinical characteristics were found among the three maintenance therapy groups (A, B, and C).

Table 3. Patient Characteristics at Enrollment, According to Maintenance Treatment Groups

Characteristics	Group A (Omeprazole) N = 16	Group B (Ranitidine) N = 16	Group C (No Treatment) N = 14	<i>P</i> Value
Male sex, N (%)	9 (56)	8 (50)	8 (57)	0.910*
Age, months, median (range)	86 (32–148)	98 (36–166)	105 (38–166)	0.700†
Endoscopic grade, N (%)				
Grade 2	11 (69)	10 (62.5)	10 (71.4)	0.864*
Grade 3	5 (31)	6 (37.5)	4 (28.6)	
Histological grade, N (%)				
Grade 1	6 (37)	5 (31)	3 (21)	0.736‡
Grade 2	7 (44)	6 (38)	5 (36)	
Grade 3	3 (19)	5 (31)	6 (43)	
Symptomatic score, median (range)§	12 (6–30)	15 (2–38)	8 (1–28)	0.305†
Symptom onset (age, months), median (range)	13 (2–96)	21 (1–104)	22 (1–120)	0.813‡

* χ^2 test.

†Kruskal-Wallis H test.

‡ χ^2 test with exact correction.

§A total symptomatic score of 1 and 2 at enrollment was reported in two patients presenting with one episode of hematemesis with minimal blood present and two recurrent minor episodes on a weekly basis, respectively.

Table 4. Distribution of the Endoscopic and Histological Scores, and Frequency of Reported Symptoms at Enrollment (T0), at the End of the Healing Phase (T1), and at 3 Months After the End of the Maintenance Therapy (T2), in the 46 Patients Studied

Characteristics	T0	T1	T2
Endoscopic score, N (%)			
Grade 0	0 (0)	27 (58.7)	37 (80.4)
Grade 1	0 (0)	19 (41.3)	8 (17.4)
Grade 2	31 (67.4)	0 (0)	1 (2.2)
Grade 3	15 (32.6)	0 (0)	0 (0)
Histological score, N (%)			
Grade 0	0 (0)	32 (69.6)	40 (87)
Grade 1	14 (30.4)	14 (30.4)	5 (10.9)
Grade 2	18 (39.1)	0 (0)	1 (2.2)
Grade 3	14 (30.4)	0 (0)	0 (0)
Endoscopic score, median (range)	2 (2–3)	0 (0–1)	0 (0–2)
Histological score, median (range)	2 (1–3)	0 (0–1)	0 (0–2)
Patients who experienced symptoms, N (%)	46 (100)	31 (67.3)	12 (26)
Frequency for each reported symptom, N (%)			
Irritability	20 (43.5)	20 (41.7)	6 (13)
Vomiting	40 (87)	24 (52.2)	8 (17.3)
Heartburn	19 (41.3)	9 (19.5)	1 (2.2)
Epigastric pain	35 (70.1)	17 (37)	4 (8.6)
Dysphagia	13 (28.3)	1 (2.2)	0 (0)
Hematemesis	2 (4.3)	0 (0)	0 (0)
Respiratory symptoms	5 (10.9)	3 (6.5)	3 (6.5)

Table 4 shows the distribution of the endoscopic and histological scores and the frequency of each reported symptom at enrollment (T0), at the end of the healing phase (T1), and 3 months after the end of the maintenance phase (T2), respectively. Moreover, the median (range) symptomatic score at enrollment (T0) was 12 (1–38), with values of 1.5 (0–11) at T1 and of 0 (0–8) at T2 (Fig. 1). The endoscopic, histological, and symptomatic scores significantly decreased from baseline (T0) to T1 and T2 ($P < 0.0001$ vs baseline, each). A significant reduction was also observed for these three scores between T1 and T2 (histological $P = 0.02$, endoscopic $P = 0.01$, and symptomatic $P = 0.004$).

By 3 months after the end of the maintenance therapy, the erosive reflux esophagitis relapsed in one (2.2%) patient only. This patient showed the following characteristics: age, 138 months; presenting symptoms, heartburn and dysphagia (score 12); completing symptoms, heartburn and epigastric pain (score 8); histological grade = 2 and endoscopic grade = 3 (at enrollment); maintenance arm of allocation: no treatment. The patient was treated with a second cycle of PPIs and was withdrawn from the further part of the study.

No statistically significant difference was found among group A, group B, and group C regarding the symptomatic, endoscopic, and histological scores at the end of the healing phase and 3 months after the end of the maintenance therapy.

All categories of reflux symptoms were improved or resolved at T2, 3 months after maintenance discontinuation. At

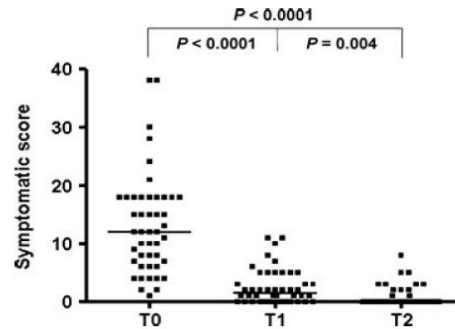


Figure 1. Scatterplot showing patients' symptomatic scores evaluated at enrollment (T0), at the end of the healing phase (T1), and 3 months after the end of maintenance therapy (T2). The thick line represents the median value.

this time point, 12 (25%) patients reported symptoms sufficiently mild to discontinue GERD therapy, excluding the only patient with relapse of macroscopic esophagitis who was treated for a further 3 months with omeprazole at the standard dose. The demographic and clinical characteristics of these 12 patients are reported in Table 5.

All patients without macroscopic esophagitis relapse at T2 were followed up for GERD symptoms every 6 months for 30 months, as scheduled. One patient did not agree to continue the study and was withdrawn. Long term, 3 of 44 (6.8%) patients showed very mild GERD symptoms: a male patient aged 38 months showed, at the 18th month of follow-up, three nonconsecutive episodes of irritability at meals and vomiting; a female patient aged 148 months showed, at the 25th month, one episode of heartburn and epigastric pain; and a male patient aged 115 months showed, at the 17th month, vomiting and epigastric pain lasting 3 days. None of them needed either endoscopy or acid-suppressant therapy; they received symptomatic treatment on demand, such as aluminum hydroxide or sodium alginate, with complete relief of their symptoms.

Significant variation in gastrin levels was observed during the study period ($P < 0.0001$). In particular, at T0, the median value of serum gastrin concentration in subjects entering the healing phase was 32 pmol/L (range 17–98) and this increased to 70 pmol/L (range 17–320, $P < 0.0001$ vs baseline) after the 3-month healing phase. Gastrin levels decreased significantly at T2, 3 months after the end of the maintenance treatment (median 38 pmol/L, range 8–211, $P < 0.0001$). Finally, there was no significant difference in gastrin concentrations among group A, group B, and group C at T2. No significant changes occurred in the other laboratory values investigated.

DISCUSSION

Studies in adults and children have reported that treatment with PPIs results in higher and faster rates of erosive

Table 5. Characteristics and Variation in the Scores at Enrollment (T0), at the End of the Healing Phase (T1), and at 3 Months After the End of the Maintenance Therapy (T2), in the 12 Patients Complaining of Mild Symptoms After Maintenance Discontinuation

Characteristics			
Male sex, N (%)	6 (50)		
Age, months (range)	124.5 (47–166)		
Symptom onset, age, months (range)	21.5 (2–120)		
Maintenance treatment, N (%)			
No therapy	6 (50)		
Ranitidine	1 (8.3)		
Omeprazole	5 (41.7)		
	T0	T1	T2
Histological score, N (%)			
Grade 0	0 (0)	5 (41.7)	11 (91.7)
Grade 1	3 (25)	7 (58.3)	0 (0)
Grade 2	6 (50)	0 (0)	1 (8.3)
Grade 3	3 (25)	0 (0)	0 (0)
Endoscopic score, N (%)			
Grade 0	0 (0)	6 (50)	10 (83.3)
Grade 1	0 (0)	6 (50)	1 (8.3)
Grade 2	10 (83.3)	0 (0)	1 (8.3)
Grade 3	2 (16.7)	0 (0)	0 (0)
Symptomatic score, median (range)	8 (4–28)	1.5 (0–8)	3 (1–8)
Patients who experienced symptoms, N (%)	12 (100)	8 (66.6)	12 (100)
Frequency for each reported symptom, N (%)			
Irritability	3 (25)	6 (50)	6 (50)
Vomiting	7 (58.3)	6 (50)	8 (66.6)
Heartburn	8 (66.6)	4 (33.3)	1 (8.3)
Epigastric pain	10 (83.3)	4 (33.3)	4 (33.3)
Dysphagia	4 (33.3)	1 (8.3)	0 (0)
Hematemesis	0 (0)	0 (0)	0 (0)
Respiratory symptoms	5 (41.6)	3 (25)	3 (25)

esophagitis healing compared with histamine-2 receptor antagonists (H₂RAs) (18–20). For this reason, these acid suppressants are considered more cost-effective than H₂RAs, and for children with severe and/or chronic GERD, the management equation becomes one of PPI therapy *versus* antireflux surgery.

Our results confirm that omeprazole is effective and safe for the healing of esophagitis and relief of symptoms in children. The 94% healing rate after the first 3 months of therapy with omeprazole was within the 40–100% range found in the pediatric literature for PPIs (5–9). However, in our patients, there was not a high rate of erosive esophagitis relapse and symptom recurrence within a follow-up period of 42 months.

Treem *et al.* (21), retrospectively reviewed the charts of 32 children (age range 3.5–16 yr) with GERD and found that 41% had improved symptoms and discontinued medication, while 47% still required medication at a follow-up 1–8 yr (mean 3.4) later. About 70% of 16 patients with esophagitis had improved symptoms, while five had persisting esophagitis at a 6-month follow-up despite treatment, and two of these needed fundoplication because of persisting GERD symp-

toms and esophagitis after 26 and 30 months of treatment. These authors mainly evaluated symptomatic long-term improvement, while the group of investigated patients affected by esophagitis was very small, and the number of patients with persisting esophagitis after long-term treatment was hardly significant. In contrast, our study group was composed only of children with erosive esophagitis and our primary aim was the healing of macroscopic lesions while symptomatic remission was a secondary concern.

Nevertheless, both our and Treem's results lead to the same consideration: if *there is not* a high rate of relapse of esophagitis and GERD symptoms after healing (as reported in our study) or if *there is* a high rate of relapse in spite of continuous use of medications (as reported by Treem *et al.*), the risk/cost of overtreatment with maintenance pharmacotherapy is probably greater than the risk/cost of undetected and unprevented relapse. In fact, we should consider that as much as 5 billion dollars may be spent annually for antireflux drugs (22).

Our data showed that at the end of the maintenance period, macroscopic esophagitis relapsed in only one patient, and that only 12 patients remained mildly symptomatic, not requiring further treatment (except for the one with esophagitis relapse), and after a further 30-month follow-up period, only three patients presented with transient mild symptoms probably related to GERD. Treem *et al.* (21) reported that, despite continuous therapy, medication could not be discontinued at a follow-up of 1–8 yr. From the above reported results, of both studies, the use of long-lasting preventive therapy is not advisable due to the fact that the risks may override the benefits. Careful clinical monitoring with treatment on demand would be a better choice.

In our study, only patients with endoscopically confirmed healing could enter the maintenance phase. Other studies have reported the value of short- and long-term PPI treatment in children with esophagitis refractory to medical treatment and GERD manifestations following fundoplication (10–12), thereby selecting patients who are more likely to relapse and therefore require more intense acid-suppressive treatment.

The long-lasting relief of mucosal lesions and the dramatic improvement in symptoms in our patients may be due to the use of the optimal dosage of PPIs during the healing phase and the optimal administration mode together with good compliance (7, 14, 15, 23). Clinical evidence indicates that the most common errors in prescribing PPIs in children are underdosing and splitting the total daily dose into twice-daily dosing. In our patients, omeprazole was administered at a dose of 1.4 mg/kg; it was given in the morning, 15–30 min before the first meal of the day, as a single daily dose. PPI pharmacokinetic studies show that, on a body weight basis, the dosages of omeprazole and lansoprazole used in the treatment of children with GERD are higher than those used in adults (14). This is because, in the first years of life, children exhibit metabolic rates that are several-fold higher than those observed in adults. A starting dose of approximately 1.4 mg/kg per day healed almost 75% of the children with

severe esophagitis and resolved or dramatically improved GERD-related symptoms (7). A single daily dose just before the first meal of the day is advisable because that is when acid pumps are generated. The conflicting conclusion by Treem *et al.* that GERD in older children is likely to be a chronic problem, requiring continuous monitoring and vigorous medical and surgical treatment, may be due to the fact that to induce the healing of GERD they used prokinetic agents (metoclopramide, bethanechol) and H₂RAs (cimetidine, ranitidine, or famotidine). Actually, it is widely recognized that: (a) there is insufficient evidence of the efficacy of prokinetic agents in the treatment of GERD, and the addition of metoclopramide to ranitidine has a better pharmacological efficacy; (b) there is evidence that treatment with PPIs results in higher and faster rates of erosive esophagitis healing compared with H₂RAs.

Some studies reporting the use of acid suppressants in children have described parietal cell hyperplasia and benign gastric polyps (5, 7, 24, 25); however, no gastric mucosal alterations were found in our study population. Gastrin levels were noted to rise and then return to normal despite continued antisecretory therapy in our study, as previously reported (5).

The safety of long-term antisecretory therapy has been shown in adults for up to 11 yr of continuous use (4) and in children for shorter periods (6, 7, 10); nevertheless, recent studies have reported that a greater risk of community-acquired pneumonia and acute gastroenteritis could be associated with the long-term use of H₂RAs and PPIs (26, 27). The authors speculate that the reduction in gastric secretion may facilitate oral acquisition of infections. Children have also recently been reported among the high-risk categories for infection, such as patients with asthma or chronic lung diseases and immunocompromised and elderly persons. However, these results are of great concern, suggesting that great caution must be used in the prescription of acid blockers, in particular for long-term therapy.

Our study suggests that, when used in adequate doses, omeprazole is highly efficacious for the healing of esophagitis and treatment of GERD symptoms in short-term use; this means that, as confirmed by our data, a considerable number of children affected by erosive reflux esophagitis likely will not relapse and so will not need maintenance treatment.

Despite the absence of a placebo group, the utility of the "no treatment" maintenance arm in our study was even more effective, if we consider that macroscopic esophagitis relapsed in only 1 of 46 patients (2.2%), and that at long-term follow-up, very mild GERD symptoms occurred in only 6.8% of our patients. On the other hand, studies in adults show high rates of relapse after short-term acid-suppressive therapy and even after antireflux surgery (28, 29), and for this reason, medical long-term treatment represents the therapy of choice. Efficacy results are comparable with those of long-term surgical results from only the best surgeons, while the morbidity and mortality rates and the costs are certainly better (30). The fact that our study population showed a low rate of endoscopic and symptomatic relapse, even without main-

tenance treatment, suggests that different pathophysiologic pathways are probably involved in the mechanisms of GERD in children.

The proven efficacy and safety of PPIs have dramatically changed the therapeutic landscape for children, becoming a possible alternative to surgery, in particular for those groups for which the failure rate, morbidity, and mortality of fundoplication are high, such as neurologically impaired children or patients with repaired esophageal atresia or chronic lung disease. These patients could represent the pediatric subset of GERD patients requiring long-term treatment.

Because a very limited amount of long-term outcome information is available in the pediatric literature, a full understanding of the prevalence of the problem, the rates of chronic *versus* limited-duration manifestations, and the likelihood of relevant morbidity related to GERD over time in pediatric subsets are not present. This information would be of great importance for the choice of the best antireflux approach while keeping in mind that the best therapeutic approach should be a safe one for children with GERD.

STUDY HIGHLIGHTS

What Is Current Knowledge

- As in adults, in children beyond 1 yr of age, gastroesophageal reflux disease (GERD) is a chronic relapsing condition.
- The available literature supports the value of sustained proton pump inhibitor (PPI) treatment (at least 6 months) in preventing symptomatic and endoscopic GERD relapse and treating adults and children refractory to other interventions.

What Is New Here

- In our pediatric study population, there was not a high rate of erosive esophagitis relapse and symptomatic recurrence within a follow-up of 42 months.
- The long-lasting relief of mucosal lesions and the dramatic improvement in symptoms in our patients may be due to the use of the optimal dosage of omeprazole and the optimal administration mode together with good compliance during the short-term healing phase, reducing the probability of GERD relapsing and the need for maintenance treatment.
- The low rate of relapse, even without maintenance treatment, suggests that different pathophysiologic pathways are probably involved in the mechanisms of GERD in children.

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Received May 19, 2006; accepted October 31, 2006.

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CONFLICT OF INTEREST

The authors have indicated no potential conflict of interest.

Functional gastrointestinal disorders in migrainous children: efficacy of flunarizine

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Cephalalgia

Boccia G, Del Giudice E, Crisanti AF, Strisciuglio C, Romano A & Staiano A. Functional gastrointestinal disorders in migrainous children: efficacy of flunarizine. *Cephalalgia* 2006; 26:1214–1219. London. ISSN 0333-1024

The aim of this study was to evaluate the prevalence of functional gastrointestinal disorders (FGIDs) in children with migraine headache and the effects of flunarizine on gastrointestinal manifestations. We studied 50 migrainous children (mean age 8.63 years). The clinical pattern and the diagnosis of FGIDs were obtained from structured questionnaires. All subjects underwent measurement of total gastric emptying time (TGET) performed by real-time ultrasonography of the gastric antrum at baseline (T0). In the second part of the study, we evaluated 10 migrainous children (mean age 9.8 years) with associated FGIDs. In these 10 patients, repeated TGET evaluation together with a detailed symptom history was obtained after 1 (T1) and 2 months (T2) of treatment with flunarizine. Control groups were composed of 10 migrainous children without FGIDs (mean age 9.2 years) and nine sex- and age-matched healthy children. Gastrointestinal disorders were present in 70% of the patients. Migrainous children with FGIDs had significantly ($P < 0.01$) more prolonged TGET than subjects without FGIDs. Prior to therapy, all migrainous children with FGIDs had prolongation of TGET compared with controls ($P < 0.05$). Patients on flunarizine had a significant decrease in TGET at both 1 ($P < 0.01$) and 2 months ($P = 0.002$) of therapy. The mean frequency of abdominal pain per month was significantly ($P < 0.001$) reduced at T1 compared with T0. The mean frequency of vomiting per month was significantly decreased at T1 ($P < 0.05$) and even more so at T2 ($P < 0.01$). Finally, the mean frequency of headache per month was significantly reduced only at T2 ($P < 0.05$), whereas the mean duration of headache was significantly decreased at T1 ($P < 0.01$) with no difference between T1 and T2. Most children with migraine report FGIDs, associated with a delayed gastric emptying. Flunarizine decreases the frequency and duration of migrainous episodes as well as the gastrointestinal symptoms. □ *Antimigraine prophylaxis, calcium antagonists, functional gastrointestinal disorders, migraine headache*

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Introduction

Migraine is the most important cause of headache both in children and adults (1). The International Headache Society (IHS) diagnostic criteria have been recently revised (2). Before puberty migraine prevalence is about 4% (3) and increases with age more rapidly in girls than in boys. The quality of life of

children with headache is significantly affected by their health condition, the impact of headaches being similar to that of other chronic illnesses (4).

It has long been recognized that migraine headaches are frequently associated with gastrointestinal symptoms. Among the functional gastrointestinal disorders (FGIDs), cyclic vomiting syndrome (CVS) and abdominal migraine are usually classified as

migraine variants or equivalents (5). This relationship is supported by several observations: there is a high prevalence of migraine headache and abdominal pain in paediatric CVS patients, with a higher prevalence of migraine among first-degree relatives of children affected by CVS; migraine headache and CVS share a similar temporal profile of disorder onset and resolution of symptoms; both syndromes share many associated gastrointestinal, sensory and vasomotor symptoms (6).

There is also evidence that the baseline sympathetic tone in children with CVS differs from that of control subjects, in a pattern similar to that found in migraine patients. Rashed et al. (7) have demonstrated that both children with CVS and adults with migraine headache manifest elevated sympathetic tone responses to postural changes. Migraine-type neural and vascular changes have been documented in CVS children, as well as a predominance of sympathetic adrenergic over parasympathetic cholinergic tone (8). Good et al. (9) have reported similar visually evoked responses in children affected with CVS and with migraine.

Therapy for CVS and abdominal migraine focuses on preventing attacks with prophylactic medication. Several studies have shown improvement using propranolol, cyproheptadine and amitriptyline, usually resulting in a $\geq 50\%$ decrease in the number and/or severity of episodes in CVS patients (10, 11). Pfau et al. have reported that 75% of children with CVS responded to antimigraine medication, as opposed to control children who suffered from chronic vomiting (12). Furthermore, another study has shown that CVS patients who also suffered from migraine improved twice as often (79% vs. 36%) as non-migrainous CVS patients when treated with antimigraine therapy (13). The shared therapeutic response of CVS and migraine implies that closely related neurally mediated pathways are responsible for some or all of the symptomatology of these two disorders.

Flunarizine is a calcium channel blocker which has been shown to reduce attack frequency in migraine headaches (14). It has been used for paediatric migraine prophylaxis and more than one randomized, placebo-controlled, double-blind study has demonstrated its effectiveness (15, 16).

Our study was designed to determine the prevalence of FGIDs in children with migraine headaches, to define clinical characteristics of migrainous children with and without associated FGIDs, and to evaluate the effect of flunarizine on gastrointestinal symptoms and gastric emptying time in migrainous children.

Methods

The first part of the study involved the evaluation of 50 consecutive patients referred to our clinic for migraine (mean age 8.63 ± 2.8 years, 21 males). Migraine was defined according to the ICHD-II criteria (2).

Questions were posed concerning migraine onset, family history, total number of episodes, frequency and duration of attacks, intervals between episodes, prodromal symptoms, precipitating and alleviating factors and associated symptoms.

For each consecutive migrainous patient, a structured questionnaire was completed which recorded symptoms and signs needed to satisfy the paediatric Rome II criteria for the following disorders: functional dyspepsia, irritable bowel syndrome, functional abdominal pain, abdominal migraine and CVS (17, 18). Furthermore, functional vomiting was diagnosed according to the adult FGID criteria (17), with the aim to include all subjects complaining of a history of recurrent but not cyclic vomiting, not explained by abnormalities of the gut or central nervous system, metabolic or biochemical disease.

Measurement of gastric emptying time was performed by real time ultrasonography (US) of the gastric antrum after ingestion of a mixed solid-liquid meal (19). All subjects were examined using a 5-MHz linear probe applied to the epigastrium, with minimal abdominal compression. Baseline scans were performed on an empty stomach and follow-up measurements were performed at 30 and 60 min, then at 15-min intervals until emptying was complete. The gastric emptying time was calculated by measuring the cross section of the gastric antrum at the sagittal plane passing through the superior mesenteric vein. The antral cross-sectional area, elliptical in shape, was calculated by the following formula: $\text{Area} = \pi \times \text{longitudinal diameter} \times \text{antero-posterior (AP) diameter} / 4$ (20) and the stomach was considered empty when the cross-sectional area returned to baseline and persisted unchanged for at least 30 min. Total gastric emptying time (TGET) was calculated in relation to the start of the meal.

In the second part of the study, we evaluated 10 migrainous children with associated FGID diagnoses (mean age 9.8 ± 1.9 years, seven males). Subjects were selected from our migrainous children who had indications of preventive treatment according to the American Academy of Neurology (AAN) Guidelines (21, 22). These children with migraine received flunarizine 5 mg, as a single daily dose, orally for a period of 2 months. A detailed symptom history was recorded and a general physical and

neurological examination performed at baseline. During the study, children's caregivers kept a weekly symptoms diary and recorded: number and duration of migraine attacks, frequency of gastrointestinal symptoms and presence of side-effects. All patients were evaluated at 1 and 2 months and the symptoms diary was checked and collected. As controls we chose a group of 10 migrainous children without FGIDs (mean age 9.2 ± 1.8 years, six males) and nine healthy children (mean age 8.6 ± 1.7 years, five males). All children underwent US measurements of gastric emptying time at baseline, 1 and 2 months. Controls were not in treatment with anti-migraine prophylaxis.

Informed consent to participate in the study was obtained from parents of all patients and the experimental design was approved by our institutional review board.

Statistical analysis

Continuous variables are shown as mean \pm SD. The unpaired *t*-test and one-way ANOVA with the Bonferroni test for multiple comparisons were used, as appropriate. Repeated measures ANOVA was applied for the analysis of data at different times of observation (T0, T1 and T2) with the Bonferroni test for the post hoc test. Categorical variables were analysed using the χ^2 test.

All analyses were performed with SPSS software, ver. 13.1 (SPSS Inc., Chicago, IL, USA).

Results

FGIDs were present in 35 (70%) of the 50 migraine patients; specifically, 35% reported functional

abdominal pain and 35% reported functional vomiting. None of the patients responded to the diagnostic criteria for functional dyspepsia, irritable bowel syndrome, abdominal migraine or CVS. Clinical criteria for definition of functional vomiting and functional abdominal pain are summarized in Table 1. A family history of migraine was reported in 84% of the study population. At baseline, the mean \pm SD duration and frequency (number of episodes/month) of migraine attacks were 8.7 ± 10.6 h and 5.4 ± 1.41 , respectively.

TGEt was significantly prolonged ($P < 0.01$) in children with migraine and associated FGIDs when compared with children with migraine alone (mean \pm SD: 183.57 ± 35.12 vs. 122 ± 37.01 , $P < 0.05$). The presence of specific triggers was not significantly different between the two groups, nor was the alleviating factor of sleep.

Gastric emptying times of migrainous children with FGIDs treated with flunarizine and of controls are summarized in Fig. 1. Prior to therapy, migrainous children with associated FGIDs had a prolonged TGEt compared with both healthy children and children affected by migraine without FGIDs ($P < 0.05$). When these patients were treated with flunarizine, they demonstrated significant decreases in TGEt at both the 1-month ($P < 0.01$) and 2-month ($P = 0.002$) evaluations. There were no significant differences in gastric emptying times between treated patients and controls at 1 or 2 months.

All patients treated with flunarizine showed a decrease in frequency and duration of migraine attacks (Table 2). A summary of gastrointestinal and headache symptomatology in treated patients is shown in Table 2.

During the study period, subjects treated with flunarizine did not suffer any relevant side-effects.

Table 1 Clinical criteria for definition of functional abdominal pain and functional vomiting

Functional abdominal pain

At least 12 weeks of:

- 1 Continuous or nearly continuous abdominal pain in a school-aged child or adolescent; and
- 2 No or only occasional relation of pain to physiological events (e.g. eating, menses or defecation); and
- 3 Some loss of daily functioning; and
- 4 The pain is not feigned (e.g. malingering); and
- 5 The patient has insufficient criteria for other functional gastrointestinal disorders that would explain the abdominal pain.

Functional vomiting

At least 12 weeks, which need not to be consecutive, in the preceding 12 months of:

- 1 Frequent episodes of vomiting, occurring on at least three separate days in a week; and
- 2 Absence of criteria for an eating disorder, rumination, or major psychiatric disease according to the DSM-IV; and
- 3 Absence of self-induced and medication-induced vomiting; and
- 4 Absence of abnormalities in the gut or central nervous system and metabolic diseases to explain the recurrent vomiting

Discussion

Published studies have shown the prevalence of migraine headache to be between 30 and 80% in children affected by CVS or abdominal migraine (5, 6, 23). However, there are few data on the prevalence of FGIDs in migrainous children. Our study shows a large prevalence of FGIDs of 70% in paediatric patients with a diagnosis of migraine. Among FGIDs, our migrainous children reported two disorders in particular: functional abdominal pain and functional vomiting. Although the latter corresponds to the adult Rome II criteria for FGID, we chose to consider it for the purpose of including all subjects complaining of a history of recurrent vomiting in the absence of organic abnormalities but not corresponding to the diagnostic criteria for CVS. In a recent study we reported that of 9960 patients,

newborn to 12 years old, 194 satisfied the Rome criteria for various FGIDs but only three (0.03%) were affected by CVS (18). Furthermore, previous studies have proposed criteria and questionnaires extensively used and validated in adults, to identify FGIDs such as functional dyspepsia and irritable bowel syndrome, in paediatric populations (24–26).

In our study, FGIDs associated with migraine appear to correlate with a prolonged gastric emptying time. The gastric emptying rate of solids in children is difficult to evaluate because the available methods are either invasive or induce a substantial radiation burden. A good correlation between scintigraphic and US parameters has been found using either liquid or solid meals (27). Hence, in our study the gastric emptying test was performed by means of a real-time US examination, a validated, non-invasive method for studying gastric motility (20, 28).

Gastrointestinal motor abnormalities are common in a variety of FGIDs (29–31). Altered myoelectrical activity of the stomach with a delayed gastric emptying time has been reported in children affected by CVS (32) and has been suggested to play a pathogenic role in functional dyspepsia (33). For this reason, on the basis of a possible link between migraine and FGIDs, we found it of interest to study this parameter in migrainous children. The finding of delayed gastric emptying in our children affected by migraine and FGIDs could be interpreted as an epiphenomenon, reflecting the overlap between inadequately defined functional syndromes, shared pathophysiology or the activation of physiological interaction at different levels of the brain and gut axis.

Much evidence points to a clinical and pathophysiological link between migraine syndromes and FGIDs, such as CVS, abdominal migraine and others. For example, a positive family history of migraine is present in 72% of paediatric patients with CVS compared with only 14% of children with chronic vomiting (13), while a positive family history is present in 65% of abdominal migraine patients

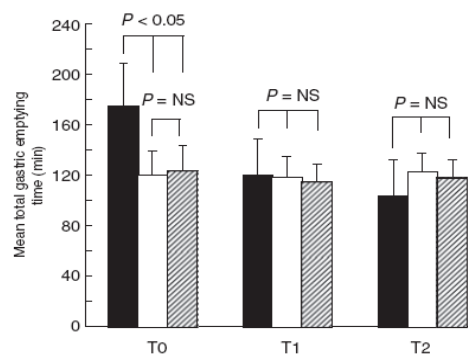


Figure 1 Total gastric emptying time measured at baseline (T0) and after 1 (T1) and 2 (T2) months of treatment with flunarizine. Migrainous children with functional gastrointestinal disorders (FGIDs): T0 vs. T1, $P < 0.01$; T0 vs. T2, $P = 0.002$; T1 vs. T2, $P = NS$. ■, Migrainous children with FGIDs; ▨, controls; hatched, migrainous children without FGIDs.

Table 2 Gastrointestinal symptoms after 1 and 2 months of treatment with flunarizine

	Baseline (mean \pm SD)	One month (mean \pm SD)	Two months (mean \pm SD)
Abdominal pain (no. episodes/month)	4.9 \pm 2.9	0.71 \pm 1.25*	0.71 \pm 1.49
Vomiting (no. episodes/month)	15.5 \pm 11.8	1.25 \pm 1.89†	0.8 \pm 1.78‡
Headache (no. episodes/month)	7.1 \pm 4.87	4 \pm 2.62	2.18 \pm 2.36†
Headache duration (h)	4.71 \pm 2.42	1.71 \pm 1.6*	1.5 \pm 1.87

* $P < 0.001$ compared with baseline; † $P < 0.05$ compared with baseline; ‡ $P < 0.01$ compared with baseline.

(23). In our study, almost all patients reported affected family members. Patients with isolated migraine as well as migraine patients with associated FGIDs also share exacerbating and alleviating factors.

In the second part of our study, we evaluated the effects of flunarizine on gastrointestinal symptoms and on gastric emptying time in children with migraine and associated FGIDs. The positive response to flunarizine lends support to the pathophysiological relationship between migraine and FGIDs.

Other antimigraine therapies are effective in CVS: Anderson et al. (11) have demonstrated that both amitriptyline and cyproheptadine are effective prophylactic treatments for CVS, with remission rates of 83–91%. In another study, migraine prophylaxis resulted in complete resolution of cyclic vomiting episodes in 75% of children (12). Abdominal migraine also responds well to migraine prophylactic medications, such as propranolol or cyproheptadine (34).

We chose to study the effects of flunarizine because it has been shown to be effective against migraine in more than one randomized, placebo-controlled, double-blind study and because it has smooth muscle-relaxing properties which may improve symptoms in FGIDs. Flunarizine is a calcium channel blocker which can modulate neurotransmission as well as vascular tone; it has minimal negative inotropic effects, and it also has antihistamine properties (H1). It was introduced into the antimigraine armamentarium because of its modulating effect on vascular tone, as well as its cytoprotective effect against cellular hypoxia (35).

Several pathophysiological pathways are potential candidates in the aetiology of both migraine and FGIDs, are intimately associated with brain–gut interactions and related to voltage-gated calcium channels (36). Recent lines of evidence favour a role for ion channel mutations in the pathogenesis of migraine. For example, four missense mutations of the α_1 subunit of the neuronal calcium channel CACNL1A4 have been described in patients affected by hemiplegic migraine and are presumed to cause this syndrome (37, 38). CVS has also been proposed to result from a similar channelopathy (39, 40).

Clinical improvement in our patients with migraine and FGIDs, when treated with flunarizine, may be due to one or more of several mechanisms of action: a calcium channel blocker in a yet undefined channelopathy, an antihistamine on the central afferent pathways, or perhaps peripherally by acting

directly to relax smooth muscle either in the vasculature or in the intestinal wall.

Whatever the mechanism, flunarizine treatment in our patients with migraine and FGIDs resulted in marked improvement in gastrointestinal as well as headache symptoms and was also quantifiable as a decrease in total gastric emptying times as measured by US. A larger, randomized, placebo-controlled trial of flunarizine in these syndromes should be the next step.

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CONCLUSIONS

WHAT THESE STUDIES ADD TO THE CURRENT KNOWLEDGE AND WHAT ARE THE RECOMMENDATIONS FOR FUTURE RESEARCH.

1) Ontogeny and normal physiology of the esophageal and gastrointestinal motility.

a) Development of Esophageal Peristalsis in Preterm and Term Neonates. (Staiano A, Boccia G et al. Gastroenterology 2007;132:1718–1725)

Our study represents the first application of high-resolution manometry to preterm and term neonates.

High-resolution manometry, is an innovative methods for the study of esophageal motility, wherein recording sites are increased in number and spaced closely in the axial direction, and interpolation of pressure data across sites is accomplished to visualize better the spacetime relationships using 3 - dimensional isobaric contour maps.

We demonstrates that the development in esophageal peristalsis occurs through late gestation into term. The 3 segments responsible for intact peristalsis in older children and adults are found in all infants and can be identified in neonates as young as 27 weeks gestational age at examination.

Differential development occurs, such that the second segment in the mid-esophagus (proximal smooth-muscle segment) is present in the majority of swallows in all preterm and term neonates while the other 2 segments at proximal and distal ends of the esophageal body lag behind, developing in tandem despite very different underlying control mechanisms.

These findings suggest a teleologic role for the second segment, possibly in enhancing clearance and preventing GERD and its complications.

The fact that only half of swallows show completely intact segmental architecture at term, however, indicates that development of esophageal peristalsis continues into infancy.

Future research should address the following areas:

- 1) The development of motor behaviour leading to the junction opening during a transient LES relaxation, (eg, crural diaphragm inhibition, esophageal shortening, and favourable pressure gradient between the stomach and esophagea lumen). This investigations could be very important for the understanding of the patophysiology of gastroesophageal reflux in neonates and children in order to provide better targets for more tailored therapeutic interventions.
- 2) Expanded investigation of the specific neuroendocrine pathways that control the three functional esophageal segments found using high resolution manometry
- 3) Focus on identification of drug targets for esophageal motility disorders that could act selectively on the specific functional segment involved.

2) Epidemiology and patophysiology of the main functional gastrointestinal disorders in children

a) Functional defecation disorders in children: Rome II criteria vs Rome III and PACCT criteria. (Boccia et al. J Pediatr 2007;151:394-8)

Our study was the first that evaluated the clinical applicability of PACCT and Rome III criteria for functional defecation disorders (FDDs) in children referred to a tertiary center for chronic constipation. The prevalence of FDDs was significantly higher when using the PACCT/Rome III criteria than when using the Rome II criteria.

Because in our study such a high percentage of constipated children reported the symptoms of defecation with straining, scybalous pebble-like stools, and painful defecation, we suggested that these symptoms be added in any revised criteria.

Future research should address the following areas:

- 1) The applicability and validity of Rome III diagnostic criteria for FC and other FGIDs in unselected populations and in both general practice and research settings.
- 2) The epidemiology, natural history and health care impact of pediatric FGIDs.

b)Dyspeptic symptoms in children: the result of a constipation- induced “cologastric brake”?
(Boccia et al. Clin Gastroenterol Hepatol,submitted with revisions, October 2007)

In this prospective study we reported that most children with functional dyspepsia (FD) also have functional constipation (FC) associated with delayed gastric emptying, and that normalization of bowel habits after laxative therapy can improve dyspeptic symptoms and gastric emptying. The enterogastric feedback activated by faecal stasis in the rectum should be considered among the possible mechanisms involved in the pathogenesis of dyspepsia.

The study is unique in pediatrics; the resolution of dyspeptic symptoms and gastric abnormalities after constipation treatment and bowel habit normalization suggests that, in a specific subset of patients, dyspepsia may be not an independent clinical entity but a clinical variant of functional constipation.

This implies that 1)dyspeptic children should be always investigated for functional constipation through appropriate validate questionnaires;2) A therapeutic trial with osmotic laxative might be advisable in dyspeptic children for the resolution to solve upper gastrointestinal symptoms.

Future research should address :

- 1)The relation between specific cluster of symptoms and different pathophysiological mechanisms, in order to provide better targets for more tailored therapeutic interventions.
- 2)More precise delineation of the relationships between sensorimotor dysfunction, individual symptoms, and individual FGIDs: conceivably, the clinical manifestations in FGID patients depend on the specific sensory and/or reflex pathways and territories affected. Improved symptom criteria, together with quantitative data relating to physiological dysfunction (eg, hypersensitivity, dysmotility, and reflex dysfunction), to mucosal inflammation/immune/endocrine activation and to autonomic dysfunction, and in the future to molecular risk factors, should enable better categorization of patient subgroups using techniques such as cluster analysis. More sophisticated techniques to assess compliance, wall tension, and accommodation and to assess more precisely the flow of luminal content and gas and the effects of dietary constituents on sensorimotor function are required. In this regard, the development of minimally or noninvasive techniques of investigation, which can function as true surrogate markers of sensorimotor dysfunction and which can be repeated in patients after various therapeutic maneuvers, is essential.

3) Diagnosis and treatment of the main pediatric gastrointestinal motility disorders.

a)Segmental characteristics of esophageal peristalsis in pediatric patients.Staiano A, Boccia G et al. (Neurogastroenterol Motil. 2007 Nov 21; [Epub ahead of print])

In this report, we successfully applied for the first time high resolution manometry to a group of subjects representing the broad age range seen in paediatrics (from neonates to children) to examine

the appearance of peristalsis using these techniques. The distinctive chain of pressure events that also characterizes oesophageal peristalsis in adults was present in all age groups with minimal variation in the relative location of defining landmarks along oesophageal length.

The identification of peristaltic segments has several important clinical implications. From a clinical standpoint the HRM appearance of peristalsis as a chain of three pressure segments with inter-segmental troughs, may facilitate recognition of normal and abnormal motor function. The distinctive peristaltic pattern also helps identify lower esophageal sphincter location. From an investigational standpoint, the segmental character to esophageal peristalsis should be taken into consideration in manometric

investigation of all age groups in particular in testing pharmacological responses and evaluating clearance mechanisms.

Future research should address:

1) The application of HRM for the study of other section of the gastrointestinal tract (such as the small intestine and the colon or rectoanal segments)

1) The investigation of the specific neuroendocrine pathways that control the three functional esophageal segments.

2) Focus on identification of drug targets for esophageal motility disorders that could act selectively on each of the three functional segment involved.

b) Chronic Cough and Gastroesophageal Reflux Disease in Children: which Test for Symptomatic Correlation? (Boccia et al. J Pediatr (submitted with revisions, September 2007))

c) Maintenance Therapy for Erosive Esophagitis in Children, After Healing by Omeprazole: Is It Advisable? (Boccia et al. Am J Gastroenterol 2007;102:1-7)

In this study children with erosive esophagitis grade 2 or 3 healed at three months with omeprazole 1.4 mg/kg/day were randomized to 3 groups for a six-month maintenance period: omeprazole in half the healing dose, or ranitidine, or placebo. In all 3 groups, very few relapsed symptomatically or endoscopically during the randomized treatment phase, or off treatment at longer-term follow-up. Three months after the completion of the maintenance phase, i.e., at 1 yr after entry into the study 12 (26%) patients had symptoms sufficiently mild to discontinue GERD therapy, and only 3 of 44 patients (6.8%) reported GERD symptoms (mild) within 30 months after discontinuation of the trial. Only a couple

patients reported a need for antacid use.

The strengths of our study lie in the study's prospective nature, its controlled and randomized character, and its relatively long duration of prospective follow-up.

The low rate of relapse, even without maintenance treatment, suggests that different pathophysiologic

pathways are probably involved in the mechanisms of GERD in children and that reflux in some children may be a transient condition. This finding has important implications for management of GERD in children..

The final message is to consider a less aggressive approach to pharmacotherapeutic maintenance of healed erosive esophagitis in children. This approach would have the merit of minimizing the potential risks of chronic acid-suppressive therapy that several studies have recently brought to light and it might also reduce the financial costs of GERD treatment, estimated at more than \$9 billion per year in the United States in 2000.

Future research should address:

- 1) The role, efficacy and safety of maintenance acid-suppressive therapy in those children who may be most apt to develop erosive esophagitis: those with chronic neurological disease, previous esophageal surgery, or chronic respiratory disease. These children have also been the most likely to develop complications of esophagitis (strictures, Barrett's esophagitis, and adenocarcinoma) and thus probably the children whom more likely will need a chronic PPI maintenance.
- 2) The existence of a "transient GERD" as a well defined entity and the possible pathophysiological mechanisms implicated.
- 3) The role of acid hypersecretion rebound in the symptomatic relapse of children with GERD, after acid-suppressive treatment.

d)Functional gastrointestinal disorders in migrainous children: efficacy of flunarizine. (Boccia et al.Cephalalgia 2006;26:1214-1219.)

In this study we reported that pediatric patients with a diagnosis of migraine show a large prevalence of FGIDs. In particular among FGIDs our patients reported functional abdominal pain and functional vomiting. These FGIDs associated with migraine appear to correlate with a prolonged gastric emptying time. Furthermore we found that flunarizine (a calcium channel blocker normally used for the migraine prophylaxis) decreased the frequency and duration of migrainous episodes as well as the gastrointestinal symptoms and gastric emptying time in children with migraine and associated FGIDs.

Future research should address:

- 1) Integration of CNS imaging technology and classic neurophysiologic and neuropharmacologic approaches for improved understanding of the neurobiology of the brain-gut axis.
2. Continued mechanistic focus on the basic science of visceral hypersensitivity and pain that includes the molecular basis for peripheral sensitization of sensory receptors by inflammatory mediators, selectivity of central pain-related transmission pathways, and higherorder central processing of nociceptive information from the viscera.
3. Expanded investigation of the neuroendocrine pathways, which connect the brain with the gut and are responsible for alteration of function during psychogenic stress.
4. Application of genomic chip technology in searches for genetic polymorphisms in receptors, enzymes, and steps in signal transduction cascades in elements of the ENS.
5. Focus on identification of drug targets on neural elements of the ENS and CNS and on non-neural cell types, such as mast cells and enterochromaffin cells,

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