Relationships among afferent neural processing, peristalsis and bolus clearance in the human oesophagus: implications for symptom perception and dysphagia

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Publication Date:
2008

DOI:
https://doi.org/10.26190/unsworks/19631

RELATIONSHIPS AMONG AFFERENT NEURAL PROCESSING, PERISTALSIS AND BOLUS CLEARANCE IN THE HUMAN OESOPHAGUS: IMPLICATIONS FOR SYMPTOM PERCEPTION AND DYSPHAGIA

by

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A Thesis submitted for the Degree of Doctor of Philosophy at the University of New South Wales

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November 2008

Certification of Originality

ACKNOWLEDGEMENTS

A number of individuals have helped me during the course of this work and deserve my sincere thanks.

First, I would like to thank my supervisor, Professor Ian Cook, for his support of my research in the department over the past 2 & 1/2 years and for providing me with an opportunity to undertake a doctorate. As my supervisor he has guided my progress and provided encouragement during the hard time to achieve valuable data in this work. I also thank Professor David deCarle for acting as my co-supervisor.

Dr Taher Omari (Adelaide Woman's and Children's Hospital) made major contribution to the design of the silicon impedance-manometry-balloon catheter, (Monster tube) which has been used for the bulk of the research studies in this thesis.

Michal Szczesniak has been a constant aid during the past two years and I thank him for his help. Sergio Fuentealba always helps me to enroll the subjects for the studies in this work, without his help it would have taken me longer to get there.

Dr Ian Cole has provided help and assistance on most of the patients enrolled in the globus study. I would also like to thank Kerrie Smyth and Julie Nelson for providing their help in contacting most of the patients for this thesis work.

I am grateful to my colleagues, Dr Jason (Chih-Hsun) Yi, Otto Chen, and Payjen Dong for their help in maintaining the research works in Taiwan during the past two years. I would also like to thank Allen Huang for his help in editing chapters in this thesis.

Tzu Chi General Hospital in Hualien has provided me with the scholarship during the last two years, without this support it would be impossible to undertake this thesis work. Dr William C Orr, my previous mentor, has provided his help whenever I am struggling with the research work.

Finally I would like to thank my wife Tiffany, while I have been writing this thesis over the last 5 months you have had the much harder task of looking after our two children, Kathryn, and William. You are amazing.

CONTENTS

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THESIS SUMMARY

The work in the body of this thesis investigated oesophageal motility and bolus clearance, as well as sensory perception of oesophaegal stimuli, in patients with several dysphagia syndromes. Central to the perception of dysphagia, is afferent processing which was evaluated in non-obstructive dysphagia syndromes and in patients with globus sensation. When compared with primary peristalsis, oesophageal secondary peristalsis has received little attention. There are published works which detail the manometric characteristics and reproducibility of initiation of secondary peristalsis in health and a variety of disease states. However, there is virtually no data on the efficiency of oesophageal bolus transport and clearance by secondary peristalsis. One novel aspect of the work in this thesis is that I have combined intraluminal impedance and manometry to study the triggering of and the effectiveness of oesophageal clearance by secondary peristalsis. This work presented in this thesis is divided into the following major sections: 1) Current advances in the application of impedance and its utility in distinguishing clearance characteristics between primary and secondary peristalsis; 2) The advances in our understanding of peristaltic motor characteristics, oesophageal bolus clearance and symptom perception in dysphagia syndromes; 3) Peristaltic dysfunction, impaired bolus clearance and symptom perception in gastro-oesophagal reflux disease (GORD) and in patients with globus; 4) TRPV1 expression in patients with GORD.

1. **Impedance and its utility in distinguishing differences in clearance characteristics between primary and secondary peristalsis**

Combined measurement of impedance and pressure in the oesophagus appears to be a useful technique to evaluate oesophageal bolus transit. From the patient's perspective, this technique is no more invasive than conventional manometry. This allows the investigator to obtain information on both oesophageal peristalsis and bolus transit during the same swallow (**Chapter 3*)***. Although impedance monitoring for oesophageal function testing has been validated, normal data regarding clearance characteristics by secondary peristalsis are sparse.

We examined, for the first time, the relationship between secondary peristalsis and bolus transport, in healthy volunteers using this combined technique. The novel finding is that secondary peristalsis appears to be somewhat less effective than primary peristalsis when it is ready for propelling a liquid bolus out of the oesophagus. Effective secondary peristaltic responses are important for ensuring more efficient oesophageal clearance by secondary peristalsis in healthy volunteers (**Chapter 7**).

2. **Peristaltic motor characteristics, bolus clearance and symptom perception in**

dysphagia syndromes

Using the impedance technique will permit researchers to measure both oesophageal peristalsis and bolus transit during the same swallow. The novelty of this part of the thesis work is the analysis of the relationship between dysphagia perception and motility of individual swallow, as assessed by both manometry and impedance, with both liquid and viscous swallows. Our work has shown that, although patients with non-obstructive dysphagia (NOD) have more oesophageal dysmotility than healthy controls, there is a poor correlation between dysphagia and oesophageal dysmotility in terms of poor contractility and impaired bolus transport (**Chapter 8**). The finding is important in the matter of the relationship of dysphagia, oesophageal contraction and oesophageal transit. The work poses the question whether oesophageal sensitivity impairment might play a role in such dysphagia complaint. Impairment due to oesophageal sensitivity has been described in patients presenting with non-cardiac chest pain (Barish, Castell et al. 1986) or association with dysphagia (Katz, Dalton et al. 1987). A recent study also showed this type of abnormality can be observed in patients complaining of isolated dysphagia without chest pain by application of balloon distension (Bohn, Bonaz et al. 2002).

Evidence exists suggesting that secondary peristalsis may be impaired in patients with NOD (Schoeman and Holloway 1994b). The hypothesis regarding the relationship between such changes and alterations in bolus transport, if any, has not been studied. In patients with NOD, we have demonstrated that triggering of secondary peristalsis is less efficient, and impedance measures also reveal impaired bolus clearance by both morphologically normal and aberrant secondary peristaltic sequences (ineffective and synchronous). From the evidence already shown herein (See **Chapter 9**), my data may suggest that abnormal secondary peristalsis with impaired bolus clearance may explain, in part, clinical presentation of dysphagia in a subset of patients with NOD.

As the pathogenesis of NOD might be multifactorial (see **Chapter 4**), hypersensitive patients may represent a different subgroup compared with normosensitive NOD patients with regard to the generation of symptoms and their treatment. In particular, restoring normal sensitivity could be an attractive target for pharmacological interventions in these selected patients. Such technique may help to select patients for future studies addressing the potentially differential clinical efficacy of visceral modulating agents in hypersensitive vs. normosensitive NOD patients.

3. Peristaltic dysfunction, impaired bolus clearance and symptom perception in GORD and globus

As discussed in **Chapter 11**, impedance can provide physiologically and clinically relevant information in reflux patients with potentially oesophageal dysmotility in whom traditional manometry would have provided less definite results. We found that whereas

manometry identified motility abnormalities in approximately one-fourth of GORD patients, impedance found that a majority of these, as well as some additional patients in whom manometry results appeared normal, had defective bolus clearance. The ultimate significance of this relatively high prevalence of defective clearance in the pathogenesis of dysphagia or GORD remains to be determined.

This work has shown that patients with erosive GORD were characterized by delayed oesophageal bolus clearance and increased oesophageal acid exposure, whereas their manometry was comparable to patients without erosive GORD (see **Chapter 12**). Our data do not definitely clarify the causal interrelationship among delayed bolus clearance, excessive acid reflux, and oesophagitis. However, because both groups of patients exhibited more motor dysfunction than normal controls, the findings would suggest that the noted differences in oesophageal bolus clearance may reflect a continuum of dysfunction consequent to increasing oesophageal mucosal damage which was paralleled by an increase in oesophageal acid exposure. These results suggest that ineffective motility alone is unlikely to be the major determinant of abnormal oesophageal acid exposure and could not be a prerequisite for the development of oesophagitis.

As discussed in **Chapter 13**, patients with globus demonstrated oesophageal hypersensitivity and aberrant referral of oesophageal nociceptive stimulation. The work confirmed the hypothesis that globus patients demonstrate hypersensitivity and aberrant viscerosomatic referral of mechanical and electrical stimuli of the oesophagus. The differential responses to stretch and electrical stimuli may indicate that globus sensation is more likely to be mediated by oesophageal mechanosensitive but not electro-sensitive afferent nerves. The exact clinical implication of our findings is still unclear and to be further investigated in a large number of patients with globus. The evidence of shifted viscerosomatic referral of oesophageal pain may implicate that the neuronal modulation of oesophageal hypersensitivity is likely to be extrinsic to the oesophagus and possibly at upper level such as spinal pathway. Therefore, therapy targeted on oesophageal visceral afferent traffic may be of potential benefit for the treatment of globus. The clinical effectiveness of this type of therapy needs to be further investigated in patients with globus.

4. TRPV1 expression in patients with GORD

TRPV1 has been previously recognized as the receptor for capsaicin, the pungent ingredient in red pepper fruits of the genus *Capsicum*. TRPV1 behaves as a multimodal nocisensor of afferent neurones and is hypothesized to be a key player in the hyperalgesia associated with inflammation. Increased expression of TRPV1 has been observed in patients with or without erosive GORD (Bhat and Bielefeldt 2006, Matthews, Aziz et al. 2004). The validity of the concept of TRPV1 as a biological marker in GORD is still unproved. Considering the potential importance of inflammatory mediators in the

modulation of structure and function of nerve terminals, it would be interesting to relate microscopic signs of inflammation to the observed changes in mucosal innervations. Experimental inflammation can trigger structural changes of nerve endings which have also been seen in humans, with an increase in TRPV1 expression in patients with other clinical disorders (Chan, Facer et al. 2003, Yiangou, Facer et al. 2001b). Similarly, we demonstrated that patients with erosive oesophagitis had greater gene expression of TRPV1 in oesophageal mucosa when compared with non-erosive reflux disease (NERD) or healthy controls (**Chapter 14**). Taken together, these findings suggest that chronic inflammation may lead to the release of mediators which may modulate the structure and/or function of primary sensory neurons.

LIST OF ABBREVIATIONS USED IN THIS THESIS

5-HT	5-hydroxytryptamine
ACC	Anterior cingulated cortex (medial pain system)
ASICs	Acid-sensing ion channels
ATP	Adenosine triphosphate
BGA	Brain-Gut axis
BHAT	Bolus head advance time
BPT	Bolus presence time
CBT	Complete bolus transit
CEPs	Cortical evoked potentials
CGRP	Calcitonin-gene related peptide
CNS	Central nervous system
CRF	Corticotropin-releasing factor
DBTT	Distal bolus transit time
DEA	Distal oesophageal amplitude
DRG	Dorsal root ganglia
EPANs	Extrinsic primary afferent neurons
ENS	Enteric nervous system
GDNF	Glial-derived neurotrophic factor
GORD	Gastro-oesophageal reflux disease
IBS	Irritable Bowel Syndrome
IEM	Ineffective oesophageal motility
IPANs	Intrinsic primary afferent neurons
LOS	Lower oesophageal sphincter

LUOS-C	Laryngo-UOS contractile reflex
MII	Multichannel intraluminal impedance
MII- EM	Multichannel intraluminal impedance and oesophageal manometry
NERD	Non-erosive reflux disease
NK-1	Neurokinin-1
NMDA	N-methyl-D-aspartate
NGF	Nerve growth factor
NOD	Non-obstructive dysphagia
NOS	Nitric oxide synthase
OGCR	Oesophagoglottal Closure Reflex
OUCR	Oesophago-UOS contractile reflex
PEIR	Pharyngooesophageal inhibitory reflex
PGCR	Pharyngo-glottal closure reflex
PGE2	Prostaglandin E2
PUCR	Pharyngo–Upper Oesophageal Sphincter Contractile Reflex
SP	Substance P
SSRIs	Selective serotonin reuptake inhibitors
TBTT	Total bolus transit time
TCAs	Ad tricyclic antidepressants
TLOSR	Transient LOS relaxation
TRPV1	Transient receptor potential vanilloid subfamily, member 1 or Vanilloid receptor transient receptor potential vanilloid type 1
TTXr	Tetrodotoxin-resistant
UOS	Upper oesophageal sphincter
VGSCs	Voltage-gated sodium channels
VIP	Vasoactive intestinal peptide

SECTION A

LITERATURE REVIEW, AIMS AND HYPOTHESES

Chapter 1

Visceral sensitivity: from basic neurophysiology to clinical investigation

1.1 Overview

Functional gastrointestinal disorders are characterised by several syndromes comprising a variety of symptoms not limited to one region of the gut, for which there is no identifiable organic or biochemical abnormality (Drossman, Richter et al. 1994). The most common and best studied of these syndromes are the irritable bowel syndrome (IBS) and functional dyspepsia. Abdominal pain is the most frequent complaint in these patients, which cannot be relieved by a specific treatment of the disease. Several mechanisms have been implicated in the pathophysiology of these disorders including psychological factors and abnormalities in gastrointestinal motility. Attention has been paid to the role of visceral sensitivity in the pathophysiology of functional gastrointestinal disorders (Mayer and Gebhart 1994). Many studies have emphasized the role of afferent nerve pathways arising from the gut to the central nerve system that trigger a number of reflexes. The afferent nerve pathways have therefore been recognized as potential targets for treatments in order to relieve pain in patients with functional gastrointestinal disorders.

In this chapter, we discuss different mechanisms, derived from human and animal studies, which provide plausible hypotheses for the development of visceral hypersensitivity in human. This review also discusses the candidate mediators involved at both peripheral and central levels in afferent nerves from the digestive tract and the effects on viscerosensitivity of pharmacology interfering with these mediators or their target receptors.

1.2 Clinical significance of visceral sensitivity

Visceral hypersensitivity is currently recognized as major pathophysiological mechanism underlying functional gastrointestinal disorders. In patients with IBS, non-cardiac chest pain and functional dyspepsia, a high prevalence of visceral hypersensitivity has been found. In these patients, luminal physiological stimuli can be perceived as unpleasant or even painful. These disorders are associated with significant health care and socioeconomic costs due to factors such as repeated visits to consultants, hospitalizations and work absenteeism.

Although the exact mechanisms contributing to such phenomenon are yet not

completely elucidated, a number of hypothesis have been proposed including an altered activation of the gut-wall receptors, an altered conduction of sensory inputs at the level of neural pathways, or an impaired processing of the sensations at the level of brain, may occur along the brain–gut axis. To date, drugs capable of attenuating hypersensitivity, that target each of the constituents of the stimuli–perception chain, have the therapeutic potential to decrease visceral hypersensitivity and thereby alleviate symptoms.

1.3 Function neuroanatomy and neuropharmacology of visceral afferents

Enteroendocrine cells located in the gut wall serve as mechanical and chemical transducers for local reflexes or in the initiation of afferent projections to the central nervous system (CNS) (Mayer and Gebhart 1994). Unlike somatic sensation, gut afferent signals arrive in conscious perception through a three-level neuron chain (Camilleri, Saslow et al. 1996). The first order neurone, with cell body located in the dorsal root ganglion, terminates in the dorsal column laminae of the spinal cord (Figure1.1). *En passant* fibres project to noradrenergic neurone in prevertebral ganglia, and this reflex center results in modulation of visceral function, including motility. Somatic and visceral afferents converge on the same dorsal horn neurons. This results in the well recognized viscerosomatic convergence and gives rise to referral of pain to the associated dermatome. Descending modulatory fibres (serotonic, adrenergic, etc.) projecting from brain stem centers (such as the periaqueductal grey) on to the dorsal horn neurons can modulate the sensitivity of the dorsal horn neurones and therebye modulate the intensity of central perception of visceral stimuli (Figure1.1).

The second order neurone projects from the dorsal horn of the spinal cord to the thalamus and reticular formation in the brain stem (Figure1.1). The ascending pathways are located in the spinoreticular and spinothalamic tracts. A nociceptive spinal pathway is also in the dorsal column in primates, which projects nociception from viscera such as the duodenum, pancreas, and colorectum (Al-Chaer, Lawand et al. 1996, Feng, Cui et al. 1998). These second neurones synapse with autonomic and satiety centers and with the third order neurone that contributes to emotional responses (limbic system) and conscious perception (sensory cortex). These connections result in changes in pulse rate, blood pressure, appetite, and emotions in response to visceral pain. The loci of projection are possibly involved with the anterior cingulate cortex, insula, and cerebellum (Mertz, Morgan et al. 2000).

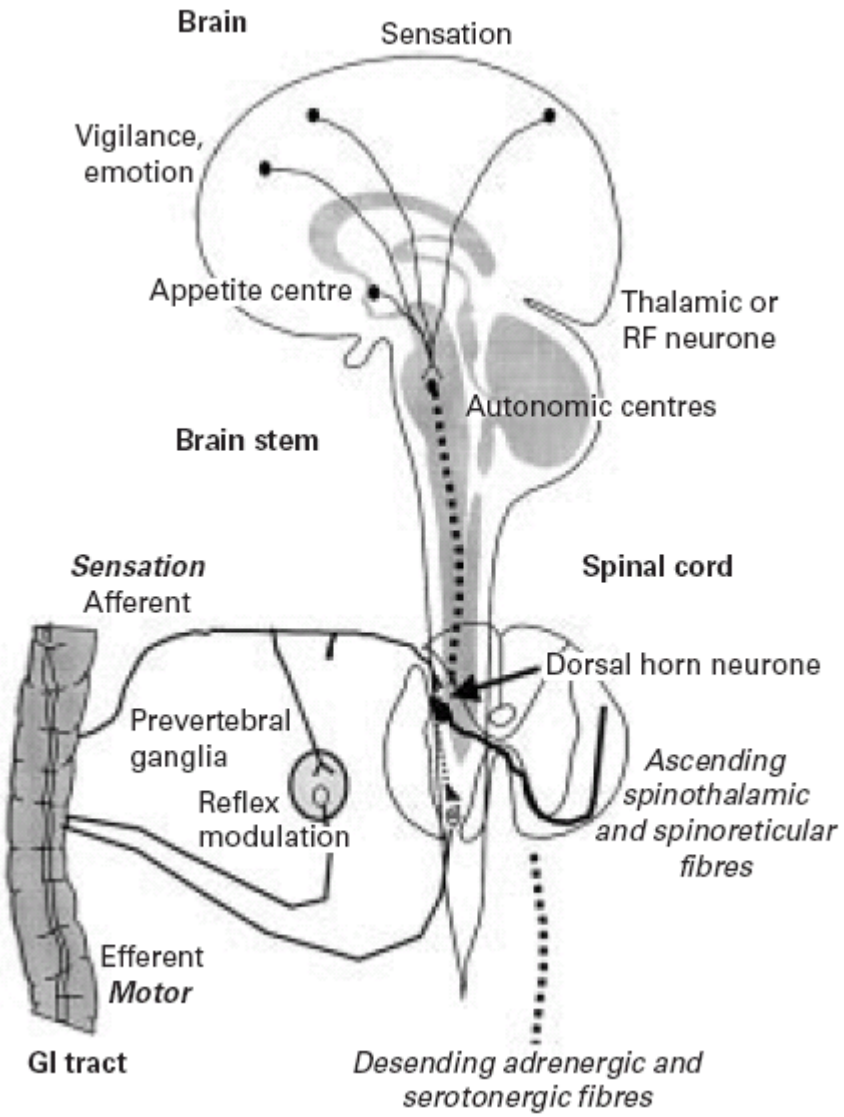


Figure 1.1: Three-level neurone chain involved in visceral perception. Note the descending inhibitory pathways converging on the dorsal horn neurone which modulates projections from this relay station to the brain. (Camilleri, Coulie et al. 2001).

At present, the concept of visceral hypersensitivity provides the most plausible hypothesis responsible for the development of symptoms in functional gastrointestinal disorders. Patients with functional gastrointestinal disorders, characterized by visceral hypersensitivity, may benefit from drugs that that attenuate visceral sensitivity. In the following, we review recent developments in neurophysiological concepts about visceral sensation and pain perception fundamental for an understanding of visceral

hypersensitivity. In addition, we review the currently available data evaluating the effect of drugs that have been suggested to interfere with visceral sensitivity in functional gastrointestinal disorders.

This review deals with the morphological nature of the afferent neurones that innervate the gastrointestinal tract. The digestive tract contains an extensive network of intrinsic neurones able to operate independently from the CNS. This ‘‘independent’’ enteric nervous system (ENS) is composed of circuits that include intrinsic afferent neurones. Therefore, there are two types of afferent neurone involved in gastrointestinal functions—namely, intrinsic primary afferent neurons (IPANs), which are part of the ENS, and extrinsic primary afferent neurons (EPANs). Their nerve endings are similar on the basis of their morphology. In addition, the nerve endings of the axons of other enteric inter- and motor neurones, as well as the endings of the extrinsic preganglionic sympathetic and parasympathetic neurones that terminate within the gut wall, are mixed with the primary afferent nerve endings.

#1.3.1 Extrinsic innervation

In vivo anterograde tracing techniques have shown that the oesophagus and stomach are innervated by primary vagal afferent neurones and that their cell bodies are located in the nodose ganglia (Berthoud and Neuhuber 2000, Fox, Phillips et al. 2000). Vagal afferent fibres form specialised nerve endings within the myenteric ganglia, named intraganglionic laminar endings (IGLE’s). These IGLEs are distributed quite homogeneously along the entire length of the oesophagus in animal models. In the guinea pig oesophagus, intraganglionic laminar endings have been demonstrated to be the mechanosensitive terminals responsible for the stretch induced afferent activity (Zagorodnyuk and Brookes 2000). The stomach is also supplied by similar afferent nerve terminals of vagal origin (Berthoud and Neuhuber 2000, Wang and Powley 2000). Intraganglionic laminar endings are responsible for the transduction for passive and active mechanical tension (Zagorodnyuk, Chen et al. 2003). Their nerve endings are mainly located on the surface of ganglia with some lamellar endings deep in the ganglia, and have a high density of mitochondria (Neuhuber 1987). An important type of primary afferent nerve ending of vagal origin is the intramuscular array (Berthoud and Neuhuber 2000, Wang and Powley 2000). These endings are found more in the pyloric sphincter region. Club-like afferent nerve endings of vagal origin are present in the thick muscle of the antrum. The stomach is

also supplied by capsaicin sensitive spinal afferent fibres, identified tentatively as containing tachykinins and calcitonin-gene related peptide (CGRP).

#1.3.2 Intrinsic innervation

The neural apparatus of ENS is composed of a large number of enteric neurones that can be identified according to their location, neurochemistry, shape, projections, proportions, connections, and function. By application of recent advance in methodology, a full description of all functional classes of enteric neurones has been recently achieved in animal models (Figure1.2) (Costa, Brookes et al. 1996).

Primary afferent neurones (also termed intrinsic primary afferent neurones [IPANs]) are present in both myenteric and submucous ganglia. IPANs respond to luminal chemical stimuli, to mechanical deformation of the mucosa, and to radial stretch and muscle tension. They represent about 30% of myenteric neurones and 14% of submucosal neurones, have a distinct Dogiel type II shape and have a long after hyperpolarisation following action potentials. All of these neurones project to the villi and branch within the submucous and myenteric ganglia locally. A proportion of these neurones (10% of primary afferent neurones) also have long descending projections to aboral myenteric ganglia (Brookes, Song et al. 1995). They project circumferentially to synapse with myenteric ascending interneurones, descending interneurones, longitudinal muscle motorneurones, excitatory circular muscle motorneurones, and inhibitory circular muscle motorneurones. They receive slow synaptic input (probably mediated by tachykinins) from other primary afferent neurones to form reciprocally innervated networks.

These Dogiel type I neurones (17%) receive fast nicotinic inputs from primary afferent neurones and non-cholinergic inputs from the long descending primary afferent neurones. They connect to the circular muscle where their axons are closely associated with those of the excitatory motorneurones in the deep muscular plexus. They use multiple mechanisms of inhibitory transmission including nitric oxide, adenosine triphosphate (ATP), and the peptides Vasoactive intestinal peptide (VIP) and pituitary activating cyclic AMP peptide acting directly on smooth muscle or indirectly via interstitial cells (Brookes, Steele et al. 1991).

This relatively large class (25%) of small neurones with short projections to the longitudinal muscle receive synaptic inputs from the enteric primary afferent neurones and from ascending and descending pathways (Brookes, Song et al. 1992).

This small (5%) but most important class of enteric neurones belongs to the Dogiel type I morphology, and receives fast synaptic inputs from other ascending interneurones which form a chain of ascending excitation. They also receive fast nicotinic and slow synaptic inputs from enteric primary afferent neurones. They project orally within the myenteric plexus to synapse with the final excitatory circular muscle motor neurones via fast nicotinic and noncholinergic slow synaptic inputs. They contain not only the enzyme for the synthesis of acetylcholine but also tachykinins and opioid peptides (Brookes, Meedeniya et al. 1997).

There are several classes of descending interneurones that comprise about 7% of the total (Costa, Brookes, et al. 1996). Three of these are probably cholinergic as they contain the enzyme for the synthesis of acetylcholine, choline acetyltransferase. Each differs in their neurochemistry. Somatostatin and Choline acetyltransferase containing descending interneurones (4%) have a filamentous shape, receive fast and slow synaptic inputs mainly from non-primary afferent neurones, and form a chain of interconnected interneurones synapsing with other somatostatin neurones and with other myenteric and submucous neurones. Serotonin and Choline acetyltransferase containing neurones (2%) project aborally to other myenteric and submucosal neurones. Whether these neurones use serotonin in addition to acetylcholine remains to be investigated. Serotonin may act via fast ion channel gated receptors or via slow G protein linked receptors. Nitric oxide synthase (NOS), VIP, and Choline acetyltransferase containing neurones also project aborally to synapse with other myenteric neurones.

There are two small classes (1% each) of secretomotor neurones in the myenteric ganglia. One is cholinergic and the other noncholinergic containing VIP. They project to the mucosa. Neurones with a similar function and neurochemistry are also present in the submucous ganglia where they represent 32% and 42%, respectively. Some of the VIP submucous neurones also project to the myenteric ganglia and may represent the basis for a functional connection between secretion and motility. The VIP secretomotor neurones receive inhibitory synaptic inputs from the extrinsic sympathetic neurones and from unidentified myenteric neurones. Most submucous neurones receive fast and slow synaptic inputs. A small submucous neurone class of submucous cholinergic neurones (12%) project to the mucosa and to the local blood vessels.

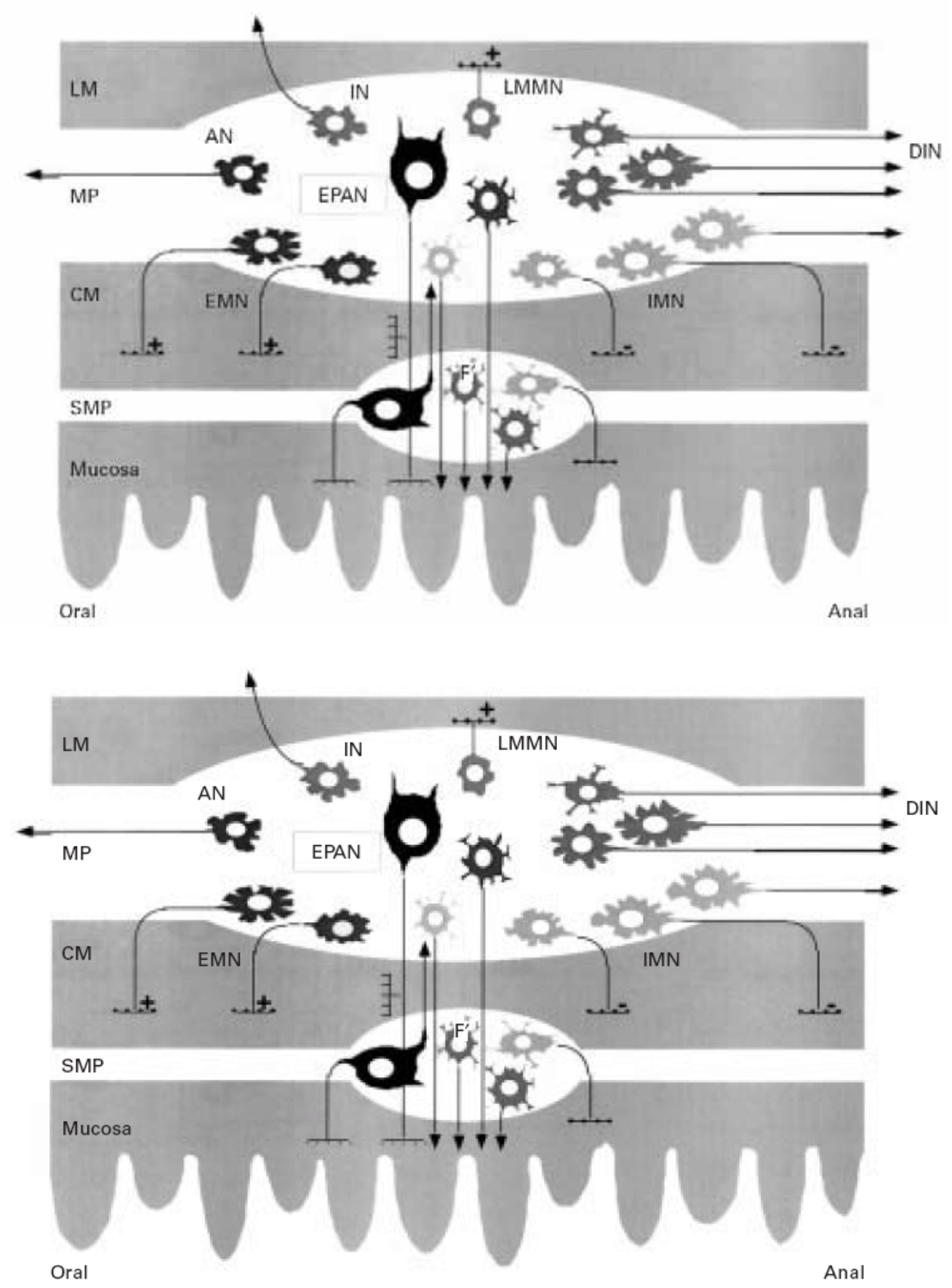


Figure 1.2: Classes of myenteric neurones. LM, longitudinal muscle; CM, circular muscle;MP,myenteric plexus; SMP, submucous plexus;AN, ascending neurones; IN, intestinofugal neurones; DIN, descending interneurones; EPAN, enteric primary afferent neurones; EMN, excitatory motorneurones; IMN, inhibitory motorneurones; LMMN, longitudinal motorneurones (Costa, Brookes, et al. 1996).

#1.3.3 Functional studies

The peripheral endings of vagal and spinal afferents can be localized within the gastrointestinal tract using neuronal tracing techniques. Studies using carbocyanine dyes such as DiI, wheatgerm agglutinin-conjugated conjugated horseradish peroxidase or dextran tracers have provided an extensive anatomical basis for understanding the vagal afferent innervation of different layers and regions of gut (Berthoud and Neuhuber 2000, Powley and Phillips 2002). Recent studies have utilized the ability of neurobiotin to be taken up by nerve fibres in vitro and transported rapidly over short distances to the nerve terminals within the gut wall (Tassicker, Hennig et al. 1999). These more restricted dye-fills of fine branches of extrinsic nerve trunks to the gut wall have allowed correlation between structure and function to be investigated (Lynn, Olsson et al. 2003).

Three distinct and characteristic patterns of terminal distribution can be observed within the gut wall. One population of afferent fibres has responsive endings in the serosal layer and in the mesenteric connections often in association with mesenteric blood vessels. Another population has been traced into the muscularis externa and forms endings either in the muscle layers (Berthoud and Powley 1992, Fox, Phillips, et al. 2000) or in the myenteric plexus, which is sandwiched between the longitudinal and circular muscle layer (Berthoud, Kressel et al. 1995). The third population makes endings in the mucosal lamina propria, where they are positioned to detect material absorbed across the mucosal epithelium or released from epithelial and subepithelial cells including enterochromaffin and immunocompetent cells (Ward, Bayguinov et al. 2003, Williams, Berthoud et al. 1997) (Figure 1.3).

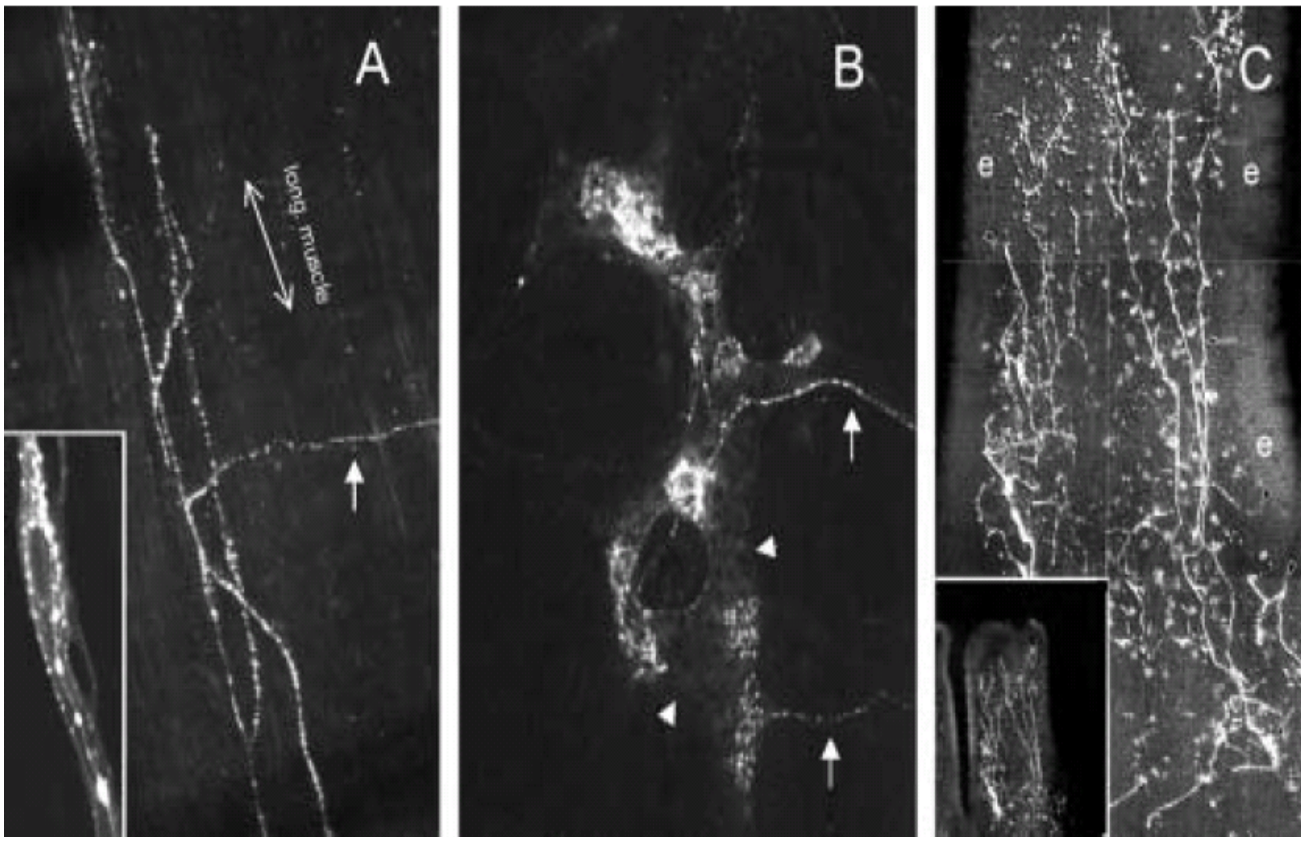


Figure 1.3: Vagal afferent endings in rat gastrointestinal tract anterogradely traced with the fluorescent dye DiI (bright white) injected into nodose ganglia. (A) Intramuscular array in longitudinal muscle layer of gastric fundus. Arrow indicates parent axon entering the muscle layer from myenteric plexus. The inset shows vagal afferent fibres in intimate anatomical contact with interstitial cell of Cajal. (B) Intraganglionic laminar endings in myenteric plexus of gastric fundus. Two different parent axons are indicated by arrows. Myenteric ganglion is indicated by arrowheads. (C) Mucosal endings close to epithelium (e) in villous of proximal duodenum (Berthoud, Blackshaw et al. 2004).

#1.3.4 Neuropharmacology targeting visceral sensitivity

This part of review will discuss the currently available studies evaluating the effects of drugs that have been proposed to interfere with visceral sensitivity in functional gastrointestinal disorders. We selected only those drug classes: (1) with visceral analgesic properties, as shown in basic experimental studies; (2) of which data were available on visceral sensitivity in humans (including studies on the normal physiology of visceral sensation carried out in healthy volunteers and studies in patients with functional gastrointestinal disorders) and (3) of which controlled data

were available addressing their clinical efficacy. The five drug classes that fullfilled these criteria were opioid substances, serotonergic agents, antidepressants, somatostatin analogues and α2-adrenergic agonists.

Opioid agonists inhibit the perception of somatic and visceral pain through their action on opioid receptors, involving the μ-, δ- and *κ*-opioid receptor subtypes. The antinociceptive effects of selective ligands acting on μ- and δ-receptors involve hyperpolarization of neurones, whereas *κ*-agonists have been shown to modulate intracellular ion conductance (Bueno, Fioramonti et al. 1997). Different opioid receptors have been demonstrated not only in the brain and the spinal cord, but also in the periphery, including in the dorsal root ganglia (DRG), on primary afferent neurones and their sensory nerve endings (Johnson and Duggan 1981, Ninkovic, Hunt et al. 1982).

In somatic pain, selective μ-, δ- and κ-opioid receptor agonists have been shown to block nociceptive responses in experimental animal models, and have been successfully applied for clinical use (Inturrisi 2002). For example, the cardiovascular reflex response to noxious balloon distension of the duodenum in the rat was inhibited by the μ-opioid agonist morphine, but also by the κ-opioid agonists fedotozine and U-50488 (Diop, Riviere et al. 1994).

There are seven known serotonergic ([5-hydroxytryptamine (5-HT)]) receptors, of which 5-HT_1, 5-HT_3 and 5-HT_4 receptors (and their subtypes) seem to play the most important role in the gut (Kim and Camilleri 2000). 5-HT is released by mucosal enteroendocrine cells in response to intraluminal stimuli and diffuses across the basal membrane. Via activation of 5-$HT_{1B/P}$/5-HT_4 receptors on the nerve endings of IPANs, 5-HT plays a key role in stimulating peristalsis and secretion (Grider 1994, Kim and Camilleri 2000). Excitatory 5-HT_3 receptors have been identified on IPANs, afferent sensory fibres and DRG neurones. Blocking these receptors reduced visceral pain in rats (Hicks, Coldwell et al. 2002, Kozlowski, Green et al. 2000). Similarly, the 5-HT_4 partial receptor agonists tegaserod reduced visceral afferent firing during colorectal distension in cats (Schikowski, Thewissen et al. 2002), whereas 5-HT_{1A} and 5-HT_{1B} receptor agonists have been shown to decrease the visceromotor response to noxious colorectal distension in rats (Danzebrink and Gebhart 1991). Several drugs targeting these receptors have been developed for their possible use in the treatment of functional gastrointestinal disorders (Gershon 1999, Kim and Camilleri 2000). These compounds include the 5-HT_3 receptor antagonists alosetron, ondansetron, granisetron,

tropisetron and cilansetron, and the 5-HT_4 receptor agonists prucalopride and tegaserod.

Antidepressants have been widely used in the treatment of functional gastrointestinal disorders, for several reasons. Firstly, many of these patients show significant levels of comorbid depression and anxiety (Whitehead, Palsson et al. 2002). Secondly, antidepressants have been shown convincingly in animals, to downregulate nociceptive afferent traffic. In addition to their psychotropic action, antidepressants have both neuromodulatory and analgesic properties, of which the most convincing clinical evidence comes from experimental models of somatic pain in various somatic pain syndromes (Coquoz, Porchet et al. 1993, Gorelick, Koshy et al. 1998). These studies have demonstrated the analgesic potency of both ad tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs), although TCAs, in particular amitriptyline, seem superior in this perspective and are certainly the best studied. The mechanisms by which antidepressants have analgesic effects are largely unknown, but may involve serotonergic, noradrenergic and opioidergic systems (Singh, Jain et al. 2001).

Studies have shown somatostatin and its synthetic analogue octreotide are effective in the treatment of different clinical pain syndromes (Chrubasik, Meynadier et al. 1985), and there is preclinical evidence that octreotide also has visceral analgesic effects (Su, Burton et al. 2001). Of the five cloned somatostatin receptors, octreotide has a high affinity for three subtypes (somatostatin receptors 2, 3 and 5). Somatostatin and its receptors have been demonstrated in the CNS such as brain and spinal cord (Kaupmann, Bruns et al. 1993, Schindler, Humphrey et al. 1996), and in the peripheral nervous system (primary afferents, DRG)(Hokfelt, Elde et al. 1976). The sites and/or mechanisms of action involved in the possible (visceral) analgesic effects of octreotide remain unclear. Although it seems unlikely that peripherally administered octreotide crosses the blood–brain barrier in significant amounts (Banks, Schally et al. 1990), the analgesic effects of octreotide in somatic pain have been demonstrated after both intrathecal and subcutaneous injection (Befon, Mystakidou et al. 2000, Penn, Paice et al. 1990). However, the involvement of somatostatin receptors on alternative peripheral afferent pathways needs to be further evaluated.

The adrenergic nervous system plays an important role in modulating nociceptive processing. α_2-Adrenergic agonist-binding sites are located along nociceptive pathways in the spinal cord, brain stem and forebrain (Unnerstall,

Kopajtic et al. 1984), and activation of spinal α_2-adrenergic receptors has been shown to play a role in antinociception (Mayer and Gebhart 1994). This event may involve modulation of spinal neurotransmission at the level of the dorsal horn and/or activation of descending, inhibitory pathways.

In summary, based on the current available evidence, the concept of targeting visceral hypersensitivity as a treatment of functional gastrointestinal disorders needs to be further explored.

#1.3.5 Nociceptive pathways

It is well established that visceral pain is mediated by spinal afferent neurons (Mayer and Gebhart 1994, Ness and Gebhart 1990), but vagal afferent fibers are of similar importance in the modulation of nociception. The sensitivity of the nociceptive nature appears to be significantly associated with both the quality and the intensity of the noxious stimulus as well as the experimental situation (Grundy 1988). When activated, chemosensitive and mechanosensitive nociceptors transmit their impulses through small, unmyelinated C-fibers or myelinated A∂ fibers. C-fibers are known to transmit their signals relatively slowly, and pain perception transmitted by them is perceived as dull, burning, gradual, and poorly localized. In contrast, A∂ fibers transmit signals quickly, and the perception of the pain transmitted is typically sharp, sudden, and well localized.

Once nociception has been activated, their impulses are transmitted peripherally and centrally (Figure1.4). Peripheral transmission involves local reflex arcs for activation of afferent and efferent signals to muscle, glands, and blood vessels, which can result in adaptive, possibly protective, responses such as altered motility, secretion, and blood flow. Central transmission is required for pain perception by peripheral spinal and vagal afferent nerves. Spinal afferents, predominant for nociception, send their signals to the spinal cord via the sympathetic nerves, and vagal afferents, which are principally involved with pain modulation, send their information to the central nerve system (medulla) via the vagus nerve (see Figure1.4) (Holzer 1998).

Spinal afferents transmit pain to the brain, including transmission via first-order neurons located in the DRG and second-order neurons in the dorsal horn of the spinal cord, each distributed over several spinal segments. After reaching the spinal cord, pain signals traverse across the midline and ascend via the contralateral spinothalamic

and spinoreticular pathways to synapse with third-order neurons in the thalamus and reticular nuclei, the latter transmitting the signals to the somatosensory cortex for localization and interpretation.

Only spinal afferents transmit pain signals to the somatosensory cortex for recognition, whereas spinal afferents and vagal afferents transmit signals to the limbic system for affective and motivational assessment and to the frontal cortex for perception of pain. Vagal afferents send impulses to the brain for pain modulation, including transmission via first-order neurons located in the nodose ganglia and second-order neurons located in the nucleus tractus solitarius within the medulla, the latter then transmitting impulses to the limbic system and frontal cortex.

There is substantial overlap between the neuroanatomic pathways for the oesophagus with those of the heart, lungs, etc. For instance, vagus nerve impulses from the cardiopulmonary region converge with those of the oesophagus before reaching the medullary centers in the brainstem (Figure1.5). Similarly, there is overlap of nociceptive pathways via sympathetic nerves from the cardiopulmonary and oesophageal regions.

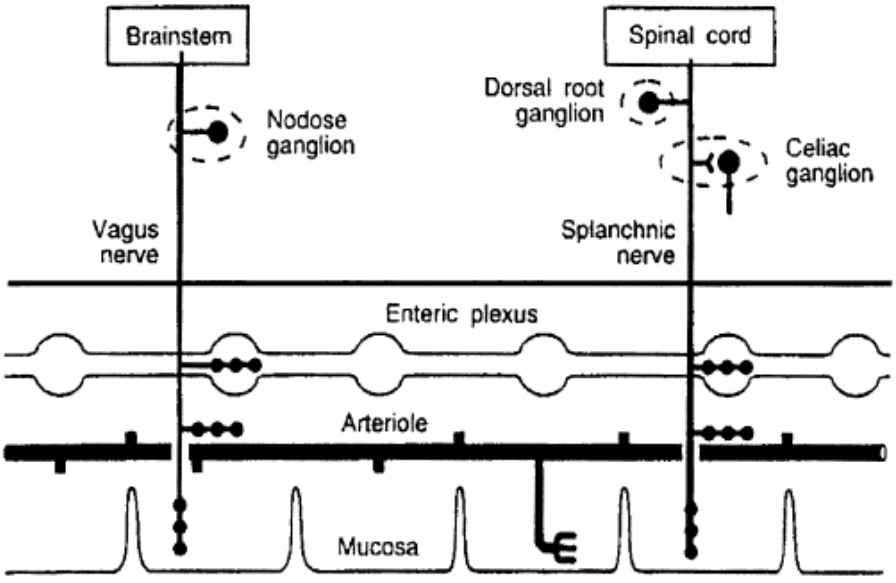


Figure 1.4: Innervation of the mammalian upper digestive tract by extrinsic primary afferent neurons. Vagal afferent neurons have their cell bodies in the nodose ganglion while splanchnic afferents have their cell bodies in the dorsal root ganglion (DRG) (Holzer 1998).

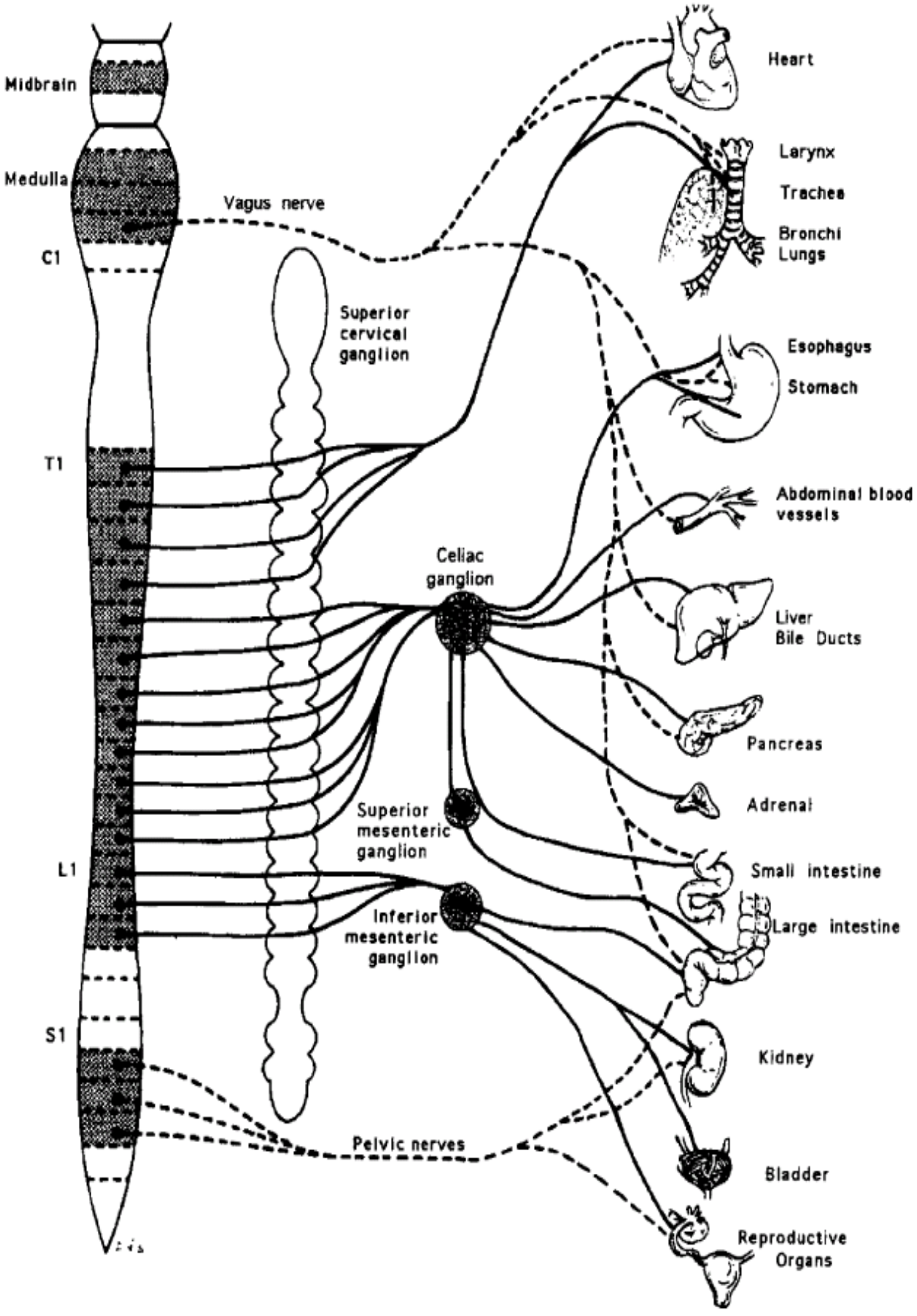

Midbrain
Medulla
C1
T1
L1
S1
Vagus nerve
Superior cervical ganglion
Celiac ganglion
Superior mesenteric ganglion
Inferior mesenteric ganglion
Pelvic nerves
Heart
Larynx
Trachea
Bronchi
Lungs
Esophagus
Stomach
Abdominal blood vessels
Liver
Bile Ducts
Pancreas
Adrenal
Small intestine
Large intestine
Kidney
Bladder
Reproductive Organs

Figure 1.5: Oesophageal pain pathways are traced from the periphery via the sympathetic nervous system and vagus nerve to the central nerve system. For the vagus nerve, there is overlap between impulses arising within the oesophagus and passing to the central nerve system and that arising in the heart, lungs, trachea, and bronchi (Heimer 1983).

1.4 Mechanisms involved in visceral sensitization

The predominant sensation from the viscera is pain. Although some non-painful sensations arise from organs of the gastrointestinal tract such as feeling of satiety, gas, or urge to defecate, the only conscious sensation from most viscera is pain. In addition, humans can perceive visceral hyperalgesia, which is a heightened painful perception to a painful stimulus after injury or disease. Patients with functional gastrointestinal disorders have reported greater pain to colonic distention than normal subjects given the same stimulus intensity (Mertz, Naliboff et al. 1995). The question is what underlying mechanisms contribute to these different sensations. Experimental evidence has supported the roles of both peripheral and central mechanisms in the etiology of visceral sensitivity and hyperalgesia. Additionally, when compared with somatic pain, there are many similarities, but there are some noticeable differences in the anatomy and physiology of viscerosensory processing. This review will discuss the mechanisms involved in visceral sensitization.

#1.4.1 Peripheral and central sensitization

Tissue damage due to inflammation results in sensitization of primary afferent nerves due to the release of inflammatory mediators, such as K^+, H^+, ATP, bradykinin, prostaglandins, serotonin and histamine (Costigan and Woolf 2000). These inflammatory mediators lower the transduction threshold of primary afferents and recruit previously silent nociceptors (Woolf and Salter 2000). Inflammation also induces increased expression of sodium channels Nav 1.8 and 1.9, the vanilloid receptor transient receptor potential vanilloid type 1 (TRPV1 or VR1), the purine receptor P2X3 and acid-sensing ion channels (ASICs) (Yiangou, Facer et al. 2001a, Yiangou, Facer, et al. 2001b, Yiangou, Facer et al. 2001c). Furthermore, cytokines secreted by macrophages and mast cells indirectly lead to nerve sensitization by up-regulating the expression of nerve growth factor (NGF) (Woolf 1996) and the release of cyclooxygenase metabolites (Sciberras, Goldenberg et al. 1987) and

sympathomimetic amines. The consequence of these changes is an increase in pain sensitivity at the site of inflammation (Woolf and Salter 2000).

Recently, immunocytochemical techniques have been used to demonstrate an upregulation of peptides, cytokines, TRPV1 receptor and neurotrophic factors in skin, urinary bladder and rectal biopsies of patients with chronic hypersensitivity states without evidence of overt inflammation (Chan, Facer, et al. 2003). It has been suggested that the TRPV1 receptor may be an important marker of afferent nerve sensitization. An upregulation of TRPV1, P2X3 receptors and ASICs has also been identified in the inflamed human GI tract (Yiangou, Facer, et al. 2001a, Yiangou, Facer, et al. 2001b, Yiangou, Facer, et al. 2001c). Recent studies have demonstrated that patients with oesophagitis have an upregulation of both TRPV1 (Matthews, Aziz, et al. 2004) and cytokine interleukin-8 (Yoshida, Uchiyama et al. 2004). They show that it is potentially possible to explore what receptors may play an important role in mediating visceral hypersensitivity peripherally.

Enhanced nociceptor input activates intracellular signaling consequences within spinal dorsal horn neurones, which results in amplified responses to both noxious and innocuous inputs, due to facilitated excitatory synaptic responses and depressed inhibition (Woolf 1995). Facilitation is triggered by the presynaptic release of neurotransmitters and neuromodulators, such as glutamate, substance P (SP), brain-derived neurotrophic factor and prostaglandins. These neurotransmitters and neuromodulators activate ligand-gated ion channels including N-methyl-D-aspartate (NMDA) and a-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid receptors, G-protein-coupled metabotropic receptors, neurokinin receptors and tyrosine kinase receptors, which then increase intracellular calcium via release from intracellular stores and calcium inflow. Thus, changes in ion channel and receptor activity via calcium-dependent activation of protein kinase A, protein kinase C and tyrosine kinases lead to phosphorylation of the NMDA receptors (Woolf and Salter 2000). This dramatically changes NMDA receptor kinetics and reduces its voltage-dependent magnesium block, thus augmenting its subsequent responsiveness to glutamate and increasing synaptic strength, enabling previously sub-threshold inputs to activate the cell (Woolf and Thompson 1991). The increase in gain changes receptive field properties and pain sensitivity, causing tissue hypersensitivity far beyond the site of injury that initiated central sensitization.

In addition to producing central sensitization, which occurs within seconds of

appropriate activation of spinal dorsal horn neurones, nociceptive input also generates an activity -dependent change in transcription in dorsal root ganglion and dorsal horn neurones (Woolf and Costigan 1999). These transcriptional changes occur in response to a complex mechanism involving the activation of transcription factors that lead to both an increase and a modification of constitutively expressed genes and also induction of novel genes. For instance, non-nociceptive afferents begin to express SP and brain-derived neurotrophic factor after inflammation (Neumann, Doubell et al. 1996) and this phenotypic shift results in allodynia, i.e. non-nociceptive tactile stimuli now induce pain. These changes take hours to manifest but, when established, result in long-lasting changes in normal stimulus response characteristics.

A striking feature of the increase in synaptic efficacy characteristic of central sensitization is that it includes not only those nociceptor central terminal synapses activated by the conditioning stimulus but also synapses made by low threshold mechanosensitive Aβ fibres on dorsal horn neurones (Woolf and King 1990). Low-threshold sensory fibres, activated by innocuous stimuli such as light touch, can then activate normally high-threshold nociceptive neurones at the dorsal horn, leading to a decrease in pain threshold such that non-painful stimuli are now perceived as pain (allodynia), which is a direct consequence of an increased excitability of central CNS neurones. Despite pain being referred to the periphery, it arises from within the CNS. This central facilitation manifests within seconds of an appropriate nociceptive conditioning stimulus and can outlast the stimulus for several hours (Woolf and Wall 1986). If the stimulus is maintained, even at low levels, the central sensitization persists. After peripheral nerve injury, for example, ongoing ectopic activity arising from sensory fibres in the injured nerve can obtain prolonged central sensitization (Gracely, Lynch et al. 1992). Such activity-dependent central sensitization is extremely robust and has been reported in dorsal horn neurones of animal models, including spinothalamic neurones (Hylden, Nahin et al. 1989, Simone, Sorkin et al. 1991, Willis 2002).

The consequences of central sensitization can be readily found in human psychophysical experiments. Intradermal injection of capsaicin, the pungent ingredient in chilli peppers which activates the TRPV1 receptor, produces an intense but transient pain due to activation of TRPV1-expressing nociceptors. This is followed by heightened sensitivity to pinprick outside the region of the capsaicin injection (secondary mechanical hyperalgesia) and to low-threshold mechanosensory

(brush) inputs (secondary mechanical allodynia), due to the induction of central sensitization (Koltzenburg, Lundberg et al. 1992, LaMotte, Lundberg et al. 1992).

#1.4.2 The Brain-Gut axis

This part of review will discuss current knowledge about the bidirectional communication system between the gut [ENS] and the brain (CNS), classically termed the Brain-Gut axis (BGA) is critical for understanding a putative influence of psychosocial factors on GI sensitivity and motor functions. The ENS and the CNS communicate through neural (autonomic nervous system), neuroendocrine (hypothalamo-pituitary-adrenal axis) and neuroimmune pathways, and these systems may highly interact.

Gastrointestinal sensory input is transmitted to the brain through vagal and spinal afferent nerves. Vagal afferents project to the nucleus of the solitary tract, which in turn projects to the thalamus (mostly via the parabrachial nucleus) and directly to regions regulating arousal and emotional, autonomic and behavioural responses including the hypothalamus, locus coeruleus, amygdala and periaqueductal grey. From the thalamus, GI sensory signals are relayed to the cortical components of the visceral sensory neuromatrix (Jones, Dilley et al. 2006).

First-order spinal afferent nerves make synapse in the dorsal horn of the spinal cord and second-order neurones project to the brain through the spinoreticular, spinomesencephalic, spinohypothalamic and spinothalamic tracts (Almeida, Roizenblatt et al. 2004). The first three of these tracts mainly functions fast, largely unconscious and/or automatic responses to visceral stimuli (arousal, orientation, autonomic responses, prototype emotional and behavioural responses), thereby playing a key role in maintaining the homeostasis of the organism (Almeida, Roizenblatt, et al. 2004). The spinothalamic tract projects to the ventral posterior lateral, medial dorsal and ventral medial posterior nuclei of the sensory thalamus, from which information is relayed to the somatosensory cortices (lateral pain system), the (Anterior cingulated cortex) ACC (medial pain system) and the insula, respectively (Almeida, Roizenblatt, et al. 2004). In these cortical regions, conscious and more complex processing takes place. The main function of somatosensory cortices is to provide information about intensity and localization of the stimulus (sensorydiscriminative pain dimension), whereas the ACC mainly processes pain affect (affective-motivational pain dimension).

The different subregions within the ACC are also important in generating autonomic, behavioural and descending antinociceptive responses to (visceral) pain (Almeida, Roizenblatt, et al. 2004), and in anticipation of or attention to aversive (visceral) stimuli (Naliboff, Berman et al. 2006). The insula is the "interoceptive cortex" where all information about the internal state of the organism is processed (Critchley, Wiens et al. 2004), playing an important role in integrating visceral sensory and emotional information and in higher order control of autonomic visceromotor responses.

Finally, the orbital prefrontal cortex is playing a key role in the integration of sensory information from different modalities (especially for food and eating) and attributing affective, motivational, reward and hedonic valence to it (Ongur and Price 2000). Additionally, this region is also involved in the generation of and choice between autonomic and behavioural response patterns (Ongur and Price 2000), and has been shown to be a putative biological substrate of cognitive influences including placebo effect and expectation of relief on emotions and the affective dimension of visceral pain (Petrovic and Ingvar 2002). Thus, different dimensions of visceral sensation and pain are processed at the different levels of the ascending part of the BGA as described. However, descending pathways originate at virtually all BGA levels to modulate the ongoing transmission of visceral sensory information, mainly at the level of the dorsal horn of the spinal cord (Jones, Dilley, et al. 2006).

The excitability of viscerosomatic afferents within the ventral horn, projecting to the anterolateral ascending pathways, can be enhanced by stimulation of the reticular formation of the nucleus raphe magnus. This is part of the excitatory spino-bulbo-spinal feedback loop, which is conveyed within the venterolateral funiculus to excite spinal cord neurones (Cervero, Meyer et al. 1994, Tattersall, Cervero et al. 1986). The role of this pathway is thought to be to activate the descending antinociceptive system to the dorsal horn via the nucleus raphe magnus and to activate arousal and emotional responses via autonomic nuclei (Haines, Milhailoff et al. 1997).

The inhibitory neurones are thought to have a modulatory role in visceral pain. For example, visceral stimulation can induce excitation of innervating neurones and inhibit non-innervating spinal neurones so that the ascending information within the cord is enhanced from this organ (Foreman 1993), therefore allowing it easier to interpret the source of afferent information for the brain. Other viscerosomatic

neurones that innervate the viscera can be inhibited by either a visceral or somatic input for up to 1 s, so that no afferent response to a further input during this period occurs (Tattersall, Cervero, et al. 1986). The activity of these viscerosomatic afferents may explain the intermittent rhythmic nature of abdominal colic. Additionally, these neurones are implicated in explaining the phenomena called counterirritation (Payne and Poulton 1928), where the pain threshold in the viscera is enhanced following noxious somatic stimulation within its segmental spinal innervation.

In addition to local spinal inhibitory pathways, it is well known that spinal nociceptive transmission is modulated by descending pathways from various supraspinal structures, including the nucleus raphe magnus, periventricular grey of the hypothalamus and the midbrain periaqueductal grey (Basbaum and Fields 1984). At cortical level, the ACC is the most important source of descending modulatory pathways, projecting to the amygdala and the periaqueductal grey, which is probably the major pain modulatory region. On a lower brainstem level, the noradrenergic locus coeruleus, the serotonergic raphe nuclei and the rostrolateral ventral medulla receive input from the amygdala and the periaqueductal grey, and project in turn to the dorsal horn of the spinal cord, where ongoing transmission of sensory information is modulated (gate mechanism) (Jones, Dilley, et al. 2006). Throughout the whole descending modulatory system, from cortex (ACC) to periaqueductal grey and spinal cord (dorsal horn), endogenous opioids are importantly involved, together with other neurotransmitters including noradrenaline and serotonin (Fields 2004).

Similar to its somatic counterpart, visceral nociceptive transmission is also subject to descending inhibitory modulation (Ness and Gebhart 1987). This is supported by the fact that the responses of dorsal horn neurones to noxious colorectal distension were inhibited by electrical or chemical stimulation applied within the periaqueductal grey (Ness and Gebhart 1987). The visceromotor response (contraction of abdominal and hind limb musculature) and the spinal dorsal horn neuronal responses to colorectal distension are modulated in a biphasic manner by chemical stimulation in the brainstem rostrolateral ventral medulla (Zhuo and Gebhart 2002). The interaction between the descending facilitatory and inhibitory systems from the rostrolateral ventral medulla appears to produce a net facilitatory effect following tissue injury, perhaps as an evolutionary defense mechanism to enable protection of the injury. The neuromodulators producing these effects are not fully understood, but it appears that activation of NMDA receptors and production of nitric oxide are

critical in the descending facilitatory pathway (Coutinho, Urban et al. 2001), while non-NMDA receptors involve the inhibitory descending pathways (Jasmin, Rabkin et al. 2003, Rosen, Lundeberg et al. 2000).

#1.4.3 Mechanisms underlying visceral pain

Alterations in the pain transduction pathways may occur throughout the BGA from the primary afferent, through the spinal cord to the brainstem and higher centres. Although visceral pain hypersensitivity has been widely described, the pathophysiological mechanisms underlying such hypersensitivity are not well characterized. Subsequent neural pathways from the brain to the gut via vagal and spinal efferents will modulate this sensory input, resulting in either a facilitatory or inhibitory response to nociceptive stimulus.

The post-inflammation development of peripheral sensitization of visceral afferent fibres has been shown to cause long-term sensorimotor disturbances of the gut (Al-Chaer, Lawand, et al. 1996). It is suggested that either persistent sensitization of primary afferent neurones or synaptic plasticity within the CNS can occur long after the resolution of the insult. Thus, it is conceivable that a disorder labeled as functional had an antecedent peripheral initiating event. Evidence for this hypothesis is documented in patients with postinfectious IBS who give a preceding history of GI infection before the onset of their symptoms. Increased mast cells, T lymphocytes and expression of interleukin-1β are detected in the large bowel in postinfectious IBS patients (Gwee, Collins et al. 2003). Furthermore, recent data in IBS have observed close proximity of mast cells and nerves, with a correlation to abdominal pain severity (Barbara, Stanghellini et al. 2004). This suggests a neuroimmune interaction in IBS, and is supported by demonstrations that SP can alter mast cell excitability and function via NK-1 receptors on mast cells (Suzuki, Furuno et al. 1999), with NK-1 receptor expression being influenced by interleukin-4 production from T lymphocytes (van der Kleij, Ma et al. 2003). Alterations in peripheral neuroimmune interactions may contribute to the pathophysiology and clinical expression of changed visceral pain hypersensitivity seen in functional gastrointestinal disorders (Barbara, De Giorgio et al. 2002).

Another possible mechanism for peripheral sensitization is nerve injury, as this is well known to cause long-lasting hyperalgesia in animal models. Studies using a model of pelvic nerve damage in the rat have shown a reduced threshold to distension

and increased spontaneous activity, suggesting that visceral nerve damage could significantly contribute to the afferent barrage arriving at the spinal cord without peripheral inflammation (Coutinho, Su et al. 2000). Peripheral inflammation could also potentially influence the phenotype of visceral afferent neurones such that an increased expression of ligand- or voltage-gated channels remains despite resolution of the inflammation. Candidate receptors are TPRV1, voltage-gated calcium or sodium channels and stretch activated potassium channels (Blackshaw and Gebhart 2002).

The TRPV1 receptor, activated by heat and capsaicin, plays an important role in visceral hypersensitivity. First cloned in 1997 (Caterina, Schumacher et al. 1997), the polymodal TRPV1 receptor belongs to the family of TRP receptors expressed particularly by small-sized afferent neurones and by mononuclear blood cells (Mezey, Toth et al. 2000). TRPV1 is activated by capsaicin and its analogues, lipids, other molecules such as resiniferatoxin, and also by endocannabinoids including anandamide (Caterina, Schumacher, et al. 1997, Zygmunt, Petersson et al. 1999). Upon activation, a sensation of burning pain is evoked, along with release of the neuropeptides SP and calcitonin gene-related peptide. The receptor is also gated by noxious heat (>43°C), and its mechanism potentiated by protons. It has been suggested that inflammatory and ischaemic hyperalgesia may in part be mediated by the enhanced TRPV1 response due to a decreased tissue pH and production of excess hydrogen ions (Caterina, Schumacher, et al. 1997).

There is increasing evidence that TRPV1 is involved in gut hypersensitivity and pain. Topical capsaicin has been shown to be effective in the treatment for idiopathic pruritus ani, with the probable mechanism being desensitization of nociceptors by capsaicin. (Lysy, Sistiery-Ittah et al. 2003). Hypersensitivity is likely to result from inflammatory products driving phenotypical changes in sensory neurones expressing TRPV1, mainly via increased NGF and/or glial-derived neurotrophic factor (GDNF). TRPV1 receptor expression changes have also been linked with other gut hypersensitivity disorders. Patients suffering from rectal hypersensitivity and faecal urgency have been found to have an upregulation in the TRPV1-expressing nerve fibres when compared with controls, and these levels correlated with a decrease in threshold to rectal heat and distension (Chan, Facer, et al. 2003). This group of patients was also found to have increased GDNF and trk-A-expressing fibres (Figure1.6)(Bar, Facer et al. 1997).

TPRV1 has been implicated in the mechanism of pain produced in gastro-oesophageal reflux disease (GORD). In oesophagitis patients, the proportion of papillae positive for these nerve fibres was increased, suggesting that acid-induced inflammation may upregulate expression of acid-sensitive receptors such as TRPV1, hence contributing to the visceral hypersensitivity often seen in patients with GORD and chest pain (Matthews, Aziz, et al. 2004). A recent study in patients with non-erosive reflux disease (NERD) has revealed an increase in TRPV1-expressing nerve fibres in the oesophageal mucosa but without inflammation, further strengthening the hypothesis (Bhat and Bielefeldt 2006). A trial of dyspeptic patients treated with red pepper resulted in patients initially complaining of epigastric pain, followed by an improvement of symptoms after prolonged treatment for a few days (Bortolotti, Coccia et al. 2002). This is similar to the effect seen with capsaicin treatment of pruritus ani described above, suggesting initial stimulation of TRPV1-expressing neurones, followed by desensitization. Capsaicin induces ileal pain when applied via ileal stomata (Drewes, Schipper et al. 2003). A study in healthy adults has revealed that perfusion of capsaicin in the human jejunum induced pain and warmth sensation indicative of activation of capsaicin-sensitive receptors, probably TRPV1 (Schmidt, Hammer et al. 2004). Furthermore, in patients with painful inflammatory bowel disease, the number of TRPV1-expressing neurones is significantly increased in colonic mucosa (Yiangou, Facer, et al. 2001b).

These studies in humans provide evidence of a role for TRPV1 in inflammation-induced pain and visceral hypersensitivity. The changes in expression are likely to be mediated by the effects of NGF, which is produced locally during inflammation. NGF sensitizes TRPV1 receptors to protons, enhancing their effect, and also increases expression of TRPV1. Increased NGF and recently trk A expression have been reported in acute inflammatory bowel disease (di Mola, Friess et al. 2000). The increase in TRPV1 levels which occur immediately after inflammation is by an NGF-mediated p38 kinase pathway (Ji, Samad et al. 2002). TRPV1 activity is modulated by inflammatory mediators including bradykinin and prostaglandins, probably by cAMP-dependent protein kinase A or protein kinase C -mediated phosphorylation of the receptor (Premkumar and Ahern 2000). Possible mechanisms by which NGF can mediate chronic pain and hypersensitivity are summarized in Figures1.7-8.

Tissue damage, whether it be a result of any cause such as trauma, infection, etc,

results in local tissue acidosis and pain. Pain may be due to modulation of receptors, such as TRPV1, by acidic pH or by direct activation. A sodium selective channel, ASIC1, expressed by sensory neurones, is closed at a pH of 7.4, but is activated once the pH falls below 7.0 (Waldmann, Champigny et al. 1997). The related ASICs have been renamed as ASIC2a, ASIC2b and ASIC3.

These channels are likely to play a role in nociception and GI visceral hypersensitivity, but experimental evidence in humans is still lacking. Yiangou et al. looked at ASIC expression in biopsies from actively inflamed Crohn's disease patients and found that ASICs 1, 2 and 3 were all expressed in the enteric neurones. Interestingly, only ASIC3 expression was significantly upregulated in the inflamed specimens when compared with controls, suggesting a role for ASIC3 in inflammation and pain/GI hypersensitivity (Yiangou, Facer, et al. 2001c). As acid-sensing channels, they would be a potential candidate for oesophageal pain provoked by acid.

Ion channels that are gated by extracellular ATP have been characterized on sensory neurones including those in the intestine of animal models. Two types of receptors exist: P2X receptors are ATP-gated and P2Y are G-protein-coupled receptors (North and Barnard 1997). In the GI tract, ATP release may occur from a variety of sources including cell damage, sympathetic and extrinsic sensory neurones, and hence ATP-gated ion channels are a likely candidate for mediating GI nociception following inflammation, infection or injury. P2X3 receptors, a subgroup of the P2X receptors, have been shown to be present in human enteric neurones (Yiangou, Facer, et al. 2001a). It was also found that in inflamed inflammatory bowel disease colonic biopsies, the levels of P2X3-expressing neurones were significantly increased. This human study implies that P2X3 have a role in inflammation, pain and dysmotility. Voltage-gated sodium channels (VGSCs), of which there are numerous in the central and peripheral nervous systems (Wood 2004), are responsible for the rising phase of the action potential (Woolf and Costigan 1999), by a voltage-dependent increase in sodium ion permeability. They are involved, along with potassium channels, in determining the excitability of sensory neurones.

VGSCs can be classified into two types: those sensitive to the potent puffer fish toxin tetrodotoxin (tetrodotoxin-sensitive) and those in the second group which are insensitive to tetrodotoxin (TTXr) (Catterall 1992). Tetrodotoxin-sensitive channels are found in all sensory neurones, but TTXr channels are preferentially expressed by

nociceptor sensory afferents (Woolf and Costigan 1999). TTXr channels are likely to play an important role in nociceptive transmission and there is particular interest in the TTXr VSGC α subunit SNS Nav 1.8. Regarding TTXr sodium channels, a number of inflammatory mediators such as prostaglandin E2 (PGE2), serotonin and adenosine increase the rates of their activation and inactivation, decrease the activation threshold and increase the size of the current, i.e. cause sensitization (Catterall 1992). Animal studies reveal that TTXr sodium channels play an established role in sensitization of afferents and development of inflammatory hyperalgesia.

Mechanisms by which primary visceral afferent neurones contribute to visceral pain hypersensitivity may therefore include (i) peripheral inflammation, defined by ongoing cytokine expression in the absence of histological changes, (ii) visceral nerve damage and (iii) changes in the number or function of several ion channels. All of these potential mechanisms could result in visceral pain hypersensitivity without direct involvement of visceral afferent input to the CNS. However, it is more likely that the peripheral input adds to the CNS mechanisms, which also contribute significantly to visceral pain hypersensitivity.

Central sensitization is a key process in the development of persistent somatic pain hypersensitivity and previous studies have highlighted the importance of SP, neurokinin B, PGE2 and the NMDA receptor in its development and maintenance at the spinal level (Woolf 1995). Animal studies have demonstrated that following somatic inflammation, a positive correlation exists between visceral pain thresholds and increased afferent discharge of dorsal horn neurones demonstrating viscerosomatic convergence (Garrison, Chandler et al. 1992, Miranda, Peles et al. 2004). Spinal cFOS expression, a marker of dorsal horn activity, has been shown to be increased following noxious colorectal distension (Zhai and Traub 1999) and this is inhibited by NMDA receptor antagonism (Traub, Zhai et al. 2002). To address the question whether inflammation/injury can induce central sensitization in the human GI tract, a human model was developed, which demonstrated that infusion of hydrochloric acid into the healthy oesophagus reduced pain threshold not only in the acid-exposed region (peripheral) but also in the adjacent unexposed region (central). This effect was prolonged, lasting up to 5 h after 30 min of acid exposure. Repeat exposure after recovery significantly enhances the effect of the first infusion, suggesting that repeated injury can induce a progressive increase in hypersensitivity.

A major limitation of most visceral hypersensitivity studies is that they rely on

subjective methods of reporting sensation (Whitehead and Palsson 1998). To overcome this, a commonly used neurophysiological technique, cortical evoked potentials (CEPs), has been developed as a more objective correlate of oesophageal sensation. CEPs allow recording of cortical neuronal electrical fields generated in response to a peripheral nerve stimulus. Using this technique before and after acid infusion, a reduction in CEP latency was demonstrated, which suggests that facilitation of afferent pathway conduction accompanies central sensitization (Sarkar, Hobson et al. 2001). A recent study showed that administration of an antagonist at the PGE2-receptor EP1 prior to acid infusion blocks the development of oesophageal hypersensitivity, suggesting that prostaglandins play an important role in mediating peripheral and central sensitization.

Evidence for a role of SP in visceral nociception comes from several animal models, including neurokinin-1 (NK-1) knockout mice, which have shown an effect of NK-1RA on reducing visceral hyperalgesia (De Felipe, Herrero et al. 1998). An oral selective NK-1 receptor antagonist was used in the oesophageal model of central sensitization to assess the role of SP in human visceral hypersensitivity (Willert, Delaney et al. 2006). This demonstrated that the hypersensitivity induced in the proximal oesophagus (secondary allodynia) by acid infusion in the distal oesophagus was not inhibited by prior treatment with the NK-1 receptor antagonist.

Hypervigilance is a normal physiological state of the nervous system in response to perceived threat and enhanced arousal. Hypervigilance can be associated with enhanced sensitivity to visceral sensations, as seen in healthy individuals with sensations of palpitations and urgency when experiencing fear. Some patients with functional gastrointestinal disorders are chronically hypervigilant to physiological visceral stimuli in that they selectively attend to normally subthreshold visceral inputs (Labus, Bolus et al. 2004). Patients with IBS often present during times of increased personal stress (Mertz, Pickens et al. 2002), and evidence for a role of stress in visceral pain hypersensitivity comes from animal models of IBS where inducing stress causes the animals to develop visceral pain hypersensitivity to colonic distension during further periods of stress (Stam, Croiset et al. 1996).

Corticotropin-releasing factor (CRF) is implicated in stress-induced visceral pain hypersensitivity as it is released by the hypothalamus during increased limbic activity (Gue, Del Rio-Lacheze et al. 1997), activates the hypothalamic-pituitary-adrenal axis and results in increased cortisol production which may then facilitate intestinal

sensitivity and in increasing general arousal (Lechner, Curtis et al. 1997). What causes some to become hypervigilant or hyperresponsive to stress while others do not remains unclear, but as with the animal models above, a previous history of childhood adversity or significant life stressor appears to modulate your responses to stress and is implicated in your future risk of developing an functional gastrointestinal disorders. In a recent study in a large sample of tertiary care functional disorders patients, factor analysis was performed on dyspepsia symptoms to define patient subgroups; associations of symptoms with gastric pathophysiological mechanisms and psychosocial factors/psychiatric comorbidity were determined. It has also been shown that help-seeking IBS patients with comorbid psychiatric disorders are more likely to develop psychiatric disorders (especially anxiety disorders) before the onset of IBS (Sykes, Blanchard et al. 2003). This may suggest that _psychiatric symptoms, especially anxiety, play a role in the development of IBS. Finally, the evidence for a beneficial effect of psychotherapy and hypnosis in functional gastrointestinal disorderss is increasing (Gonsalkorale, Miller et al. 2003, Whorwell, Prior et al. 1984), and these point towards central mechanisms playing an important role in visceral pain hypersensitivity as they are probably acting on the limbic system, to reduce the effects of stress on the BGA.

Descending CNS pathways from the rostrolateral ventral medulla to the dorsal horn of the spinal cord are well described in somatic nociception (Urban and Gebhart 1999) where they have a tonic inhibitory effect (Sandkuhler and Gebhart 1984). Conversely, spinal visceral nociceptive transmission has both descending facilitatory and inhibitory inputs that produce a net facilitatory effect (Zhuo and Gebhart 2002). Alterations in this dynamic equilibrium between facilitatory and inhibitory inputs from the midbrain to the spinal dorsal horn neurones following central stress or peripheral inflammation lead to enhanced visceral pain perception, due to enhanced descending facilitatory influences or reduced inhibitory inputs (Mayer 2000).

The “visceral sensation/pain neuromatrix” was outlined by numerous functional brain imaging studies assessing brain responses during visceral stimulation. It consists of the cortical and subcortical regions described above (Derbyshire 2003). A recent study confirmed the involvement of several distinct brainstem regions, including the periaqueductal grey and rostrolateral ventral medulla regions, in the processing of visceral sensation (Dunckley, Wise et al. 2005). In a recent positron emission tomography study, Damasio et al. induced four different emotions in healthy

volunteers using autobiographical memory scripts, providing a neurobiological link between emotions and visceral sensation (Damasio, Grabowski et al. 2000). Several recent studies have compared brain responses during GI distension between patients and healthy controls, providing evidence for abnormalities in the affective and/or cognitive dimension of the pain experience, which might be one aspect of a more generalized state of negative affectivity (Jones, Dilley, et al. 2006). In addition, IBS patients showed higher ACC activity during painful rectal distension when compared with healthy volunteers (Mertz, Morgan, et al. 2000). Upregulation of visceral afferent input or increased ACC response may account for these findings. However, lower or absent ACC activity was found as a brain response to rectal distension in IBS patients when compared with controls (Wilder-Smith, Schindler et al. 2004). It should be noted that heterogeneity in patient samples, stimuli applied and imaging methods used may at least partly account for the discrepancies in brain imaging findings in IBS (Hobson and Aziz 2004). In conclusion, despite these discrepancies, there is a growing body of evidence supporting abnormal affective processing of visceral sensation in patients with IBS and non-cardiac chest pain. Furthermore, it has recently been shown that cognitive behavioural therapy is associated with a reduction of baseline activity in the right subgenual ACC and the left medial temporal lobe (including the amygdala) of IBS patients, which was accompanied by improvements in GI symptoms, anxiety and worry. These changes in brain activity may be the consequences of reduced attention to visceral stimuli or visceral-specific anxiety as results of cognitive behavioural therapy in these patients (Lackner, Lou Coad et al. 2006).

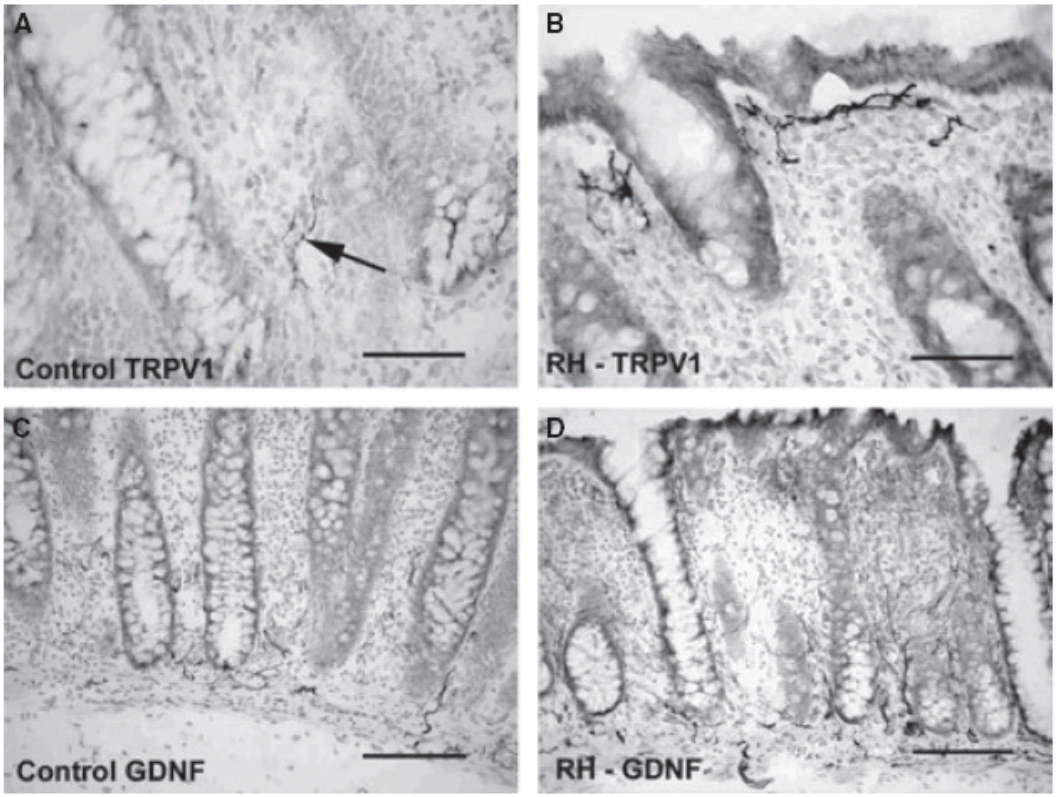


Figure 1.6: Immunohistochemical staining positive for TRPV1 and GDNF in rectal hypersensitivity. Capsaicin receptor (TRPV1) immunoreactive nerve fibres within rectal mucosa from healthy controls (A), and patients with rectal hypersensitivity (B); GDNF-immunoreactive fibres within rectal mucosa from healthy controls (C), and patients with rectal hypersensitivity (D). Scale bars: (a, b) 50 μm; (c, d) 100 μm (Bar, Facer, et al. 1997).

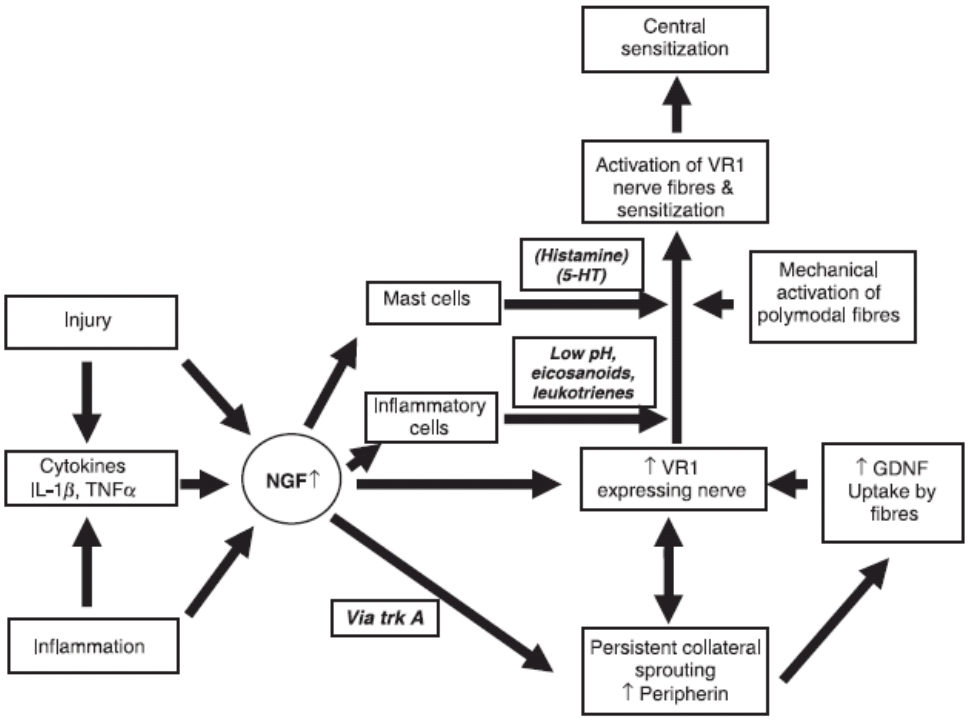


Figure 1.7: Proposed molecular mechanism for visceral hypersensitivity (Chan, Facer, et al. 2003).

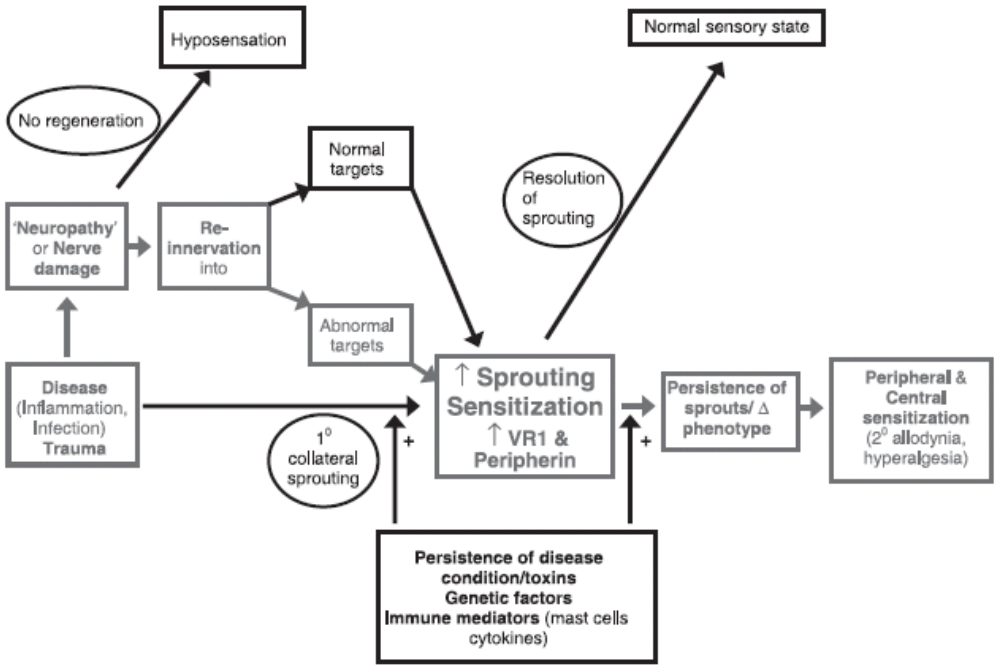


Figure 1.8: Proposed pathways leading to chronic pain and hypersensitivity (Chan, Facer, et al. 2003).

1.5 Evaluation of and validity of techniques for measurement of sensitivity and hypersensitivity

In human, there are two widely published techniques used in the evaluation of visceral sensation *in vivo* (Figure1.9). The first measures the perception of mechanical, electrical, or other stimuli applied within the gut, and quantifies this by applying a standardized symptom based questionnaires (visual analogue scale or adjectival scale) to determine thresholds or severity of symptoms induced. The second measures alterations in cerebral blood flow using positron emission tomography (PET), functional magnetic resonance imaging (fMRI), or single photon emission computed tomography (SPECT) during visceral stimulation. These techniques permit identification of the regions of the brain that are activated during the stimulus.

 # 1.5.1 Methods of measuring visceral thresholds

Methods to assess the threshold for initial perception or discomfort/pain include the ascending method of limits, tracking, or a random staircase design (Distrutti, Azpiroz et al. 1999, Whitehead and Delvaux 1997). In these studies, progressively increased pressure or volume distensions are performed until the subject perceives either first sensation or the symptom of discomfort/pain. After the threshold is achieved, a computer program randomly produces a pressure or volume stimulus which is either above or below the previously identified threshold. This allows fine tuning of the level of the threshold for either volume or pressure distensions. The stimulus paradigm, in the random staircase method, does not necessarily increase continually as in ascending method of limits but randomly applies the stimulus either of greater or lower intensity to try to eliminate response bias (Whitehead and Palsson 1998).

While these methods have been widely applied in the literature, their sensitivity and potential for response bias have not been adequately assessed. For example, when ascending method of limits and tracking are used to evaluate first perception and pain, it is likely that the subject will be interrogated 40 or 50 times while assessing the thresholds. This clearly could lead to an element of response bias which is probably worse with increasing number of distensions. In order to avoid the potential inaccuracies produced by response bias, recent studies have used a restricted number (three to five) of distensions using pressure based mechanical stimuli which are performed in a randomized order (Bharucha, Camilleri et al. 1996, Ford, Camilleri et

al. 1995, Thumshirn, Camilleri et al. 1999). During distensions, the subject is asked to complete a visual analogue scale pertaining to the symptoms that are of interest, for example, pain and gas in the colon; pain and urgency in the rectum; or bloating, nausea, and pain in the stomach.

 # 1.5.2 Cerebral blood flow measurements

Cerebral blood flow measurements are designed to identify the projections in the brain of visceral stimuli originated in the gut. It is important to understand that alterations in cerebral blood flow detected by these methods vary from 2% to 5%. Sensitivity to detect increases in cerebral blood flow over background activity, with variations unrelated to the specific stimulus, is somewhat vulnerable due to the relatively low absolute changes in blood flow that can be expected (Kern, Birn et al. 1998, Silverman, Munakata et al. 1997). Therefore, there is a low signal to noise ratio which renders interpretation difficult.

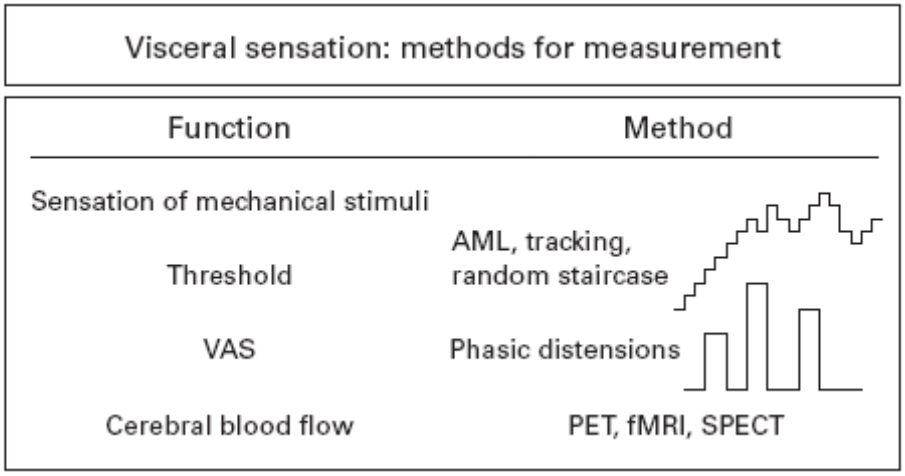


Figure 1.9: Distension paradigms used in visceral sensation studies. PET, positron emission tomography; fMRI, functional magnetic resonance imaging; SPECT, single photon emission computed tomography; VAS, visual analogue scale; AML, ascending method of limits (Camilleri, Coulie, et al. 2001).

Chapter 2

Oesophageal protective reflexes and secondary peristalsis

2.1 Overview

The oesophagus is a hollow muscular tube with close ends proximally and distally by muscular sphincters. The oesophageal wall has 2 distinct layers, of which the inner mucosal layer consists of squamous epithelium and underlying connective tissue, within which lies a longitudinally oriented muscle layer called the muscularis mucosa. The outer muscular coat, known as the muscularis propria, is involved in bolus transport and consists of an inner layer of circularly oriented muscle fibers and an outer layer of longitudinally oriented fibers. The myenteric plexus lies between these two muscle layers, controlling the motor function of these muscles. The upper oesophageal sphincter (UOS) and proximal one third of oesophageal body is composed of striated muscle. There is then a transition zone where striated and smooth muscle mix together. The lower oesophageal sphincter (LOS) and the distal one half to two thirds of the oesophageal body are composed of smooth muscle.

Oesophageal peristalsis results from sequential contraction of circular muscle, which serves to push the ingested food bolus toward the stomach. Oesophageal longitudinal muscle may also play a role in peristalsis. Swallow-induced peristalsis is called primary peristalsis, and the peristalsis elicited by oesophageal distention is called secondary peristalsis. Through an elaborate reflex mechanism, a close functional relationship exists among the pharynx, larynx, and oesophagus during both anterograde and retrograde transit, which also helps protect the airway against aspiration. These protective reflexes (1) enhance the UOS pressure, such as oesophago-UOS contractile reflex (OUCR), pharyngo–Upper Oesophageal Sphincter Contractile Reflex (PUCR), and laryngo-UOS contractile reflex (LUOS-C); (2) close the vocal cord, such as oesophagoglottal closure Reflex (OGCR), pharyngo-glottal closure reflex (PGCR), and laryngeal adductor reflex; or (3) clear the contents from the pharynx and oesophagus, including secondary oesophageal peristalsis and pharyngeal reflexive swallow.

This chapter will discuss current knowledge about oesophageal protective reflexes and secondary peristalsis. The review will specially focus on the physiological mechanisms underlying secondary peristalsis. The clinical and functional significance of secondary peristalsis is discussed as well.

2.2 Mechanisms of oesophago-pharyngo-laryngeal protective reflexes

This chapter will discuss the mechanisms of the reflexes responsible for oesophago-pharyngo-laryngeal protective function.

#2.2.1 Oesophago–Upper Oesophageal Sphincter Contractile Reflex

The UOS is one of the major components of the airway protective mechanisms against entry of gastrooesophageal refluxate into the pharynx and larynx (Shaker, Ren et al. 1993). UOS during gastro-oesophageal reflux (GOR) events has been of interest and the subject of several previous studies. However, the results are controversial (Gerhardt, Shuck et al. 1978, Vakil and Sparberg 1989). So far, the UOS function during GOR events was not completely understood. This difficulty is due to the fact that GOR results in both intraluminal pressure increase and pH changes. The most critical time for the UOS protective function during a reflux event begins with the onset of the entry of refluxate into the oesophagus until its clearance from the oesophagus by a secondary or primary peristalsis. In normal controls and patients with reflux oesophagitis, UOS pressure change at the onset of reflux events was evaluated by Torrico et al. (Torrico, Kern et al. 2000). A total of 321 reflux events were identified by the development of abrupt reflux-induced intraoesophageal pressure increase; 285 events occurred in patients and 36 in controls. In control subjects, 33 of 36 and in patients 252 of 285 intraoesophageal pressure increase events were associated with a pH drop. In patients and controls, 99% and 100%, respectively, of all intraoesophageal pressure increase events irrespective of a pH drop were associated with an abrupt increase in UOS pressure. The average percentage of maximum UOS pressure increase over pre-reflux values ranged between 66% and 96% (control subjects) and 34% and 122% (patients). These pressure increases lasted 5 to 25 seconds. These findings suggest the existence of a strong positive relationship between UOS tone and intraoesophageal pressure increase induced by GOR events, shown previously by experimental distention. Although both acidic and nonacidic reflux events induce UOS contraction, an intraluminal pH below 4 seems to modulate this contractile response.

#2.2.2 Oesophagoglottal Closure Reflex (OGCR)

Some GOR episodes, especially those of large volume, may cause an instantaneous increase in intra-oesophageal pressure that might overcome the UOS.

This circumstance could potentially leave the upper airway vulnerable to aspiration. Studies have documented the existence of an OGCR in humans (Shaker, Dodds et al. 1992) as well as in the feline models (Shaker, Ren et al. 1994a). Stimulation of OGCR reflex results in adduction of the vocal cords and closure of the introitus to the trachea. To generate this reflex, oesophageal distention may involve the entire body of the oesophagus, such as distentions induced by air insufflation, or it may be regional, such as those caused by a short balloon.

The OGCR is an example of close coordination between digestive and respiratory systems during retrograde oesophageal transit. The physiologic role of the OGCR could be postulated to be one of the airway protective mechanisms during retrograde oesophageal and pharyngeal transit, such as those occurring during belching, GOR, regurgitation, and possibly vomiting. Studies have documented that this reflex is evoked during spontaneous GOR episodes, however, this reflex is absent in about half of the patients over the age of 70 years (Ren, Shaker et al. 1991).

Under experimental conditions there is a direct relationship between the duration of vocal cords closure and magnitude of the oesophageal distention by a balloon. Also this reflex is triggered more frequently by proximal oesophageal than distal oesophageal distention (Shaker, Dodds et al. 1991). This could be attributed to differential distribution and the phenotype of the vagal afferent fibers innervating the different regions of the oesophagus (Kressel and Radespiel-Troger 1999). Another possible explanation for this phenomenon could be that the proximal oesophagus also receives innervation from the recurrent laryngeal nerve (Kobler, Datta et al. 1994). Therefore, stimulation of the richly innervated proximal oesophagus by two branches of the vagus, that is, the cervical vagus and recurrent laryngeal nerve, can further facilitate activation of OGCR.

#2.2.3 Pharyngeal Reflexive Swallow (PS)

Mechanical stimulation of the pharynx can trigger an irrepressible swallow, the pharyngeal reflexive swallow (Nishino 1993, Paterson, Rattan et al. 1986). This local stimulus for the initiation of swallowing may play a role in airway protection from pharyngeal reflux of gastric contents and inadvertent spillage of oral contents into the pharynx during the preparatory phase of swallowing.

The threshold volume to trigger pharyngeal reflexive swallow in healthy elderly is significantly larger than that required for young volunteers (Shaker, Ren et al. 1994b). Swallows triggered by direct stimulation of the pharynx are different from primary swallows by not inducing sequential contact of the proximal tongue with the hard palate known to occur during primary swallows (Shaker, Ren, et al. 1994b). Therefore, pharyngeal reflexive swallow does not result in transit of the oral bolus while it clears the pharynx. In this regard, pharyngeal reflexive swallow is similar to secondary oesophageal peristalsis, which usually spares the activation of the peristaltic wave from areas proximal to the point of stimulation (Paterson, Hynna-Liepert et al. 1991). Except for lingual peristalsis and transit of oral bolus, the rest of the deglutitive biomechanical events during both types of swallows were found to be similar.

From a functional point of view, therefore, pharyngeal reflexive swallow may help prevent aspiration by two mechanisms: (1) Activating the swallow-induced glottal closure (which, in turn, seals off the airway and prevents possible aspiration of material that may either fall into the pharynx inadvertently during the preparatory phase of swallowing or enter the pharynx during GOR episodes); and (2) clearing the pharynx of materials that enter it during reflux from the oesophagus.

#2.2.4 Pharyngo–Upper Oesophageal Sphincter Contractile Reflex (PUCR)

Pharyngeal mechanical stimulation in cats (Medda, Lang et al. 1994) and water stimulation in humans (Shaker, Ren et al. 1997) induce an increase in the resting tone of the UOS—the PUCR. This reflex has been suggested to be an airway-protective mechanism whereby retrograde entry of small volumes of liquid into the pharynx from the stomach can result in augmentation of UOS tone, reducing the chance of further regurgitation into the pharynx (Shaker, Ren, et al. 1997).

Contrary to rapid water stimulation that results in an abrupt UOS pressure increase, during slow, continuous water injection into the pharynx, the UOS pressure increases gradually before the occurrence of the pharyngeal swallow(Shaker, Ren, et al. 1997). The afferent limb of PUCR is the glossopharyngeal nerve. In animal studies (Medda, Lang, et al. 1994), cutting the glossopharyngeal nerves blocked the PUCR but did not block the OUCR or the responses of the thyropharyngeus or cricopharyngeus muscles during swallowing (Medda, Lang, et al. 1994). The efferent

limb of the PUCR is the pharyngooesophageal nerve that branch from the vagal trunk just rostral to the nodose ganglion (McClure, Dallman et al. 1973). Transection of the pharyngooesophageal nerve eliminates basal tone of the cricopharyngeus muscle and blocks its response to all reflex stimuli—PUCR, OUCR, and swallowing (Medda, Lang, et al. 1994). Transection of the vagus nerves at the cervical level (i.e., below the nodose ganglion) had no effect on the PUCR, but blocked the OUCR, which indicates that the recurrent laryngeal nerve (that branches from the vagal trunk in the thoracic cavity) serves no role in this reflex (Medda, Lang, et al. 1994). Topical anesthesia of the pharyngeal mucosa completely abolished this reflex (Ren, Xie et al. 2000). Application of local anesthetics (2% lidocaine) to the pharyngeal mucosa blocks the contractile responses of the cricopharyngeus muscle to pharyngeal stimulation, but not to its response to oesophageal distention (Medda, Lang, et al. 1994). The results suggest the involvement of pharyngeal mucosal mechanoreceptors in eliciting the PUCR.

#2.2.5 Pharyngo-glottal Closure Reflex (PGCR)

Injection of minute amounts of water into the pharynx can lead to brief closure of the vocal cords (Shaker, Ren et al. 2003). Gradual entry of liquid into the pharynx leads to partial adduction, whereas rapid injection causes complete closure of the cords. It is suggested that this reflex is part of a complex mechanisms that protect the airway from aspiration. The threshold volume for inducing this reflex is reported to be significantly smaller than that required to trigger a pharyngeal (reflexive) swallow, but similar to that required to induce a PUCR (Shaker, Ren, et al. 2003).

#2.2.6 Laryngo–Upper Oesophageal Sphincter Contractile Reflex (LUOS-C)

Direct stimulation of the larynx induces a brief adduction of the vocal cords and arytenoids, closing the introitus to the trachea—the vagovagal laryngeal adductors reflex. Because the UOS and larynx both are innervated by the vagus, it is conceivable that stimulation of the larynx may induce contraction of the UOS (Kawamura, Easterling et al. 2004). Using an air stimulation technique, recent studies have shown that afferent signals originating from the larynx induce contraction of the UOS—the LUOS-C (Kawamura, Easterling, et al. 2004). The afferent arm of this reflex in humans includes the laryngeal mechanoreceptor and internal division of the

superior laryngeal nerve (Sasaki and Weaver 1997), a branch of the vagus nerve. The efferent arm is undoubtedly the vagus nerve (Sasaki 2000), including the superior laryngeal nerve and recurrent laryngeal nerve, although the glossopharyngeal nerve cannot be excluded because it serves branches into the pharyngeal plexus. The central control for this reflex is probably different from those of swallowing because the contractile response to the stimulation of this reflex is the opposite of the relaxation response of the UOS to a volitional, subconscious, and reflexive pharyngeal swallow.

LUOS-C is different from the PUCR that is triggered by stimulation of mechanoreceptors in the posterior pharyngeal wall (Shaker, Ren, et al. 1997). Although the effector organ and efferent arc are the same for both reflexes, the sensory field and the afferent arc are different, in that the PUCR is mediated via the glossopharyngeal nerve with possible contribution from the superior laryngeal nerve.

#2.2.7 Pharyngooesophageal Inhibitory Reflex(PEIR)

In humans as well as experimental animals, it has been shown that pharyngeal mechanical stimulation by air or water injection produces a general inhibition of both primary and secondary peristalsis—the PEIR (Lang, Medda et al. 1998, Ren, Shaker et al. 1995).

Inhibition of the progression of primary oesophageal peristalsis by sensory impulses initiated form the pharynx by water injection would inhibit oesophageal bolus transit (Bardan, Xie et al. 1997). This inhibitory effect can overcome the facilitating effect that the presence of a bolus induces on the swallowing apparatus. The threshold volume for inhibiting the progression of peristalsis induced by swallowing liquid boluses is significantly higher than that induced by the dry swallows (Bardan, Xie, et al. 1997). Inhibition of oesophageal peristalsis by PEIR is not followed by generation of a new peristatic pressure wave. This phenomenon is different from inhibition of an ongoing peristaltic wave by a closely timed second swallow, which results in inhibition of peristalsis due to the first swallow and generation of peristalsis by the second (Lang, Medda, et al. 1998, Ren, Shaker, et al. 1995). The PEIR occurs in both the striated and smooth muscle portion of the oesophagus, but its effect is relatively stronger in the striated muscle compared to smooth muscle (Bardan, Xie, et al. 1997). This difference is attributed to the different nature of innervation between the two types of oesophageal muscles.

In summary, there are a large number of anterograde and retrograde vagovagal

reflexes that provide oesophago-pharyngo-laryngeal protective function. Although most of these reflexes are well defined and extensively studied under different conditions, a few have been reported recently and need to be further investigated. Hyper- or hypo-responsiveness of any of these reflexes can potentially harm the coordination between the upper gastrointestinal and the aero-digestive systems.

2.3 Current understanding of normal secondary peristalsis

Secondary peristalsis refers to peristalsis or reflex activated by oesophageal distension. This can occur physiologically by food left behind after the primary peristaltic wave has passed, or by an episode of GOR. Unlike primary peristalsis, secondary peristalsis is not accompanied by deglutition with associated UOS and pharyngeal motor function. Although primary peristalsis is the most important motor event to respond to acid reflux, secondary peristalsis may be important during sleep when swallowing is suppressed.

In this chapter, we will discuss the currently available knowledge on the determination and functional significance of secondary peristalsis. The neural pathways that mediate secondary peristalsis will also be described. By focusing on oesophageal motor disorders, we will present evidences that link disordered second peristalsis to the pathophysiology in patients with a variety of oesophageal disorders.

#2.3.1 Determination of functional significance of secondary peristalsis

Secondary peristalsis occurs in response to oesophageal distension. Physiologically, it occurs if food, liquid or air is retained in the oesophagus after a failed primary peristaltic event or after a reflux from the stomach. Functionally, it is of protective importance in maintaining an empty oesophagus by clearing the bulk of the volume of the refluxate after a reflux event (Helm, Dodds et al. 1984). This helps the return to normal values of oesophageal pH by primary peristalsis and swallowed saliva (Helm, Dodds, et al. 1984) by preventing prolonged contact time between the refluxed gastric acid and the oesophageal mucosa (Corazziari, Pozzessere et al. 1978).

Meltzer first defined secondary peristalsis in animal experiments in 1906 (Meltzer 1906). From then on, several studies have used different stimuli such as oesophageal balloon distension and infusion with air or water, but their results are inconsistent due to technical differences in distention volume, level of infusion, or duration of distension (Creamer and Schlegel 1957, Fleshler, Hendrix et al. 1959, Paterson, Rattan et al. 1988). Earlier studies recorded data of few recording sites

inside the oesophagus and used manometric techniques with low recording fidelity that limit the validity of data on peristaltic parameters.

A previous study has evaluated the triggering and characteristics of secondary oesophageal peristalsis (Schoeman and Holloway 1994b). In this study, secondary peristalsis was stimulated by rapid intraoesophageal injections of air and water, and by a five-second oesophageal distension with a balloon. The authors found that both air and water boluses produced complete secondary peristaltic responses that, regardless of the level of injection, started at the most proximal oesophageal recording site and traversed the entire length of the oesophageal body (Figure 2.1). However, balloon distension produced a different pattern of secondary peristalsis from that of air or water boluses (Figure 2.2). Characteristically, during distension there was a high amplitude synchronous contraction above the balloon while below there was motor quiescence. After distension, the synchronous contraction above the balloon subsided and a peristaltic contraction wave progressed distally from the level of the balloon. The variability in the response to oesophageal distension may reflect the conflicting results of previous studies, and shows that oesophageal responses to distension can only be considered in the context of a specified stimulus.

The balloon provides a focal stimulus that cannot be moved by any induced motor response. Conversely, air and water disperse along the oesophagus and can be moved forward by any induced propagated wave. The moving bolus may also serve to reinforce the response in a manner similar to that of water swallows in primary peristalsis (Hollis and Castell 1975, Janssens, Valembois et al. 1974). Previous study indicate that stimulation of the striated muscle segment of the oesophagus may be an important factor in triggering secondary peristalsis through activation of a central reflex pathway (Blank, Greenwood et al. 1989, Paterson, Hynna-Liepert, et al. 1991). In addition, the air and water boluses invariably induced a small common cavity pressure rise throughout the oesophagus at the time of injection showing that the striated muscle segment was distended whatever the site of injection (Schoeman and Holloway 1994b). However, the effect could not been observed with balloon distension, whereas the balloon distension at the mid-oesophagus, was significantly better at stimulating peristalsis than at the upper oesophagus (Schoeman and Holloway 1994b)). The discrepancy may relate the transition from striated to smooth muscle at mid-oesophagus.

In summary, secondary peristalsis is an important mechanism for the clearance of

retained refluxate or material from the oesophagus and impaired secondary peristalsis may possibly be a mechanism contributing to the pathogenesis of reflux disease or dysphagia. Therefore, determination and measurement of secondary peristalsis could therefore useful in assessing these problems. It can be easily and reliably tested by injecting air or water boluses through the manometric catheter (Schoeman and Holloway 1994b). However, it appears that balloon distension is less ideal when compared to air or water injection for testing secondary peristalsis.

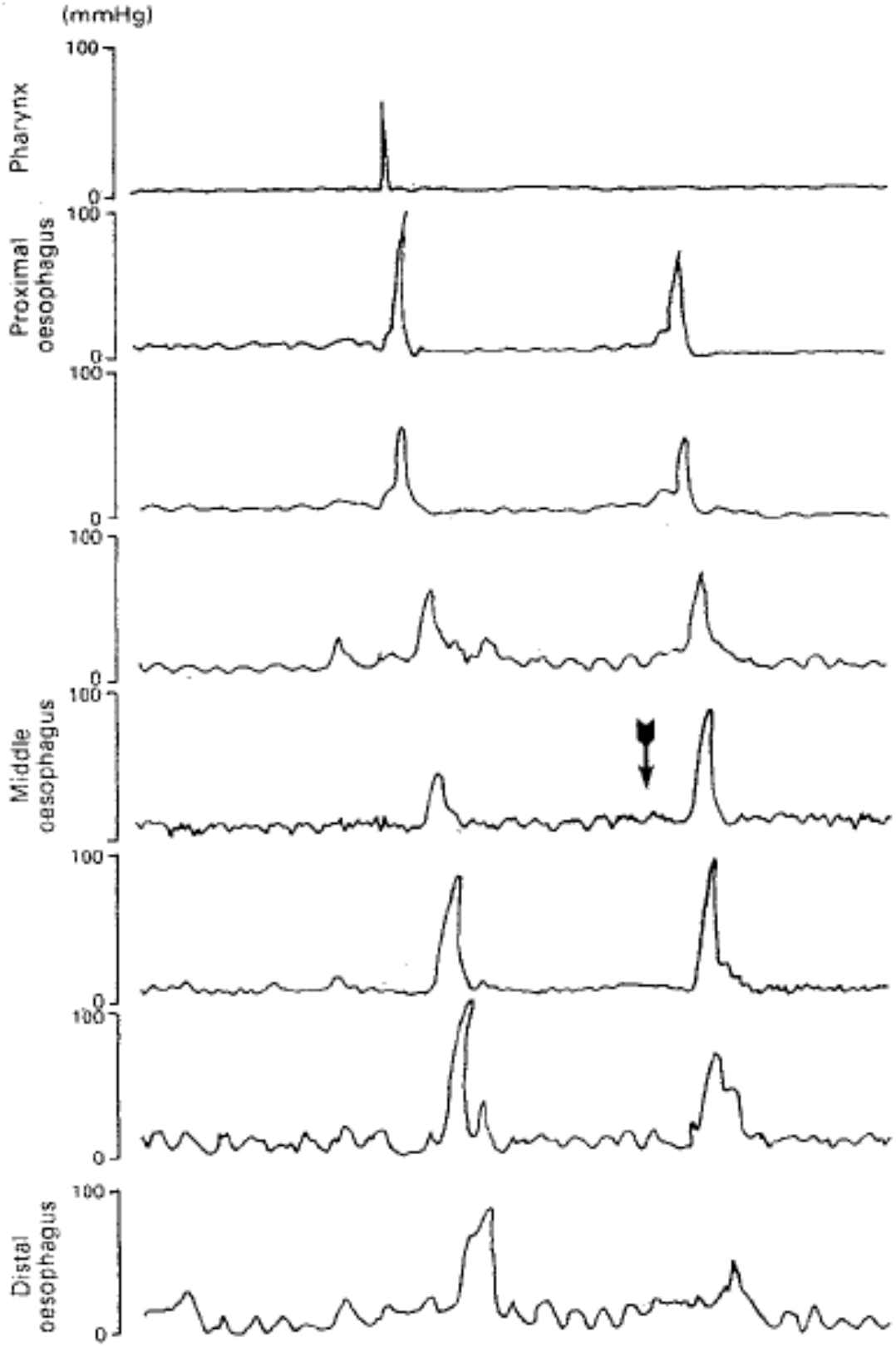


Figure 2.1: The tracing shows primary peristalsis triggered by a water swallow and secondary peristalsis triggered by a 10-ml air bolus injection. The position of the arrow shows the time and level of bolus injection (Schoeman and Holloway 1994b).

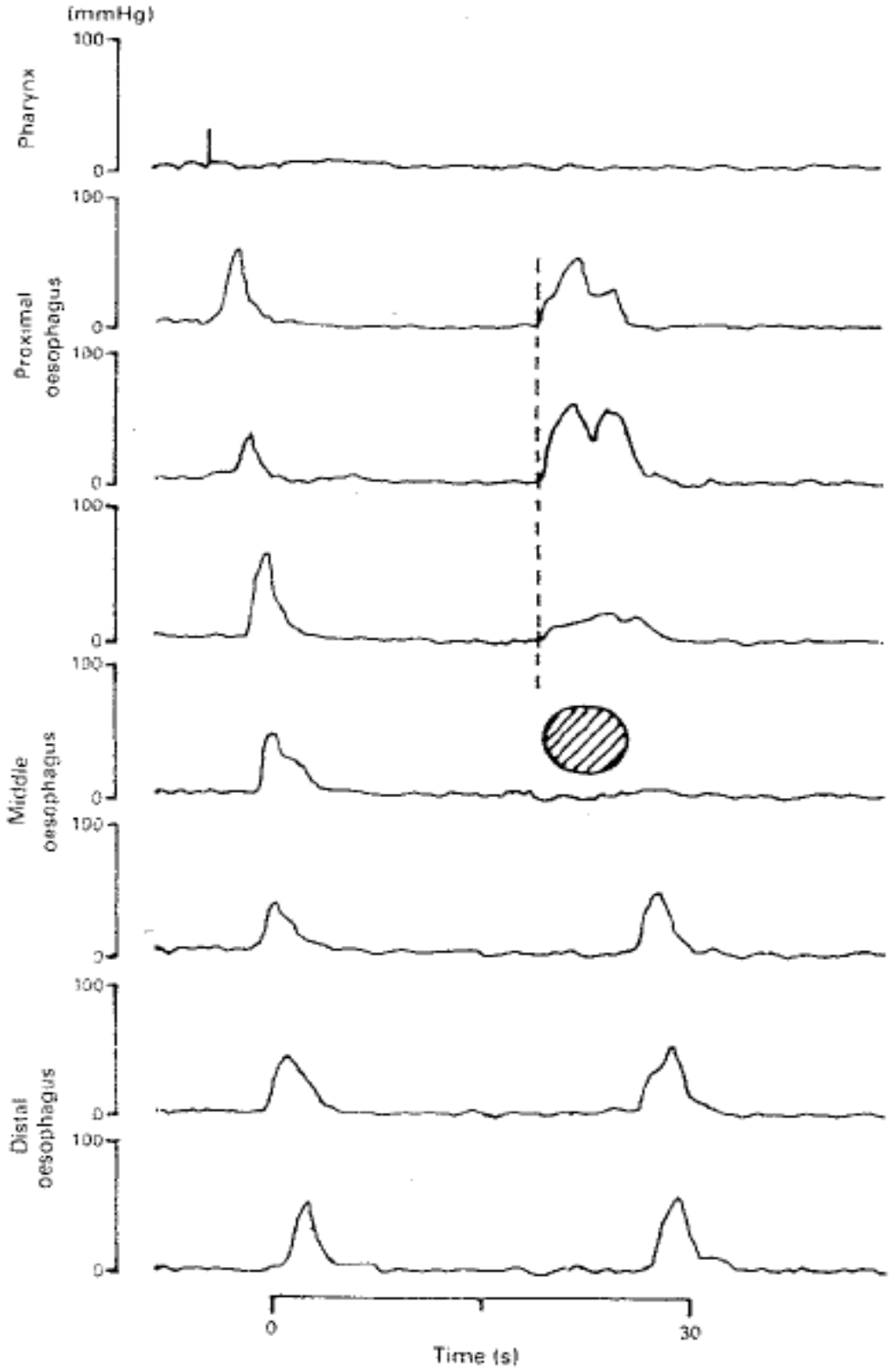


Figure 2.2: Pressure tracing showing primary peristalsis triggered by a water swallow and secondary peristalsis triggered by balloon distension. The position of the schematic balloon illustrated shows the level and duration of balloon distension (Schoeman and Holloway 1994b).

#2.3.2 Manometric characteristics of secondary peristalsis

Oesophageal peristalsis is traditionally separated into two types: primary, set off by swallowing and propagated by stimuli directing from a swallowing center; and secondary, initiated by oesophageal distention and propagated by sequential short reflex arcs stimulated by the moving bolus. The studies underlying this concept of secondary peristalsis made use, however, of a moving bolus, a circumstance which makes it impossible to tell whether secondary peristalsis, once elicited, would similarly pass down the oesophagus were no bolus present.

In an earlier study by Fleshler et al. in 1958 (Fleshler, Hendrix, et al. 1959), secondary peristalsis was initiated by distending an oesophageal balloon, and by measuring the resultant motor and inhibitory phenomena above and below the balloon with the aid of manometric devices too small to interfere motility. When the balloon was distended momentarily, the high resting pressure in the zone of the LOS fell, and a pressure wave travelled down the oesophagus. The total sequence was identical to that produced when primary peristalsis is initiated by a dry swallow (Figure 2.3). By varying the method of balloon inflation, motor and inhibitory phenomena identical to those produced by rapidly or slowly repeated swallows were obtained. Above the distending balloon, non-propulsive contractions were the usual reactions to balloon distention. No evidence was obtained to indicate that a nervous swallowing center was controlling the secondary peristaltic phenomena elicited.

The propagation of secondary peristalsis thus does not require a moving bolus to stimulate sequential motor reflexes, nor does it appear to be dominated by a swallowing center. The orderly progress of secondary peristalsis presumably depends, therefore, on impulses released by the contraction itself and transmitted via vago-vagal, myenteric plexus, and perhaps intermuscular activity.

Until recently, Schoeman systemically evaluated the triggering characteristics of secondary peristalsis and defined the distension induced oesophageal motor responses in healthy subjects (Schoeman and Holloway 1994b). They observed air and water boluses triggered secondary peristalsis that started in the proximal oesophagus regardless of injection site. Response rates were volume dependent with 83% of the 20 ml air boluses triggering secondary peristalsis compared with 2% for the 2 ml water bolus. Response rates for air and water were similar for equal bolus volumes and were not affected by the site of the injection. Secondary peristaltic amplitude was less than that of primary peristalsis. In addition, secondary peristaltic amplitude was

less than that of primary peristalsis. It was concluded that the manometric characteristics of complete secondary peristalsis were similar to those of primary peristalsis.

However, a previous study has demonstrated contrary finding by showing different oesophageal responses between swallowing and balloon distension in human (Paterson, Hynna-Liepert, et al. 1991). In comparison to the swallow-induced contractions, contractions induced by balloon distension aboral to the distending balloon are of low amplitude, and more often nonperistaltic. In addition, when balloon distension-induced contractions propagate in a sequential fashion, the speed of peristalsis is significantly faster in the mid-oesophagus than the corresponding swallow-induced peristaltic wave.

The explanation for these discrepancies can be possibly addressed by the fact that when distension occurs in the striated muscle segment, the peristaltic response is mediated by central mechanism and therefore mimics primary peristalsis; the response to distension in the smooth muscle segment, on the other hand, may be different because it is mediated by enteric nervous system (ENS). The notion is further supported by a study in opossum, which revealed a significant difference between swallow-induced peristalsis and secondary peristalsis when the aboral distension-induced response was performed in smooth muscle part of the oesophagus (Paterson, Rattan, et al. 1988).

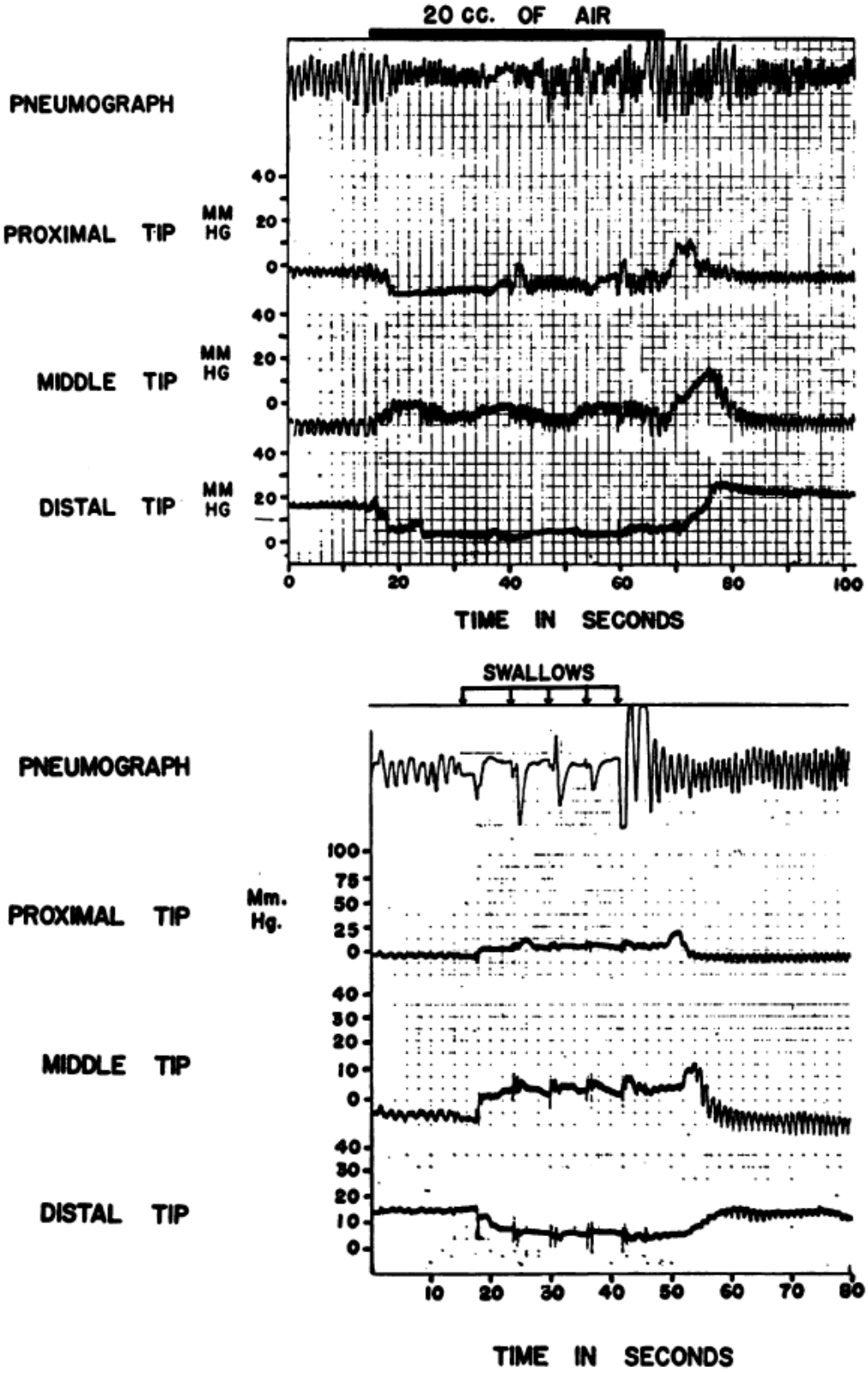


Figure 2.3: Pressure tracings during prolonged distension (Upper) (secondary peristalsis) and during frequent swallowings (Lower) (primary peristalsis) in the oesophagus. Both motor sequences, primary vs. secondary peristalsis, are identical.

#2.3.3 Current knowledge of the responsible neural pathways

Unlike primary peristalsis, secondary peristalsis is not accompanied by deglutition with associated pharyngeal and UOS motor function. In the striated muscle oesophagus, distention activates a peristaltic reflex that is mediated by central mechanisms; distention activates vagal afferents, which consequently turn leads to sequential vagal efferent discharge to the striated musculature of the proximal oesophagus (Hwang 1954). On the other hand, secondary peristalsis in the smooth muscle oesophagus is mainly an intrinsic neuromuscular reflex. Indeed, luminal distention of an oesophagus excised and placed in a tissue bath results in a peristaltic contraction (Christensen 1970, Paterson and Indrakrishnan 1995). The luminal distention triggers an immediate contraction just at and proximal to the distending stimulus, which occurs in the presence of tetrodotoxin, suggesting that it is a purely myogenic contractile reflex (Paterson, Hynna-Liepert, et al. 1991)). In the intact animal, however, contractions mediated by a central vagal reflex occur for a few centimeters proximal to the distending stimulus (Paterson, Hynna-Liepert, et al. 1991). Direct electrical recordings from the circular smooth muscle proximal to the distending stimulus in the opossum model have demonstrated a direct depolarization in response to balloon distention that is blocked by either bilateral cervical vagotomy or the administration of atropine (Paterson, Hynna-Liepert, et al. 1991). This indicates a direct evidence of separate vagal innervation of excitatory and inhibitory motor neurons.

A similar, atropine-sensitive contraction orad to the balloon is also seen in humans (Paterson, Hynna-Liepert, et al. 1991). Aboral to the distending stimulus, central mechanisms do not appear to play a role. During the distending stimulus there is a descending inhibitory discharge, mediated predominantly by nitrous oxide, which results in hyperpolarization and inhibition of the circular smooth muscle (Anand and Paterson 1994, Sifrim and Janssens 1996). This is then followed by rebound depolarization, spike bursts, and contraction. This peristaltic reflex is quite different from that described in the intestine, where the proximal excitation does not involve extrinsic innervation (Bornstein, Costa et al. 2004). In the opossum oesophagus, interneurons do not appear to be involved. Neverthless, descending nitrergic neurons appear to be activated directly by the distending stimulus and send long descending inhibitory neural connections to the distal oesophagus (Muinuddin, Ji et al. 2004, Paterson and Indrakrishnan 1995).

#2.3.4 Clearance capability of secondary peristalsis

Studies have not directly evaluated clearance capability of secondary peristalsis. However, previous studies have examined the relationship between oesophageal body motility and clearance of spontaneous acid reflux episodes. A study done in recumbent normal subjects showed that peristaltic failure or incomplete peristaltic sequences were associated with prolongation of acid clearance and a smaller increment in oesophageal pH than were complete peristaltic sequences (Dent, Dodds et al. 1980). Similar findings were established in a comparable study in patients with reflux disease (Dodds, Kahrilas et al. 1990).

The interrelationship between oesophageal volume clearance and peristaltic function has been extensively assessed by a previous study using concurrent videofluoroscopic and manometric recordings in patients with non-obstructive dysphagia (NOD) or heartburn (Kahrilas, Dodds et al. 1988b). A single normal peristaltic wave resulted in complete clearance of a barium bolus from the oesophagus, whereas absent or incomplete peristaltic contractions invariably resulted in little or no volume clearance. Regional hypotensive peristalsis was associated with incomplete volume clearance by the mechanism of retrograde escape of barium through the region of hypotensive contraction. The mean peristaltic amplitude associated with instances of retrograde escape was 25 mmHg in the distal oesophagus compared with 12 mmHg in the proximal oesophageal segments. The authors concluded that the peristaltic dysfunction commonly seen in patients with oesophagitis likely leads to impaired volume clearance.

Since there are minimal data regarding the characteristics of oesophageal bolus transport and clearance by secondary peristalsis, we will determine oesophageal bolus transit and clearance by secondary peristalsis in Chapter 8.

#2.3.5 Clinical significance of secondary peristalsis

Oesophageal dysphagia is a common presentation that indicates impaired transport of a swallowed bolus along the oesophagus. In patients with NOD, there is usually no evidence of mechanical obstruction and this presentation is more relevant to oesophageal motor dysfunction (Jacob, Kahrilas et al. 1990). The link between such symptom and impaired primary peristalsis is still unclear (Katz, Dalton, et al. 1987, Ott, Richter et al. 1987)). The possible implication for these discrepancies is that there is an undetected oesophageal motor abnormality. Therefore, a possible

explanation for such presentation could be an abnormality in secondary peristalsis. In 1994, Schoeman et al. have systematically examined the motor characteristics of secondary peristalsis in patients with NOD (Schoeman and Holloway 1994b). Secondary peristalsis was stimulated with either 10-ml air or water injected in the mid-oesophagus and by a 3-cm balloon distension. The major finding is that patients with NOD had significantly less response rate to both air and water distensions when compared with normal age matched healthy subjects, however, the group difference could not be observed in balloon distension. These authors concluded that NOD patients show a defect in the triggering of secondary peristalsis which might consequently lead to impaired oesophageal bolus clearance as well as dysphagia in this condition.

Abnormal secondary peristalsis is thus a potential mechanism that might account for these discrepancies, as secondary peristalsis is important for oesophageal volume clearance (Dent, Dodds, et al. 1980). The implication from their results (Schoeman and Holloway 1994b) is that the patients would similarly fail to respond to the distension induced by a retained bolus in the oesophagus. Although standard manometry detected abnormal primary peristalsis in 63% of their patients, about 80% of their patients had abnormal secondary peristalsis when air or water distension was applied. As secondary peristalsis is a reflex response to oesophageal distension, the defect may lie either in the oesophageal motor part, or sensory function, or perhaps both. For example, in patients with normal primary peristalsis and abnormal secondary peristalsis, the efferent pathways may be intact and this suggests that there is a defect in the afferent limb of the reflex pathway with impaired oesophageal sensitivity to distension. Conversely, patients with abnormal primary peristalsis and normal secondary peristalsis may have a defect in the central control mechanisms rather than in peripheral neural pathways.

The defect in inducing secondary peristalsis may well result in delayed bolus transit along the oesophagus and contribute to the development of the patient’s symptoms. Although oesophageal bolus clearance by secondary peristalsis has not been extensively evaluated, manometric examination is therefore suggested to be useful in the diagnostic evaluation of patients with NOD.

Similar to their results observed in patients with NOD, patients with reflux disease have considerably lower secondary peristaltic response rates than healthy controls (Schoeman and Holloway 1995). Although their findings are at variance with

previous studies that did not find any difference between reflux patients and normal subjects (Corazziari, Materia et al. 1986, Corazziari, Pozzessere, et al. 1978), the discrepancy could be attributed to different methodology and speed of bolus injections. The significance of defective triggering of secondary peristalsis to the pathogenesis of reflux disease remains to be fully elucidated. During the day patients are awake, any effect of defective secondary peristalsis on acid clearance may be minimized by frequent primary peristalsis. Secondary peristalsis is likely to be more important during sleep when the rate of primary peristalsis is significantly reduced (Orr, Robinson et al. 1981). This notion is supported by a previous investigation with concurrent ambulatory manometry and pH monitoring in which primary peristalsis was the most common initial oesophageal clearance event overall, secondary peristalsis was the important initial motor event when the subjects were supine or asleep, or both (Schoeman, Tippett et al. 1995).

A recent study has further designed to assess the status of secondary oesophageal peristalsis in reflux patients and to evaluate the effect of healing of oesophagitis on these changes (Pai 2000). Although the results support previous observations on the status of secondary oesophageal peristalsis in reflux patients, data regarding secondary peristalsis did not differ significantly for the values before and after therapy with proton pump inhibitors. The fact that the abnormalities do not regress after complete endoscopic healing of oesophagitis suggests that the abnormalities are important in the pathogenesis (Howard, Reynolds et al. 1994). Although the small numbers involved in the study need to be carefully interpreted, the lack in normalized secondary peristalsis after macroscopic healing supports the role of secondary peristalsis in causation of reflux disease.

Since secondary oesophageal peristalsis has been demonstrated to be impaired in patients with reflux disease, the effect of anti-reflux surgery on its characteristics is unclear. A previous study by Tew et al., who investigated the effect of Nissen fundoplication on oesophageal secondary peristalsis by comparing reflux patients with healthy volunteers as well as other patients with dysphagia after fundoplication (Tew, Jamieson et al. 1997). In addition to similar observation of lower secondary peristaltic response rate in the patient group, fundoplication did not change the initiation or propagation rate of secondary peristalsis. Furthermore, fundoplication was not associated with any change of oesophageal motility parameters even in patients with post-fundoplication dysphagia. They conclude that there is no

improvement in secondary peristalsis after fundoplication and dysphagia after fundoplication is not due to altered peristalsis.

The other study has also investigated the triggering of secondary peristalsis in surgery (Rydberg, Ruth et al. 2000). Secondary peristalsis was elicited by oesophageal distension by a 10-ml bolus of air injected rapidly into the mid-oesophagus. Secondary peristalsis occurred more in healthy subjects than in the reflux patients. In patients after successful anti-reflux surgery, a secondary peristaltic wave was elicited significantly lower than in non-operated reflux patients. It is suggested that the triggering of secondary peristalsis appears to be impaired in chronic reflux patients, and even lower in patients after successful anti-reflux surgery. This study may implicate persistence of the abnormality after surgery and support the notion that GORD is associated with a primary defect in oesophageal motor function.

Chapter 3

Development of intraluminal impedance as a powerful complimentary tool to evaluate oesophageal disorders

3.1 Introduction

In 1991, impedance monitoring was introduced by Silny as a new technique to detect flow of liquids and gas through hollow viscera (Silny 1991). The landmark publication of Silny triggered various studies in which the possible applications of this technique were investigated. Subsequently, it has now become apparent that impedance monitoring offers new opportunities in the field of oesophageal transit testing and gastro-oesophageal reflux (GOR) monitoring.

The impedance technique alone can not offer the measurements of the contraction amplitude and other important parameters of oesophageal function, which may limit some observations of the relationships between oesophageal wall movement and bolus motion, especially in patents with suspected oesophageal motor disorder or dysphagia. Therefore, the catheter integrating impedance monitoring and manometry in a single device has been developed. Both tests can be performed simultaneously and the relationships between the dynamics of bolus transport and wall motion can be evaluated well, while the quality of recording is maintained. In this review, we will focus on the clinical applications of this emerging new technique and summarize current results regarding this novel technique.

3.2 Principles and scientific basis

The method is based on the oesophageal intraluminal measurement of electrical impedance and pressure between a number of arranged electrodes and pressure sensors during a bolus transit using an intraluminal probe (Figure 3.1). The electrical impedance is inversely proportional to the electrical conductivity of the luminal contents and the cross-sectional area (Figure 3.1). Saliva or nutrients show a higher conductivity and therefore induce an impedance drop at the corresponding measurement segments, whereas air has a lower electrical conductivity and yields increased impedance. On the other hand, luminal dilatation results in an impedance drop, whereas luminal narrowing causes an increase in impedance (Silny 1991).

The bolus passage along each measured segment allows the alteration of the typical tracing of impedance, which includes a maximum of five phases (Figure 3.2,

upper panel): (1) phase 1 is the resting stage of the organ; (2) phase 2 represents the facultative arrival and passage of an air volume ahead of the bolus; (3) phase 3 is associated with the arrival and the passage of a bolus. The initial rapid fall of impedance is associated with the arrival of the bolus front as bolus entry (F-Point). During the subsequent nearly plateau phase the bolus is mainly located within the measuring segment; the minimum impedance during this phase represents the bolus body (B-Point); (4) during phase 4 the bolus leaves the measuring segment as bolus exit due to wall contraction with facultative lumen occlusion, which can be represented by the maximum impedance (C-Point); (5) phase 5 is the transitory stage to resting stage. This characteristic impedance wave form may change in the case of absence of air in front of the bolus or absence of a lumen-occluding contraction wave (Figure 3.2, upper panel). For visualization of the maximum and minimum impedance values an individual scaling (Figure 3.2, lower panel, left side) can be used instead of the standard scaling (Figure 3.2, lower panel, right side).

The F-Point, B-Point and C-Point can be determined according to the resumed definitions, as shown in Figure 3.3, left panel. Alternatively, bolus entry and exit have been defined as follows (Tutuian, Vela et al. 2003): Bolus entry is considered to occur at the 50% point between impedance baseline and impedance nadir during bolus passage, and bolus exit is determined as 50% point on the impedance recovery curve, as shown in Figure 3.3, right panel.

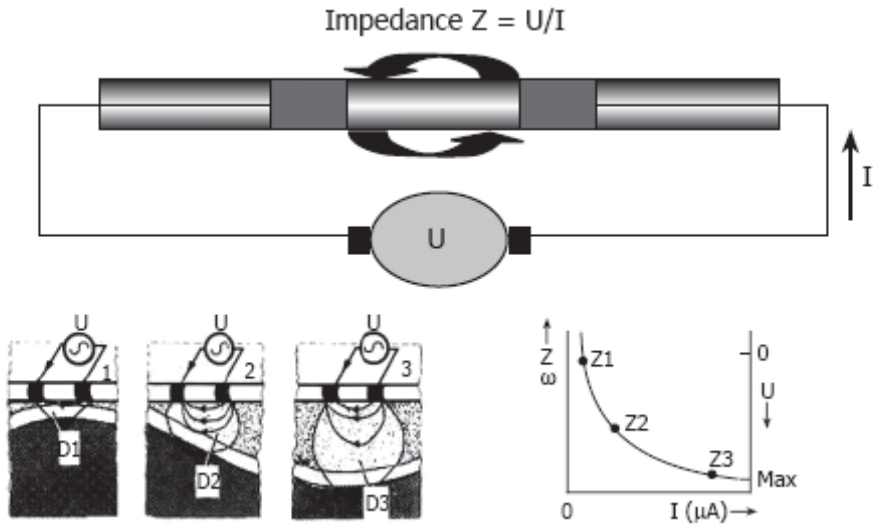


Figure 3.1: Upper: The electrical impedance (Z) of an electric field between 2 electrodes is the ratio between applied voltage (U) and resulting current (I). Lower: Impedance is non-linearly inversely dependent on bolus diameter and electrical conductivity of luminal content (Nguyen, Domingues et al. 2006).

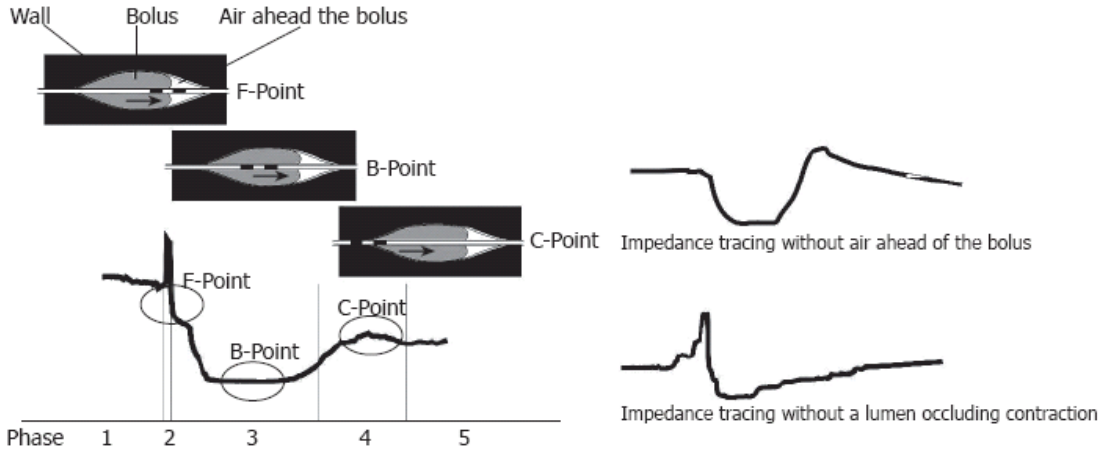


Figure 3.2: Characteristics of the impedance tracing during bolus passage in the oesophagus of healthy persons (Nguyen, Domingues, et al. 2006).

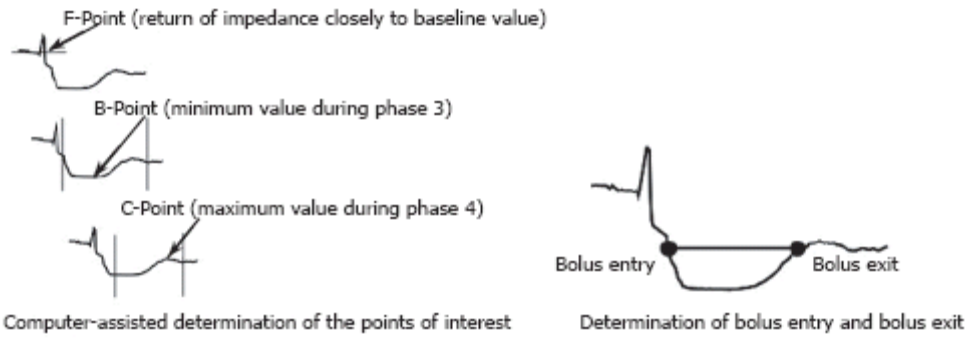


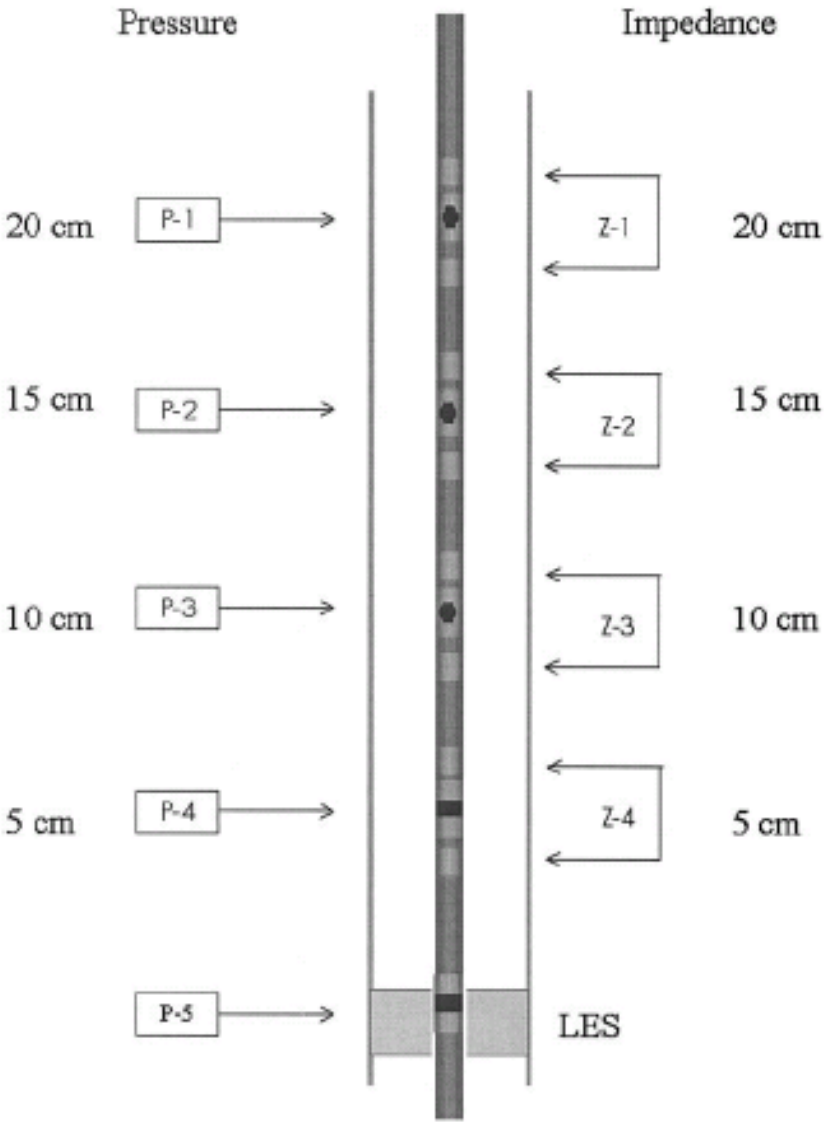
Figure 3.3: Lt: computer assisted determination of the points of interest according to presumed definitions (Nguyen, Silny et al. 1997); Rt: bolus entry and bolus exit can be considered to be 50% of the basal impedance as compared to nadir impedance as suggested by Tutuian et al. (Tutuian, Elton et al. 2003).

3.3 Equipment and technique

Fisher et al. first described the technique for measuring intraluminal impedance in 1978 (Fisher, Hendrix et al. 1978). An intraluminal probe is used to measure the electrical impedance between closely arranged electrodes during a bolus passage. Cylindrical metal electrodes are mounted along the length of a thin plastic catheter, which is passed through the nose into the oesophagus (Figure 3.4). The impedance could be designed to integrate with either pH sensors (impedance-Ph) or manometry (combined impedance and manometry).

Each neighbouring pair of electrodes (known as an impedance segment or impedance channel) is connected to an impedance voltage transducer, which delivers a measuring current. The measurement represents the electrical impedance around the catheter in the section between each pair of electrodes. The impedance is inversely proportional to the electrical conductivity of the luminal contents and the cross-sectional area between the two electrodes. Air has a low conductivity and, therefore, yields an impedance increase, whereas swallowed or refluxed material has a high conductivity and yields an impedance decrease. Furthermore, luminal dilation (i.e. induced by bolus entry in the measuring segment) results in an impedance decrease, whereas luminal narrowing (i.e. during an occlusive contraction) causes an impedance increase (Kahrilas, Clouse et al. 1994). Changes in temporal–spatial patterns in impedance are identified at various levels within the oesophagus, allowing

differentiation between antegrade (swallow) and retrograde (reflux) bolus movement (Richter 2001).



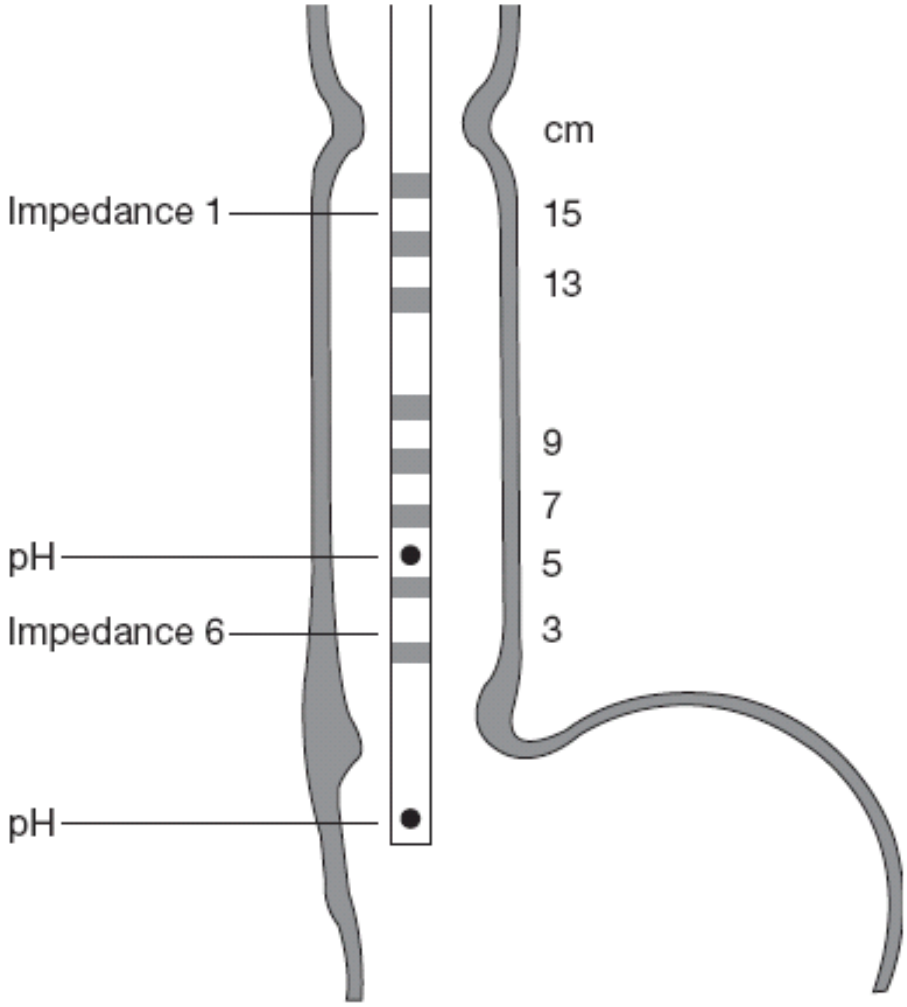


Figure 3.4: A nine-channel combined impedance and manometry catheter (Upper), and a combined impedance-pH catheter consisting of six impedance channels and two pH sensors (Lower) (Sifrim and Blondeau 2006). Circumferential solid-state pressure sensors located in the lower oesophageal sphincter (LOS) high-pressure zone (P5) and 5 cm above it (P4); unidirectional solid state pressure sensors located 10 (P3), 15 (P2), and 20 cm (P1) above the LOS. Impedance-measuring segments centered 5 (Z4), 10 (Z3), 15 (Z2), and 20 cm (Z1) above the LOS (Rt) (Tutuian and Castell 2004a).

3.4 Physiological observation of oesophageal transport

#3.4.1 Validation studies

During oesophageal manometry intraluminal pressure sensors (either water perfused or solid state) are used to record pressures generated within the oesophageal

body and the resting and residual LOS pressure during standardized swallows. Manometry offers information on the amplitude and peristaltic progression of oesophageal contractions but limited information on the bolus transit (Frieling, Hermann et al. 1996). Early studies combining manometry and videofluoroscopy have determined that oesophageal contractions with the amplitude greater than 30 mmHg are accompanied by complete bolus transit (CBT) (Kahrilas, Dodds, et al. 1988b). Combined impedance monitoring and manometry is not subject can offer information on oesophageal pressure and bolus transit without the use of radiation (Figure3.8) (Fass, Silny et al. 1994).

The accuracy of impedance to determine bolus transit was validated by studies combining impedance monitoring and videofluoroscopy. A study in healthy volunteers by Simren et al. found a good correlation between videofluoroscopy and impedance measurements to estimate the time to oesophageal filling ($r^2 = 0.89$; $p < 0.0001$) and time to oesophageal emptying ($r^2 = 0.79$; $p < 0.0001$) (Simren, Silny et al. 2003). More recently, Imam et al. reported on the correlation between bolus transit parameters as assessed by impedance measurements and fluoroscopy in 13 healthy volunteers indicating that the two techniques yielded concordant results in 97% (72/74) of swallows (Imam, Shay et al. 2005).

#3.4.2 Normal data for oesophageal bolus transit

Oesophageal function testing using combined impedance–manometry in healthy volunteers has been reported by several groups. It is mostly performed with liquid and viscous or semisolid boluses. Nguyen et al. reported on the dynamics of oesophageal bolus transit in 10 healthy subjects who received liquid boluses in the supine and upright positions and semisolid boluses in the supine position (Nguyen, Silny, et al. 1997). Their analysis focused predominantly on bolus head, body, and tail velocities in the pharynx, proximal, middle, and distal third of the oesophagus. It was suggested that bolus propagation velocities decreased from proximally to distally and that upright position and bolus consistency influenced bolus transit patterns. In a similar study the role of gravity and bolus consistency on oesophageal contractions and bolus transit pattern was studied by evaluating these parameters in 10 healthy volunteers positioned at inclinations of 0, 30, 60, and 90 degrees (Tutuian, Elton, et al. 2003). The authors found that the distal oesophageal contraction amplitude and bolus transit times declined with increasing inclination with an almost perfect negative correlation

between the angle of inclination and bolus transit time.

Currently, normal values for combined impedance and manometry have been reported by three groups. Tutuian and coworkers reported normal data from a multi-center study, in which each subject received 10 liquid and 10 viscous swallows at intervals of 20–30 seconds (Tutuian, Vela, et al. 2003). Swallows were classified by manometry as (i) *normal peristaltic* (defined as contraction amplitude at both 5 and 10 cm above the LOS of at least 30 mmHg and onset velocity in the distal oesophagus not greater than 8 cm/s), (ii) *simultaneous* (defined as contraction with an onset velocity greater than 8 cm/s or retrograde onset and an amplitude>30 mmHg at both 5 and 10 cm above the LOS) and (iii) *ineffective* (defined as contraction amplitude in the distal part of the oesophagus less than 30 mmHg). Swallows were classified by impedance monitoring as having either (a) *CBT* (defined as detection of bolus exit in all three distal impedance channels located at 15, 10, and 5 cm above the LOS) or (b) *incomplete bolus transit* (defined as bolus retention in at least one of the three distal impedance channels). Using these definitions, more than 93% of normal individuals were found to have at least 80% swallows with complete liquid or at least 70% swallows with complete viscous bolus transit.

Another study in 42 healthy volunteers, similar results were found (Nguyen, Rigda et al. 2005) with combined water-perfused manometry-impedance catheters. The authors proposed a more liberal definition of normal bolus clearance, namely, complete bolus clearances of at least 70% of liquid swallows and at least 60% of viscous swallows. The other set of normal data of combined impedance–manometry testing was reported by Nguyen et al. in a group of 25 healthy subjects (Nguyen, Domingues et al. 2003). The authors also reported on normal value of the oesophageal baseline impedance and deglutitive impedance gradient during saline and yogurt swallows.

#3.4.3 Oesophageal bolus transit in pathological conditions

Using the established normal values (≥80% complete liquid bolus transit and ≥70% complete viscous bolus transit), oesophageal function testing was investigated in a group of 350 patients presenting with various oesophageal symptoms and having various manometric findings (Tutuian and Castell 2004b). Abnormal bolus transit was found in all patients with achalasia and scleroderma, proving the principle that impedance can assess bolus transit in patients with severe oesophageal motility

abnormalities. On the other hand, almost all (*i.e.*, ≥95%) patients with normal oesophageal manometry, nutcracker oesophagus, and isolated LOS abnormalities (*i.e.*,hypertensive, hypotensive, and poorly relaxing LOS) had normal bolus transit for liquid. In the groups of patients with ineffective oesophageal motility (IEM) and diffuse oesophageal spasm, approximately half of the patients had normal bolus transit.

Conchillo et al. reported on the results of combined impedance–manometry testing in 40 patients with non-obstructive dysphagia (NOD) (Conchillo, Nguyen et al. 2005). In this group of patients, abnormal transit for liquid and/or viscous boluses was found in 35.3% of patients with normal motility and in 100% of achalasia patients. It was concluded that the addition of impedance to manometry identifies oesophageal function abnormalities in patients with NOD in which manometry would have been normal or unspecific.

A more detailed study in 70 patients with ineffective motility identified that there is no perfect (*i.e.*, highly sensitive and highly specific) manometric cutoff that would predict CBT and that the current manometric criteria for diagnosing ineffective motility (*i.e.*, ≥30% manometric ineffective swallows) is too sensitive and lacks the specificity of identifying patients with abnormal bolus transit (Tutuian and Castell 2004a). Normal bolus transit in this patient group was likely to be dependent on the distal oesophageal contraction amplitude (*i.e.*, average amplitude at the oesophageal sites 5 and 10 cm above the LOS), the number of sites with low contraction amplitudes, and the overall number of manometrically ineffective swallows. Of another important finding was that approximately one third of their patients had normal bolus transit for liquid and viscous (suggesting a mild functional defect), approximately one-third had abnormal bolus transit for either liquid or viscous (*i.e.*, moderate functional defect), and the remaining third had abnormal bolus transit for both liquid and viscous (*i.e.*, severe functional defect).

Although fluoroscopy has the disadvantage of exposing the patient to ionizing radiation, it provides both functional and anatomical information, while with impedance monitoring only functional information is attained. Furthermore, swallows of solid material can be studied fluoroscopically, which is not possible with impedance monitoring. Impedance monitoring does not seem to be very useful for the diagnosis of achalasia and for the follow-up evaluation of oesophageal emptying in achalasia patients. Because 100% of the manometrically diagnosed achalasia patients

have an abnormal emptying pattern during oesophageal function testing and no achalasia-specific impedance abnormalities have yet been reported, impedance monitoring does not contribute to the diagnosis of achalasia (Conchillo, Nguyen, et al. 2005), the value of impedance monitoring for assessment of oesophageal emptying in achalasia patients appears to be limited.

In summary, current data support the concept that combined impedance monitoring and manometry can be used in research and clinical settings to provide more detailed information on oesophageal function. The next step in evaluating the clinical utility of the additional information provided by impedance monitoring is using this technique in clinical conditions such as disordered swallowing or dysphagia, and interventional outcome studies. These studies would allow a critical evaluation of the proposed parameters and allow quantification of the predictive value of the information provided by impedance measurements.

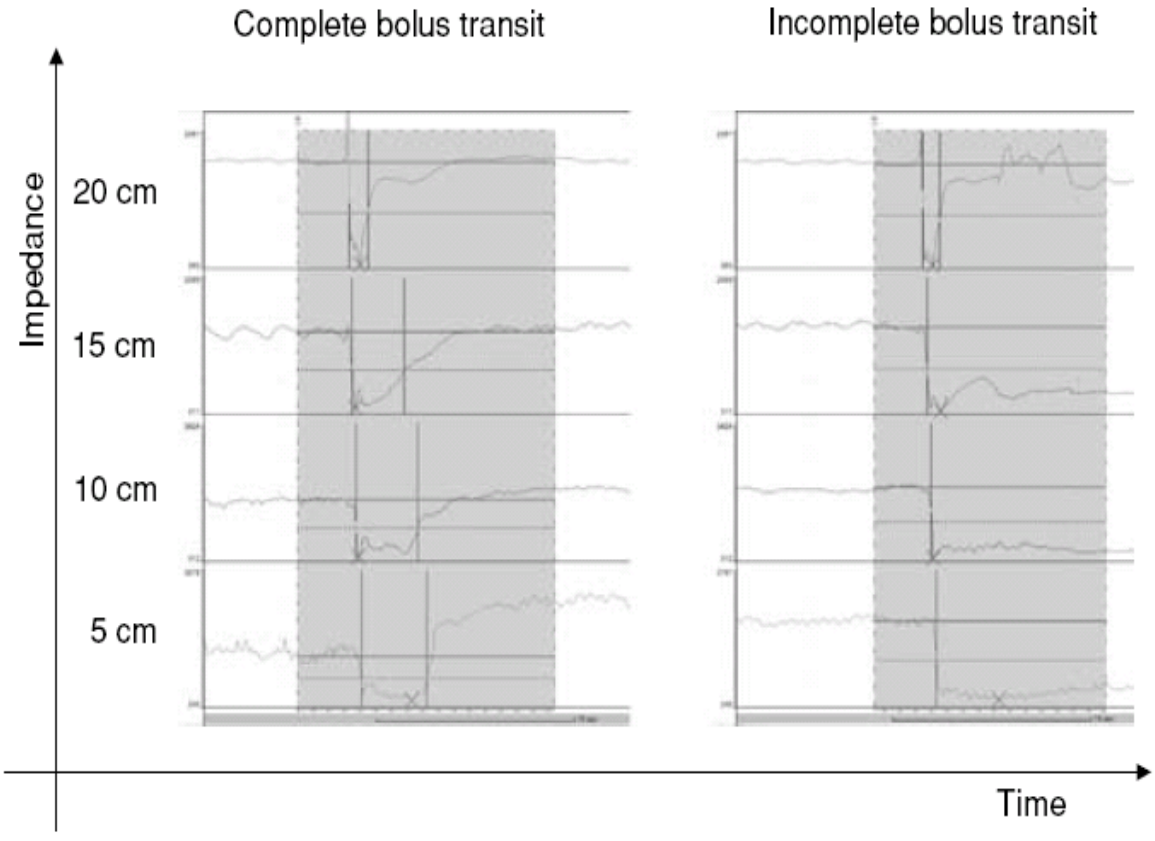


Figure 3.5: Example of impedance tracings of complete (Lt) and incomplete bolus transit (Rt) (Bredenoord, Tutuian et al. 2007).

3.5 Clinical relevance for impedance monitoring

According to the discussion earlier in this chapter, combined impedance and manometry can be applicable and particularly suitable for physiological investigations of oesophageal motor function as well as bolus transport patterns. Therefore, different aspects of oesophageal bolus transport can be obtained: (1) normal and pathological bolus transport patterns including bolus escape and retrograde bolus transport can be monitored, (2) several parameters of bolus transit can be calculated allowing differentiation between normal and abnormal bolus transport, (3) parameters related to bolus clearance and bolus transit completion can be determined, and (4) swallow-associated events such as normal and pathological air movement as well as pathological reflux can be monitored. Thus, detailed information regarding oesophageal motor function and associated bolus transport can be obtained by combined impedance and manometry during a single investigation.

In patients with suspected oesophageal motor disorder, the technique provides additional information about the functional status of the oesophagus and may explain some symptoms in these patients. However, the gold standard for diagnosis of achalasia remains manometry due to its diagnostic criteria and the unique manometry patterns. In patients with reflux disease, combined impedance and manometry may provide additional information about mechanisms related to impaired bolus transit and bolus clearance. In patients with IEM, it helps clarifying the associated functional abnormalities (Tutuian and Castell 2004a). Therefore, combined impedance and manometry is emerging as an important tool for obtaining detailed information about the physiology and pathophysiology of oesophageal motility. The potential clinical implications of this technique may include (1) the functional classification of oesophageal motor disturbances in patients with NOD; (2) the perioperative management of laparoscopic fundoplication and other endoscopic procedures which could impact oesophageal motility and (3) the physiological characteristics of oesophageal bolus transport caused by oesophageal stimulation such as secondary peristalsis.

Chapter 4

Oesophageal dysphagia

4.1 Overview

The term “Dysphagia”, from the Greek words *dys* (with difficulty) and *phagia* (to eat), means either difficulty initiating a swallow (oropharyngeal dysphagia) or the sensation that foods, liquids, or both are stuck in their transport from mouth to stomach (oesophageal dysphagia). It is important to distinguish dysphagia from odynophagia (painful swallowing), although it is often difficult to separate the two symptoms clearly. Dysphagia is common in all age groups, increasing in prevalence with advancing age. It is a significant cause of disability and a contributor to health care costs. Studies in chronic nursery unit have shown that a significant proportion of elderly patients have swallowing disorders, resulting in a high incidence of co-mobility such as aspiration pneumonia (Marik and Kaplan 2003). Table 4.1 outlines the common causes of dysphagia; however, many disorders overlap, producing both oropharyngeal and oesophageal dysphagia.

The aim of this chapter is to describe integrated function of swallowing and clinical presentation of oesohageal dysphagia. The presentation can be categorized as non-obstructive dysphagia (NOD) and other type of dysphagia such as in reflux disease or post-operative condition (post-fundoplication). The pathophyiology underlying these conditions will be discussed.

Table 4.1. (Richter 2000).

- Oropharyngeal dysphagia
- Neuromuscular diseases
  - Cerebrovascular accidents
  - Parkinson's disease
  - Brain stem tumors
  - Multiple sclerosis
  - Amyotrophic lateral sclerosis
  - Peripheral neuropathies (diphtheria, diabetes, polio)
- Skeletal muscle disorders
  - Polymyositis/dermatomyositis
  - Muscular dystrophies (myotonic dystrophy, oculopharyngeal dystrophy)
  - Myasthenia gravis
  - Metabolic myopathies
- Mechanical obstruction
  - Inflammatory status
  - Extrinsic compression (thyromegaly, cervical osteophyte)
  - Postsurgical changes
  - Zenker's diverticulum
  - Cricopharyngeal bar
- Miscellaneous
  - Decreased saliva (medications, radiation, Sjögren's syndrome)
  - Alzheimer's disease
  - Depression
- Esophageal dysphagia
- Mechanical obstruction
  - Benign strictures (peptic, corrosive, radiation)
  - Webs and rings (Schatzki's)
  - Neoplasm
  - External compression (vascular, thyroid, mediastinal mass)
  - Diverticulum
- Motility disorders
  - Achalasia
  - Spastic motility disorders
  - Scleroderma
  - Miscellaneous (diabetes, alcohol, gastroesophageal reflux disease)

4.2 Integrated function of swallowing

Swallowing, or deglutition, has been divided into three stages—oral, pharyngeal, oesophageal—all regulated and coordinated by the swallowing center in the medulla. The oral stage is largely voluntary and highly variable, depending on taste, environment, degree of hunger, and motivation. The preparatory stage entails the chewing of food and the forming of it into an oral bolus. Aided by adequate dentition, food is broken down to a size and a consistency appropriate for swallowing and mixed with saliva. Fine tongue movement is critical for confining the food bolus to the midline and pushing it up and back toward the palate while propelling the bolus into the pharynx. This process requires proper function of the striated muscles of the tongue and pharynx, and is the stage of swallowing most likely to be abnormal in patients with neurologic or skeletal muscle disease.

The pharyngeal stage requires the fine-tuned, coordinated sequence of contractions and relaxations resulting in the transfer of the bolus material from the pharynx to the oesophagus. Food in the pharynx stimulates sensory receptors, sending impulses to the swallowing center in the brain stem. The central nervous system (CNS) then initiates a series of involuntary responses occurring over a 1-second period. The soft palate is elevated and retracted with complete closure of the nasal pharynx, preventing swallowed material from entering the nasal cavity. The vocal cords are closed, and the epiglottis swings back and down to close the larynx. The larynx is pulled up and forward by the muscles attached to the hyoid bone, stretching the opening of the oesophagus and the upper oesophageal sphincter (UOS). The UOS relaxes during elevation of the larynx. Contractions of the pharyngeal constrictor muscles propel the bolus into the open mouth of the oesophagus. Respiration is suspended during the swallow. The pharyngeal swallowing response is complex. Sensory information is carried along cranial nerves V, VII, IX, and X; the motor responses are carried along cranial nerves V, VII, X, and XII.

In the oesophageal stage, digested material from the mouth is transported to the stomach. This active process requires contraction of both longitudinal and circular muscles of the tubular oesophagus and coordinated relaxation of the sphincters. Swallowing shortens the longitudinal muscles, providing a structural base where the circular muscle contraction forms the peristaltic wave. This primary wave moves from the striated muscle of the upper oesophagus through the oesophageal body at 2 to 4 cm per second. Primary peristalsis is initiated by a swallow; secondary peristalsis can

be initiated at any level of the oesophagus in response to local luminal distention. This type of peristalsis allows clear ingested material incompletely transported by a primary peristaltic wave and material regurgitated from the stomach.

4.3 Definition of oesophageal dysphagia

The patients with oesophageal dysphagia have difficulty *transporting* food down the oesophagus once the bolus has been successfully transferred through the pharynx. Normally, food travels through the oesophagus quickly; the peristaltic wave takes approximately 8 seconds to pass from the UOS to the oesophagogastric junction. In the erect position, a liquid bolus will traverse the oesophagus in less than 3 seconds. Solids take longer and may require a series of peristaltic waves - primary followed by secondary peristalsis - to clear the oesophagus. Any difficulty with the coordinated contractions of the oesophagus (motility disorder) or any kind of mechanical obstruction may cause a transport problem. The patient most often reports this problem as food "hanging up" somewhere behind the sternum. If the symptom is localized to the lower part of the sternum or the epigastric area, the lesion is most likely in the distal oesophagus, although the symptom may be referred and the patient may locate the level of dysphagia to the lower part of the neck (Edwards 1982). For example, a patient with a carcinoma of the distal end of the oesophagus may sometimes indicate that the food stops at the suprasternal notch. When a patient reports that dysphagia occurs with both solids and liquids and that even water sometimes seems to stop, they may have a motility problem or primary neuromuscular abnormality of the oesophagus. Conversely, if the dysphagia occurs only after swallowing a fairly large piece of meat or other solid food, never when the patient drinks any kind of beverage, the possibility of a mechanical obstruction may be favored. The history regarding the nature of food inducing the symptoms is often very helpful.

4.4 Presentations of dysphagia and difficulty distinguishing it from odynophagia

Dysphagia, difficulty swallowing, and odynophagia, painful swallowing, are symptoms that can be difficult to be differentiated. Difficulty with swallowing can be caused by functional or mechanical disorders. An imaging or endoscopy study is needed to rule out structural lesions. Contrast radiography may be valuable in some instances to localize or characterize pathologic changes. Endoscopy is useful because

it allows direct visualization, tissue sampling, and dilation therapy for mechanical processes. Benign mechanical causes of dysphagia include peptic stricture, Schatzki's ring, congenital oesophageal mucosal webs and rings, and strictures caused by radiation therapy, caustic ingestion, and surgical anastomoses. Dysphagia is the most common symptom of oesophageal cancer. Achalasia is an acquired neuromuscular abnormality in which the oesophagus loses normal peristaltic function and the lower oesophageal sphincter (LOS) is hypertensive and does not relax. These features can be demonstrated at oesophageal manometry.

Odynophagia can occur when injury to the oesophageal mucosa causes ulceration. Because it may be a symptom of a serious pathologic condition, odynophagia should be investigated with endoscopy. Odynophagia may be caused by acid reflux, chemical injury, infection, or neoplasia. Among immunocompromised patients, Candida, herpes, or cytomegalovirus infection can cause infectious oesophagitis. In addition to these, idiopathic AIDS ulceration may occur among patients with HIV infection.

4.5 Non-obstructive dysphagia: Is non-obstructive dysphagia different from other forms of oesophageal dysphagia?

As discussed earlier, dysphagia means difficulty in swallowing and should not be confused with odynophagia or painful swallowing. A patient with true dysphagia will describe either problems initiating a swallow or a sensation of food stopping or "sticking" somewhere behind the sternum or perhaps in the neck region. Swallowing usually is not painful, however, so odynophagia is not present. When a patient complains of true difficulty with swallowing that the food does not pass on into the stomach in the normal way, it almost always indicates some kind of organic lesion and not a functional problem.

The patients with oesophageal dysphagia have difficulty *transporting* food down the oesophagus once the bolus has been successfully transferred through the pharynx. However, NOD is defined as difficulty in swallowing liquids and/or solids in the absence of endoscopically or radiologically demonstrable lesion in the oesophagus. The symptom usually indicates impaired transport of a swallowed bolus along the oesophagus. The etiology of NOD is thought primarily to be related to an oesophageal motor disorder (Schoeman and Holloway 1994b, Singh, Stein et al. 1992). Nevertheless, an oesophageal cause can be found in less than 50% of NOD patients

using stationary oesophageal manometry (Katz, Dalton, et al. 1987). In addition, NOD has been reported in patients with reflux oesophagitis in whom the symptoms was found to correlate well with oesophageal pH (Triadafilopoulos 1989).

#4.5.1 Oesophageal motility in non-obstructive dysphagia

Dysphagia is a relatively common symptom and may reflect a true oesophageal disorder. It has been reported that the prevalence of dysphagia varies between 1.6% and 15% in the middle-aged and elderly general population (Lindgren and Janzon 1991). After diagnostic possibilities have been excluded by endoscopic and/or radiographic diagnoses, motility disorders should be considered as a potential cause of dysphagia. It has been shown that manometric abnormalities are common in patients with dysphagia as the principal symptom (Katz, Dalton, et al. 1987). Abnormalities have been grouped into the following diagnostic categories: achalasia, diffuse oesophageal spasm, nutcracker oesophagus, and ineffective motility disorder. Among these, ineffective motility was the most common motility disturbance diagnosed by oesophageal manometry (Katz, Dalton, et al. 1987). Furthermore, ineffective motility has been linked to functional abnormalities of incomplete bolus transit (Kahrilas, Dodds, et al. 1988b). Although our previous investigation reconfirms the notion that ineffective motility is the most common pattern of motility abnormality in NOD, we do observe that a normal motility study is the most frequent finding in NOD patients (Chen and Orr 2005).

The term “non-obstructive dysphagia” (NOD) is used to describe the presence of the sensation of difficulty in swallowing solids or liquids in the absence of endoscopically or radiologically demonstrable oesophageal lesion (Parkman, Maurer et al. 1996, Richter, Baldi et al. 1992). Motility disorders may be found manometrically in NOD patients but do not necessarily temporally correlate or explain the symptom of dysphagia under usual conditions with water swallows (Benjamin, Castell et al. 1983, Benjamin, Gerhardt et al. 1979, Clouse and Ferney 1986, Howard, Pryde et al. 1989). Conflicting results have been published in NOD patients examined by oesophageal manometry with solid or viscous swallows (Cordier, Bohn et al. 1999, Sears, Castell et al. 1990). A recent study has noted both oesophageal motor and sensitivity impairments in some of NOD patients (Bohn, Bonaz, et al. 2002). The hypothesis of impaired secondary peristalsis rather than primary peristalsis in NOD patients has therefore been posed by Schoeman et al., who

have demonstrated a noticeable defect in secondary peristalsis in NOD patients, and have implicated that this defect may further lead to delayed bolus transit along the oesophagus (Schoeman and Holloway 1994b).

Multichannel intraluminal impedance (MII) is a technique that allows detection of oesophageal bolus transport and real-time quantification of bolus movement without radiation (Silny 1991). Multichannel intraluminal impedance and oesophageal (MII-EM) is also able to enhance the diagnostic capability and clarify functional abnormalities in patients with disordered oesophageal clearance (Tutuian and Castell 2004b). In a group of 40 patients with NOD studied with MII-EM, Conchillo et al. have found that impedance identifies oesophageal function abnormalities in NOD patients with normal manometry, ineffective motility, and diffuse oesophageal spasm (Conchillo, Nguyen, et al. 2005), although they conclude that the MII technique seems to be less suitable for the diagnosis of most severe form of dysphagia such as achalasia (Conchillo, Nguyen, et al. 2005).

The pathophysiological implication of dysphagia is that there is some resistance or delay to the passage of a bolus, the final form of which is bolus impaction (Conchillo, Nguyen, et al. 2005). A recent study applying 24-h oesophageal manometry was able to detect and characterize abnormal oesophageal motor activity with NOD, and found that the prevalence of meal-related peristaltic contractions correlated best with the presence of dysphagia (Stein, Singh et al. 2004). Deschner et al. noted that a reproduction of dysphagia sensation could occur with balloon distension in a majority of subjects with NOD (Deschner, Maher et al. 1989). These authors found that repeated simultaneous contractions occurred distal to the balloon in many of those patients with reproduced symptoms, and suggested that the development of abnormal distal motility was a principal even leading to dysphagia. Other studies using intra-oesophageal balloon distention have indicated that oesophageal sensory dysfunction only partially overlaps with motor dysfunction, and has an association with dysphagia that is independent of motor abnormality on baseline manometry (Clouse, McCord et al. 1991). It appears that dysphagia can be another manifestation of oesophageal sensory dysfunction.

#4.5.2 Impaired oesophageal sensitivity in non-obstructive dysphagia

In patients with NOD, oesophageal manometry with water swallows is usually normal or nearly normal, and dysphagia is rarely generated during this examination

(Keren, Argaman et al. 1992). Although dysphagia is more often experienced when swallowing solid food than liquids, the findings between NOD patients and healthy subjects are still conflicting (Cordier, Bohn, et al. 1999, Howard, Pryde, et al. 1989, Sears, Castell, et al. 1990). In addition, there is a lack in the constant relation between dysphagia and oesophageal motility, suggest that oesophageal sensory dysfunction may play a role in NOD. Additionally, Clouse et al. have demonstrated a good correlation between the presence of dysphagia and the response to balloon distension, and suggested that dysphagia may be representative of sensory dysfunction in patients referred oesophageal motility testing (Clouse, McCord, et al. 1991).

A recent study has investigated mechanical oesophageal sensitivity in patients with NOD, and attempted to evaluate the relationships between oesophageal motor abnormalities and sensitivity impairment (Bohn, Bonaz, et al. 2002). These authors observed the threshold volume for oesophageal balloon distension was significantly lower in NOD patients than healthy controls. A total of 8/19 (42%) patients presented with the association of an abnormal sensitivity threshold and an abnormal motor pattern; 5/19 (26%) presented with isolated motor abnormalities; 4/19 (21%) patients presented with isolated abnormal sensitivity thresholds; and 2/19 (11%) patients presented without any abnormality. Furthermore, dysphagia was reproduced in 15/19 (78.9%) patients during manometry with solid swallows.

Therefore, the authors proposed that the sensitivity impairment may play a part in the origin of the symptomology given that symptoms were reproduced during oesophageal distension in a majority of their patients, although they did not find any correlation between oesopahgeal mechanical sensitivity and any parameter of oesophageal motility. It has been suggested that oesophageal sensitivity impairment could be responsible for starting up motor response impairment. This notion is supported by the finding of a positive correlation in human between oesophageal sensitivity and wall tension, which implicates that oesophageal sensation depends on the force generated by the muscle wall (Patel and Rao 1998).

In summary, the addition of oesophageal sensitivity testing to traditional motility examination may help understand more precisely the pathophysiology of dysphagia in NOD patients. Further studies with investigation on the temporal correlation between oesophageal sensation/perception and motility could offer original perspectives for clinical approach of this condition.

4.6 Dysphagia in non-stricturing GORD

#4.6.1 Introduction

Gastro-oesophageal reflux (GOR) may present with a variety of symptoms, such as heartburn, chest pain, dysphagia, odynophagia, and regurgitation; these symptoms may vary in frequency and intensity from patient to patient or with different stages of the disease (Richter and Castell 1982). Even in the absence of oesophagitis or stricture, NOD has been described in patients with GOR (Schlesinger, Donahue et al. 1985). Such patients may have a fullness in the throat, disturbed swallowing, or excessive mucus secretion. NOD may be experienced at any level, is usually intermittent for solids or liquids, and becomes progressive only if stricture develops. However, the intermittent presentation of NOD leads to the difficulty in understanding its pathogenesis. This chapter will briefly review current theories about NOD in gastro-oesophageal reflux disease (GORD).

#4.6.2 Oesophageal dysmotility in GORD

Hypotensive peristaltic contractions are low-amplitude (<30 mmHg) peristaltic contractions detected on oesophageal manometry during water swallowing. They are associated with ineffective oesophageal transit (Kahrilas, Dodds, et al. 1988b). In patients with dysphagia or GORD, the frequency of this disturbance increases (≧ 30%). Often, the hypotensive peristaltic contractions are associated with hypotensive nonperistaltic contractions. By combining manometry with either videofluoroscopy or impedance recording, it has been shown that with contraction amplitudes of <30 mmHg, bolus transport, as well as clearance of any refluxed contents, is often impaired. Hypotensive oesophageal contractions have been called 'ineffective oesophageal motility (IEM)'. Whether it is a result of or the cause of reflux oesophagitis is not known.

The pathophysiology of hypotensive peristaltic or nonperistaltic contractions may involve suppression of cholinergic excitatory activity or impaired force of the circular muscle contraction. Decreased cholinergic influence causes both reductions in the force of contraction and the loss of peristaltic sequence. In animal models of acid-induced oesophagitis, inflammatory mediators have been shown to impair release of acetylcholine and also directly impair smooth muscle contraction. Thus, in many patients, hypotensive oesophageal contractions may be secondary to inflammation caused by reflux or other etiologies. Hypotensive oesophageal contractions are often

associated with hypotensive LOS. Hypotensive LOS tone and weak distal oesophageal contractions can lead to increased reflux and impaired oesophageal acid clearance, respectively, thereby promoting the development of reflux oesophagitis.

Incompetent LOS is one of most important causes of impaired anti-reflux barrier mechanisms at the oesophagogastric junction that normally prevent GOR and the development of GORD. Incompetence of the LOS may be due to its hypotension, increased intra-abdominal pressure that overwhelms a near normal LOS and inappropriate transient LOS relaxation (TLOSR). Other factors such as decreased contractile response of the diaphragmatic sphincter and a hiatal hernia also play an important role in GOR.

The TLOSR reflex that inappropriately occurs in the absence of swallowing or oesophageal peristalsis or other activities such as belching is called in appropriate TLOSR or simply TLOSR. It may be a part of the belch reflex without obvious belch. This is a centrally mediated vagovagal reflex, with the vagal afferents arising from the gastric fundus and vagal inhibitory efferent pathway projecting to the LOS. This reflex is mediated via medullary neurons in the nucleus tractus solitarius and the caudal portion of the dorsal motor nucleus of the vagus nerve that provide inhibitory vagal innervation to the LOS. The inhibitory neural pathway consists of cholinergic preganglionic neurons and nitrergic postganglionic neurons in the myenteric plexus innervating the LOS. Gastric distention increases the frequency of these reflex episodes.

The TLOSRs are accompanied by episodes of GOR that may lead to GORD. In many patients, GORD occurs owing to increased episodes of acid reflux episodes occurring during TLOSRs. This is particularly true for patients with milder grades of reflux disease. The TLOSRs may be more often associated with GOR episodes in the presence of hypotensive LOS.

#4.6.3 Factors contributing to dysphagia in non-stricturing GORD

Recent studies have demonstrated that failed peristalsis and hypotensive body peristalsis are typical finding among reflux patients, and such peristaltic sequences are associated with impaired oesophageal clearance (Kahrilas, Dodds, et al. 1988b). Therefore, a previous study has attempted to determine whether oesophageal dysphagia among GORD patients is associated with peristaltic dysfunction (Jacob, Kahrilas, et al. 1990). The study was performed on 325 patients who underwent

oesophageal manometry to investigate the relationship between dysphagia and the incidence of peristaltic dysfunction (failed or hypotensive peristaltic sequences). The main finding is that the severity of manometrically demonstrated peristaltic dysfunction in reflux patients correlated with the prevalence of dysphagia. The overall prevalence of dysphagia was 39% among the 157 reflux patients. Within this group, 29% of patients with minimal peristaltic dysfunction experienced dysphagia compared to 78% of patients with severe peristaltic dysfunction. Thus, these authors concluded that peristaltic dysfunction should be considered as a potential cause of dysphagia in patients with reflux disease.

Dysphagia was reported by 78% of patients with severe peristaltic dysfunction which was known to impair oesophageal transit. However, it is interesting that some of those patients did not experience any dysphagia, i.e., patient's perception is not completely reflective of oesophageal transit. Conversely, dysphagia was reported by 29% patients with mild peristaltic dysfunction. Dysphagia in this group was unlikely related to impaired oesophageal clearance since there were no differences in peristaltic variables distinguishing patients with dysphagia from those without or from controls without dysphagia. The etiology of dysphagia in this group is that increased oesophageal sensitivity may play a role leading to dysphagia. In summary, severe peristaltic dysfunction is suggested to be a potential explanation for dysphagia in GORD patients. The mechanism underlying the dysphagia is likely related to impaired oesophageal bolus transport, and the overall relationship between peristaltic dysfunction and dysphagia regarding GORD is similar to that seen in other disorder characterized by impaired oesophageal transport.

The other study applied stationary and ambulatory motility tests to elucidate the mechanism of dysphagia (Singh, Stein, et al. 1992). During stationary studies, there was almost no difference in oesophageal body motility among healthy controls, reflux patients without dysphagia, and reflux patients with dysphagia. LOS didn't differ between patients with and without dysphagia. However, on ambulatory motility, the rate of simultaneous contractions decreased in the upright position and at mealtimes in healthy controls and patients without dysphagia, but not in patients with dysphagia. The rate of intraprandial simultaneous wave activity was higher in patients with dysphagia (38%) than in those without dysphagia (23%) or healthy controls (13%). The authors concluded that reflux patients with dysphagia were characterized by an increase in non-peristaltic activity during mealtimes.

The technique of ambulatory motility allows the investigators to study the motility characteristics over prolonged periods and under more physiological conditions, i.e, supine, upright, or at mealtimes. Therefore, this technique is able to detect manometric abnormality during meals which could not be identified by stationary motility. This study showed few changes on stationary motility, nevertheless, a distinct inability to initiate peristaltic activity in patients with NOD, particularly at mealtimes, during ambulatory motility studies. It has been shown that patients with motility disorders and NOD can have their symptom reproduced with balloon distension of the oesophagus, and that these symptoms are associated with the presence of simultaneous waves in the distal oesophagus (Deschner, Maher, et al. 1989). In another study on patients with a motility disorder, a significant proportion of patients experienced dysphagia along with nonperistaltic activity in the oesophagus by a test meal as a provocative test (Allen, Orr et al. 1988). On the finding of these results, the intraprandial dysmotility could contribute to dysphagia experiencing in a subgroup of reflux patients.

From the above discussion regarding dysphagia in reflux disease, the mechanism underlying NOD could be related to transient oesophageal dysmotility. A previous study has attempted to determine the frequency of NOD in patients with erosive oesophagitis and correlate it with oesophageal pH and motility changes (Triadafilopoulos 1989). The study demonstrated a significant correlation (near 90%) between acid reflux and the sensation of dysphagia. In addition, their observation showed that intermittent dysphagia may occur in up to 46.8% of moderate to severe GORD. Therefore, the authors suggest that intermittent acid reflux may lead to transient dysphagia even in the absence of peptic oesophageal stricture. The study provides a potential clue about the nature of dysphagia.

GOR may present with a variety of symptoms, such as heartburn, chest pain, dysphagia, odynophagia, and regurgitation, which may vary in frequency and intensity from patient to patient or with different stages of the disease (Richter and Castell 1982). Even in the absence of oesophagitis or stricture, NOD has been described in patients with GORD. Such patients may experience fullness in the throat, difficulty initiating a swallow, or excessive mucus secretion. In addition, NOD may be felt at any level, is usually transitory for solids or liquids, and becomes progressive only if stricture develops. Thus, recognition of acid reflux in these patients and its eradication by adequate anti-reflux treatment may benefit patients and contribute to

their improvements in dysphagia (Vakil, Traxler et al. 2004).

4.7 Dysphagia in post-fundoplicaton

#4.7.1 Background

Since laparoscopic Nissen fundoplication has been first reported in 1991 (Dallemagne, Weerts et al. 1991), the frequency of this procedure has increased globally, as fundoplication appears to be an effective, economical, and minimally invasive treatment alternative to the long-term medical treatment in patients with GORD (Hinder, Filipi et al. 1994, Schwab, Blum et al. 1997).

Postoperative temporary dysphagia is one of the most common complications after antireflux surgery (DeMeester, Bonavina et al. 1986, Stein, Feussner et al. 1996). Persistent postoperative dysphagia can be the result of a variety of surgical and physiological factors (Hunter, Swanstrom et al. 1996, Schwab, Blum, et al. 1997), whereas the temporary inability to swallow normally is related to initial adaptation problems resulting from the patient's eating patterns (Glise, Hallerback et al. 1995).

Antireflux surgery for GORD treatment can be regarded as successful when the patient is able to have a normal swallow after the operation (Schwab, Blum, et al. 1997). The frequency and intensity of persistent dysphagia may differ clinically (DeMeester, Bonavina, et al. 1986, Swanstrom and Wayne 1994). Postoperative dysphagia has a direct relation to surgical and physiological factors, the surgeons' experience with laparoscopic and antireflux surgery, and the type of wrap performed (Schwab, Blum, et al. 1997). Additionally, immediate postoperative and short-term dysphagia is caused mainly by two factors: firstly, trauma related to edema at the gastro-oesophageal junction, and secondly, by adaptation problems related to individual eating and drinking patterns (Glise, Hallerback, et al. 1995). Quoting DeMeester, "dysphagia is a symptom that you can really adjust yourself to in the way you eat, your eating habits" [from the discussion with Glise et al. (Glise, Hallerback, et al. 1995)]. Dysphagia is common after fundoplication. It is usually mild, intermittent, and improves with time. At the day of hospital discharge approximately 50% of patients haven been reported to suffer from mild to severe dysphagia, with about 16% having severe swallowing difficulties. In the course of convalescence the percentage decreased to 2% after 3 months (Kamolz, Bammer et al. 2000).

#4.7.2 Oesophageal motility and clearance mechanisms in GORD

GOR can be a physiologic event and occur in almost all individuals to some degree. Reflux disease is characterized by an increased exposure of the oesophageal mucosa to acid, resulting primarily from an increased rate of reflux episodes. Approximately 50% of patients have abnormally slow clearance of the refluxed acid, and prolonged oesophageal acidification is more damaging to the oesophageal mucosa than are frequent short periods. Acid refluxate also extends more proximally up the oesophageal body in patients with reflux disease compared with controls (Weusten, Akkermans et al. 1995). The oesophageal body is a major component of the anti-reflux mechanism. Once reflux has occurred, up to 90% of the refluxate volume can be cleared by one or two peristaltic sequences (Helm, Dodds, et al. 1984), leaving just a small residue for neutralization by swallowed saliva. Therefore, an intact peristaltic mechanism is essential for effective acid clearance; disruption of oesophageal peristalsis affects not only volume clearance (Kahrilas, Dodds, et al. 1988b), but also delivery of swallowed saliva to the distal oesophageal body.

#4.7.3 Oesophageal peristalsis in response to acid reflux

In normal subjects, the initial response to acid reflux is usually primary peristalsis (Figure 4.1), which occurs in 41% to 57% of occasions (Dent, Dodds, et al. 1980, Schoeman, Tippett, et al. 1995). This is probably because of the high rate of swallowing during the awake state and the stimulation of swallowing by acid reflux (Dent, Dodds, et al. 1980). Secondary peristalsis (Figure 4.2) is less common and occurs on only 27% to 57% of occasions (Dent, Dodds, et al. 1980, Schoeman, Tippett, et al. 1995). However, secondary peristalsis may be more important when subjects are supine and asleep. During that period, secondary peristalsis has been reported to be the initial clearance event on 86% of occasions (Schoeman, Tippett, et al. 1995). It is likely that suppression of salivation and swallowing by sleep (Orr, Johnson et al. 1984) reduces the occurrence of primary peristalsis and thereby increases the relative frequency and importance of secondary peristalsis under those conditions. In patients with reflux disease, primary peristalsis is also the most common initial response and has been reported to account for 80% to 90% of initial clearance events (Anggiansah, Taylor et al. 1994), whereas secondary peristalsis accounts for only a minority (12% to 17%) of initial clearance events. Patients with reflux disease also exhibit a greater delay between the onset of reflux and the

occurrence of the initial clearance event than do normal subjects. This time may be up to twice that in normal subjects (Barham, Gotley et al. 1992).

Patterns of oesophageal motility during acid reflux events mirror those of the initial events. Primary peristalsis remains the most prevalent activity when oesophageal pH is below 4. In healthy subjects, it accounts for 70% to 90% of all activity and approximately 90% in patients with reflux disease (Anggiansah, Taylor et al. 1997, Dent, Dodds, et al. 1980). Consequently, secondary peristalsis accounts for only a small proportion of oesophageal body activity both in healthy subjects and in patients with reflux disease, ranging from 10% to 25% of all activity. Reflux patients have impaired oesophageal motor responses to reflux, as evidenced by an increased time to the first peristaltic response, fewer responses in general and fewer peristaltic responses, an increased interval between peristaltic responses, and a lower proportion of complete peristaltic sequences than do healthy controls (Anggiansah, Taylor, et al. 1997, Dodds, Kahrilas, et al. 1990).

#4.7.4 Osopahgeal body motility and acid clearance

Oesophageal peristalsis has an integral and dual role in oesophageal acid clearance: primarily in the initial clearance of the bulk of the refluxate volume but also in the transport of saliva for the subsequent neutralization of the residual acid. An inverse relationship between the integrity of the oesophageal body response to reflux and acid clearance time has been suggested. Acid clearance in reflux patients is two to three times longer than in controls (Booth, Kemmerer et al. 1968, Johnson 1980). Impaired acid clearance is found in approximately 50% of patients with reflux disease and is more common in patients with severe oesophagitis. The two main patterns of peristaltic dysfunction commonly encountered in reflux disease, failed peristalsis, and hypotensive peristalsis (Kahrilas, Dodds et al. 1986) impair oesophageal volume clearance (Kahrilas, Dodds, et al. 1986) and are found in 20% of patients with mild oesophagitis and in 50% of patients with severe oesophagitis (Kahrilas, Dodds, et al. 1986). Defective peristalsis is the major factor underlying abnormal acid clearance in patients with severe oesophageal motor disorders.

Studies that have examined directly the relationship between oesophageal body motility and clearance of spontaneous acid reflux episodes are relatively few. A static laboratory study done in recumbent normal subjects showed that peristaltic failure or incomplete peristaltic sequences were associated with prolongation of acid clearance

and a smaller increment in oesophageal pH than were complete peristaltic sequences (Dent, Dodds, et al. 1980). Similar findings were noted in a comparable study in patients with reflux disease (Dodds, Kahrilas, et al. 1990). Subsequent studies using 24-hour ambulatory manometry and pH monitoring have tended to support these findings (Barham, Gotley et al. 1995). As a group, patients with reflux disease had more prolonged acid clearance. This was associated with a longer time from the onset of reflux to the first peristaltic sequence, less frequent motor activity while pH was below 4, and a smaller proportion of peristaltic sequences. Compared with control subjects, patients with reflux disease required a greater number of peristaltic sequences to restore oesophageal pH to above 4, and each sequence was associated with a smaller increment in oesophageal pH. These differences suggest that other factors, perhaps defective salivary bicarbonate, were contributing significantly to impaired acid clearance (Anggiansah A, Gut 1997). Timmer et al. were unable to demonstrate any impairment of oesophageal body motility in reflux disease despite similar findings with regard to acid clearance (Timmer, Breumelhof et al. 1993). A potential problem with all of these ambulatory studies is that superimposed reflux, that is, reflux that occurs when oesophageal pH is below 4, was not taken into account. Superimposed reflux can artifactually prolong the measured clearance time of individual reflux episodes by combining separate reflux episodes. Much of this reflux probably occurs from a hiatus hernia (Mittal, Lange et al. 1987).

#4.7.5 The impact of fundoplication on oesophageal motility

It has been suggested that laparoscopic fundoplication reduce GOR by changing the mechanical properties and action of the gastro-oesophageal junction that result in incomplete abolition of the high-pressure zone during LOS relaxation and reduced triggering of transient sphincter relaxations (Ireland, Holloway et al. 1993)). Manometry has been widely used because it allows accurate measurement of oesophageal peristaltic characteristics. Manometry has also been used in the postoperative period but often falls shorts of providing insight into the cause of dysphagia under certain conditions (Wills and Hunt 2001). A previous investigation has undertaken to determine whether oesophageal motor function changes postoperatively and whether oesophageal dysmotility affects clinical outcome after laparoscopic fundoplication (Fibbe, Layer et al. 2001). Oesophageal motility was assessed twice before and 4 months after the operation in 200 patients. The study

showed oesophageal motility remained unchanged in 85% of patients and changed from pathologic to normal in 20 and vice versa in 9 patients. Preoperative oesophageal dysmotility was associated with more severe reflux symptoms compared with normal motility. It was concluded that oesophageal dysmotility is not corrected by fundoplication and may occur as a result of fundoplication.

The findings have implications for the question of whether oesophageal dysmotility is a primary event or a consequence of reflux-induced damage. It could be argued that oesophageal dysmotility persisted in this study because it is a primary event in GORD. However, the finding of a favorable clinical outcome independent of preoperative oesophagus body motility rather supports the assumption that once severe impairment in oesophageal function has occurred, the damage done by long-term reflux is permanent (Deschamps, Allen et al. 1998). Hence, it may implicate that a defective sphincter is the primary event in GORD and that loss of oesophageal contractility follows with time.

One of the striking observations of this study was the higher prevalence of new-onset dysphagia failure after Nissen fundoplication in the normal motility group. This clearly contradicts the rationale of the tailored concept. Additionally, the authors found a significant increase in primary peristalsis failure and worsening of dysphagia. Nevertheless, both preoperative dysphagia and postoperative new-onset dysphagia were not always associated with a deterioration of oesophageal motility. It may suggest that the assessment of oesophageal motility by standard manometry is not sensitive enough to detect underlying motor abnormalities that can cause dysphagia.

#4.7.6 Relationship between oesophageal bolus clearance and post-fundoplication dysphagia

Antireflux surgery is effective treatment for GORD (Watson, Jamieson et al. 1996), but up to 30% of patients develop dysphagia postoperatively (Stein, Feussner, et al. 1996). A number of potential causes are considered as contributing to this complication (Pandolfino, Curry et al. 2005, Watson, Jamieson, et al. 1996). The relationship between symptoms, anatomic abnormalities and oesophageal function in term of integrity of peristalsis and acid exposure is unclear. Nevertheless, dysphagia is usually either the result of anatomic abnormalities of the fundoplication and/or inadequate oesophageal motility to propel food or liquid past fundoplication.

MII is a technique that allows direct evaluation of the transit and clearance of

swallowed air, liquid and viscous material from and within the oesophagus. A recent study has characterized oesophageal bolus transit and clearance in a population with post-fundoplication dysphagia. Yigit et al. evaluated 80 patients after fundoplication, who underwent simultaneous manometry and MII, 24-hour pH monitoring, and endocopy (Yigit, Quiroga et al. 2006). For analysis, patients were divided into the following groups based on the presence of dysphagia and fundoplication anatomy (by UGI/endoscopy): (1) Dysphagia and normal anatomy; (2) Dysphagia and abnormal anatomy; (3) No dysphagia and abnormal anatomy; and (4) No dysphagia and normal anatomy. Patients with dysphagia (Groups 1 & 2) had similar peristalsis (manometry), but were more likely to have impaired clearance by MII (32 pts, 62%) than those without dysphagia (9 pts, 32%). Patients with abnormal anatomy (Groups 2 & 3) were also significantly more likely to have impaired oesophageal clearance (66% *vs.* 38%). Finally, of patients that had normal post-operative anatomy, those with dysphagia were much more likely to have impaired clearance (12 pts, 52%) than those with dysphagia (4 pts, 21%). The authors suggest that MII can provide objective evidence of disturbed oesophageal clearance after fundoplication, and is common in patients with abnormal postoperative anatomy and/or dysphagia. Oesophageal clearance is impaired in the majority of patients with postoperative dysphagia, even those with normal fundoplication anatomy and normal peristalsis. They concluded that MII can detect oesophageal dysmotility not detected by conventional manometry. This suggest that's there are abnormalities in oesophageal motility that are only detectable by MII, which contribute to post-fundoplication dysphagia.

In this study, abnormal post-operative anatomy impaired oesophageal clearance, and most of these patients experienced dysphagia, supporting a previous notion that 25-40% of redo fundoplications are performed to repair anatomic problems causing dysphagia (Granderath, Kamolz et al. 2002). It is assumed that this dysphagia is from impaired oesophageal clearance consequent to the abnormal fundoplication. On the other hand, it is suggested that the dysphagia is due to an intrinsic oesophageal motility abnormality in those patients who have post-fundoplication dysphagia without any anatomic abnormality. This is due to the fact that the oesophagus fails to generate the conditions necessary to propel the swallowed bolus past the resistance provided by the fundoplication, even when the latter is normal. Tatum et al. used timed barium fluoroscopy and showed that post-fundoplication dysphagia correlated with impaired oesophageal clearance for liquid, semisolid, and solid boluses (Tatum,

Shi et al. 2000). Similarly, among patients with anatomically normal fundoplication in the study (Yigit, Quiroga, et al. 2006), there was a significant difference in oesophageal clearance between patients with and without dysphagia, although some patients with post-fundoplication dysphagia had normal oesophageal clearance measured by MII.

In summary, there is substantially more impaired clearance in patients with anatomic abnormalities of the fundoplication, as well as in patients with dysphagia and normal anatomy. Future works of patients both before and after fundoplication with MII may help elucidate the mechanisms affecting oesophageal clearance and post-fundoplication dysphagia.

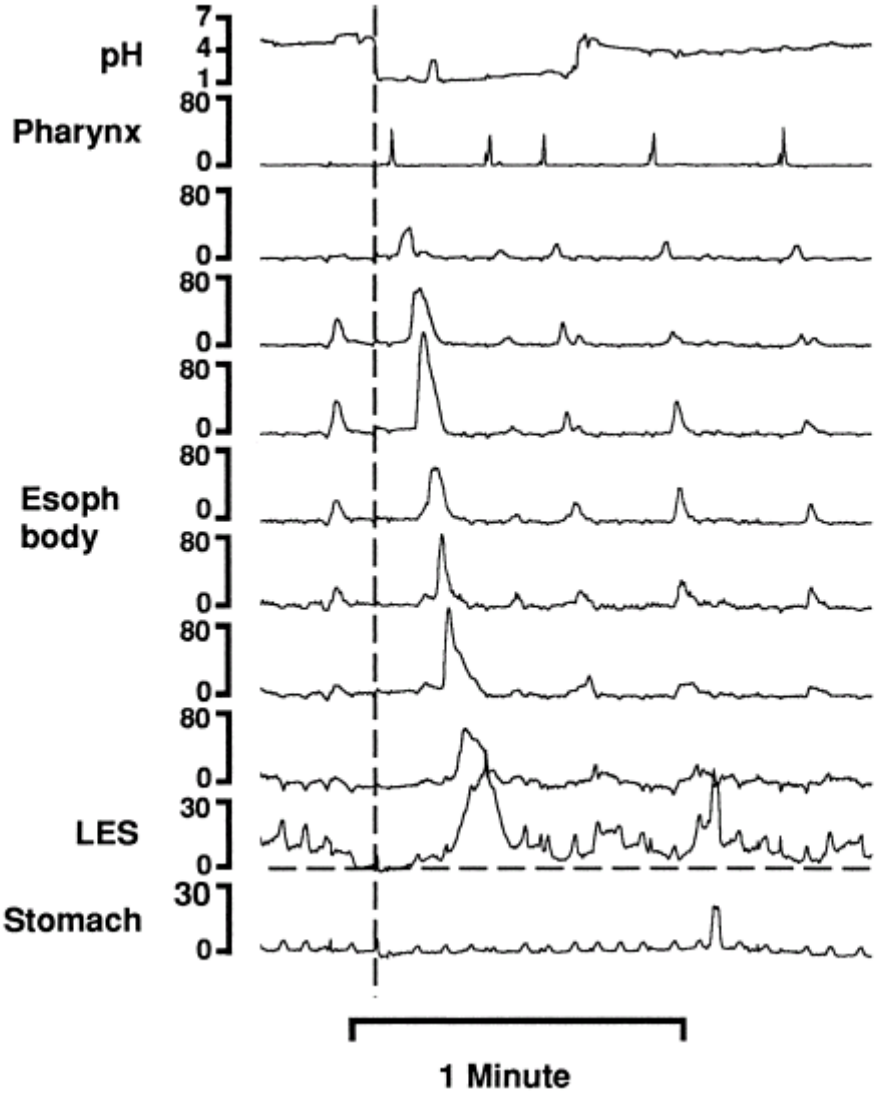


Figure 4.1: GORD occurring during an episode of TLOSR. The occurrence of reflux is indicated by the vertical dashed line. The first response after reflux is primary peristalsis that terminates.

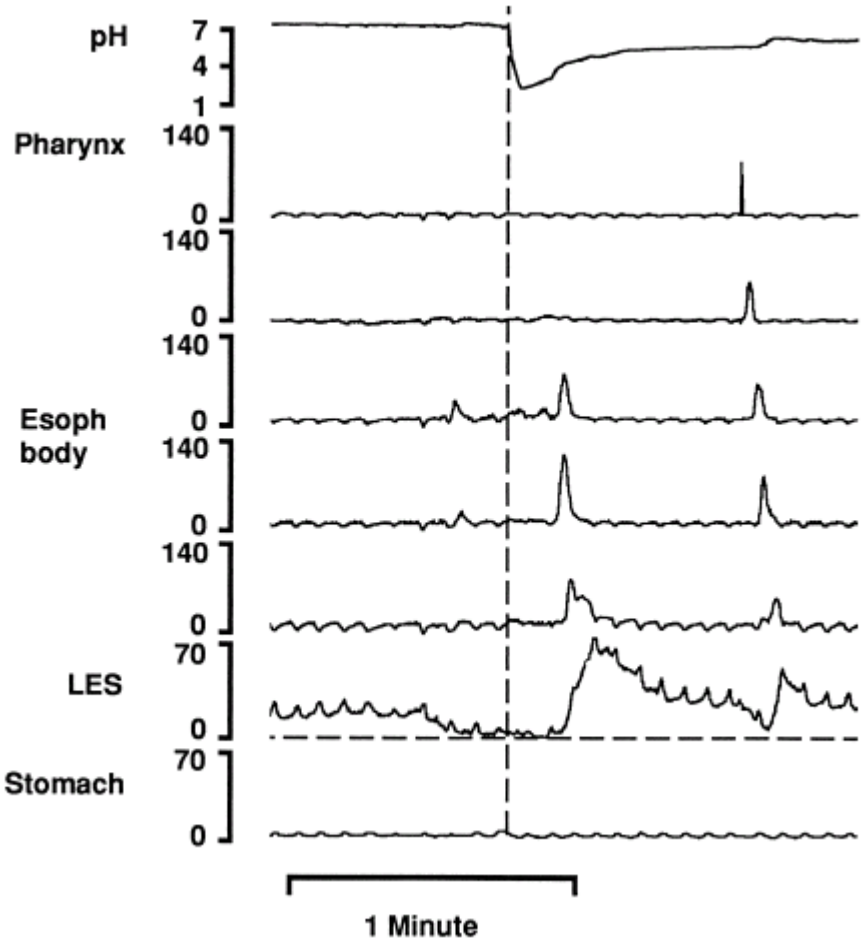


Figure 4.2: GORD occurring during an episode of TLOSR. The occurrence of reflux is indicated by the vertical dashed line. The first response after reflux is an abnormal peristaltic consequence.

4.8. Globus sensation

#4.8.1 Definition

Globus sensation, derived from the Latin word for a ball, is classically defined as a sense of a lump, a retained food bolus, or tightness in the throat. The symptom is non-painful, frequently improves with eating, commonly is episodic, and is not associated with dysphagia or odynophagia. Globus is unexplained by structural lesions, GORD, or histopathology-based oesophageal motility disorders. A range of other foreign body-like descriptors are reported by patients including a sense of

retained particulate matter, mucus accumulation or a restrictive or choking sensation (Cook, Shaker et al. 1991). This sensation is usually perceived in the midline between the thyroid cartilage and the manubriosternal notch, such condition is not a disease but rather than a symptom.

#4.8.2 Current diagnostic criteria for globus

The diagnosis is made from a compatible clinical history, including clarification that dysphagia is absent. Physical examination of the neck followed by nasolaryngoscopic examinations of the pharynx and larynx are recommended, although routine use of nasolaryngoscopy in patients with typical symptoms remains debated. There are grounds for a therapeutic trial of a proton-pump inhibitors when uninvestigated patients present with the symptom of globus, particularly when typical reflux symptoms coexist. However, positive response in symptomatic improvement after proton-pump inhibitors trial may indicate the diagnosis of GORD, even in the absence of objective evidence of GORD.

According to Rome III criteria for functional oesophageal disorders (Galmiche, Clouse et al. 2006), the diagnostic criteria for globus must include all of the following presentations for the last 3 months with symptom onset at least 6 months before diagnosis:

1. Persistent or intermittent, non-painful sensation of a lump or foreign body in the throat.
2. Occurrence of sensation between meals.
3. Absence of dysphagia or odynophagia.
4. Absence of evidence that GOR is the cause of the symptoms.
5. Absence of histolopathology-based oesophageal motility disorders.

#4.8.3 Aetiological and pathogenetic factors implicated in globus

There have been many plausible theories reported on the etiology of globus. These include cricopharyngeal spasm (Watson and Sullivan 1974), temporomandibular joint dysfunction (Puhakka and Kirveskari 1988), pharyngeal dysmotility (Wilson, Pryde et al. 1989), and GOR (Batch 1988a)). A relationship has been established with GOR which was detected in a significant proportion of globus patients (Batch 1988a, Batch 1988b, Hill, Stuart et al. 1997), but this evidence is still conflicting (Wilson, Heading et al. 1987, Wilson, Pryde, et al. 1989). Moreover, it has

been suggested that life stress might has a role in symptom genesis or exacerbation relevant to globus sensation (Harris, Deary et al. 1996). Therefore, the cause of this sensation remains intangible and the disorder is probably multi-factorial in origin (Wilson, Deary et al. 1991).

Whilst much has been investigated regarding potential factors contributing to globus, few studies have focused on the neurophysiological role in globus sensation. In 1989, Cook et al. (Cook 1989) first noted that typical globus sensation was elicited by balloon distension of the oesophagus in a majority of patients with globus who appeared to be hypersensitive to oesophageal stretch rather than acidic exposure. It was implicated that globus is primarily sensory disorder. Since visceral hypersensitivity with aberrant somatic referral of intestinal pain or discomfort is highly prevalent in all functional bowel disorders (Mertz 2003), it is unclear whether similar finding could be found in globus patients. Therefore, part of our thesis work will aim to test the hypotheses that globus patients may have heightened visceral perception to different oesophageal stimuli with balloon distension and electrical stimulation, and that the characteristics of viscerosomatic referral of oesophageal nociceptive stimuli may differ between globus patients and healthy subjects.

Chapter 5

Aims and hypotheses addressed in this thesis

This body of work can be divided into 4 broad areas: 1) methodology regarding impedance technique and its utility; 2) the relationships among peristalsis, bolus clearance and symptom perception in various dysphagia syndromes; 3) the relationships among peristalsis, bolus clearance and symptom perception in gastro-oesophageal reflux disease (GORD) and globus; and 4) transient receptor potential vanilloid subfamily, member 1(TRPV1) expression in the oesophageal mucosa in patients with GORD. Each will be dealt with separately in this chapter although the methodological techniques will overlap among the different pathophysiological sections.

5. 1 Impedance and its utility in distinguishing differences in clearance characteristics between primary and secondary peristalsis.

#5.1.1 Combined manometry-impedance in the evaluation of primary peristalsis in healthy controls

In **Chapter 3** I discussed the significant role of impedance in the measurement of oesophageal transit and in the sensitive detection of acid, non-acid and gaseous gastro-oeesophageal reflux (GOR). In patients with non-specific motor disorders, combined MII-EM helps to further clarify the functional significance of so called ineffective motility (IEM) (Tutuian and Castell 2004a). Therefore, combined multichannel intraluminal impedance and oesophageal manometry (MII-EM) is emerging as an important tool for obtaining detailed information about the physiology and pathophysiology of oesophageal motility. The potential clinical importance of this technique may include: (1) a better functional classification of oesophageal motor disturbances in patients with non-obstructive dysphagia (NOD); (2) potentially better pre-operartive assessment of patients undergoing laparoscopic fundoplication and perhaps other endoscopic or surgical procedures which could adversely impact oesophageal motility and swallow function (eg, laparoscopic banding); and (3) a better understanding of the determinants of oesophageal bolus transport by primary and secondary peristalsis.

- **Aims:** To establish normative data for combined impedance-manometry (MII-EM) and to characterize normal liquid and viscous bolus transit by

primary peristalsis in a Chinese population.

- **Hypothesis:** That combined MII-EM expands the diagnostic capability by detecting oesophageal bolus transport and clearance during primary peristalsis

The studies examining the relationship between bolus transit and secondary peristalsis, measured using the impedance technique, are summarized in ***section 5.1.2***. Data and its interpretation arising from this work are presented in **Chapter 6**.

#5.1.2 Combined manometry-impedance in the evaluation of secondary peristalsisin healthy controls

In **Chapter 2** I discussed the mechanisms underlying and clinical significance of secondary peristalsis. Primary peristalsis transports the oesophageal bolus during the volitional swallow, whereas distension-induced or secondary peristalsis maintains an empty oesophagus by clearing persistent residual swallowed material incompletely cleared by the primary swallow as well as refluxed gastric content (Helm, Dodds, et al. 1984). Ample published data exist regarding the reproducibility, manometric characteristics, and abnormalities in disease states of secondary peristalsis (Pandolfino, Shi et al. 2005, Schoeman and Holloway 1994b, Schoeman and Holloway 1995, Sifrim and Janssens 1996). Recent studies have shown in normal subjects that secondary peristalsis can be triggered reliably by different intra-oesophageal stimuli (Schoeman and Holloway 1994b). Additionally, secondary peristalsis may be important for acid clearance during sleep when salivation and swallowing are suppressed (Orr, Johnson, et al. 1984). However, there is very little published data systematically examining the characteristics of oesophageal bolus transport and clearance by secondary peristalsis. Therefore, the recording of oesophageal bolus transit and clearance by this motor activity should be important.

- **Aim:** Characterization of oesophageal bolus transit and clearance by secondary peristalsis in healthy subjects using combined MII-EM.
- **Hypotheses:** 1) Combined MII-EM is feasible in detecting oesophageal bolus clearance by secondary peristalsis; 2) Secondary peristalsis differs from primary peristalsis in efficiency of oesophageal bolus transport and clearance.

The specific pathophysiological characteristics I aim to demonstrate in different patient groups will be summarized in ***section 5.2.2 & 5.2.3.*** Data from these

studies are detailed in **Chapters 7, 9, and 10**.

5.2 Relationships among peristaltic characteristics, bolus clearance and symptom perception in dysphagia syndromes.

#5.2.1 Oesophageal motor dysfunction in NOD may play a limited role in the generation of dysphagia.

In **Chapter 4** the clinical presentation and proposed pathothopysiology of non-obstructive dysphagia (NOD) are discussed. The term "NOD" is used to describe the presence of the sensation of difficulty in swallowing solids or liquids in the absence of an endoscopically or radiologically demonstrable oesophageal lesion or a dysmotility syndrome detected by standard manometric techniques that is recognized to cause dysphagia (Parkman, Maurer, et al. 1996, Richter, Baldi, et al. 1992). Aberrant motility patterns may be detected manometrically in NOD patients. However, these patterns detected during standard water swallows, do not necessarily correlate temporally with nor explain the symptom of dysphagia during routine food consumption (Benjamin, Castell, et al. 1983, Howard, Pryde, et al. 1989). This observation has lead to studies examining boluses more accurately reflecting food ingestion. Conflicting results have been published in NOD patients with regard to manometric characteristics after administration of solid or viscous boluses during standard oesophageal manometry testing (Cordier, Bohn, et al. 1999, Sears, Castell, et al. 1990).

It is well recognized that a food bolus may dwell within the oesophagus for some time without the subject being aware of it. Hence, a crucial component in any dysphagia syndrome is the level of central perception of bolus transport or arrest. A recent study investigated oesophageal sensitivity to mechanical stimuli in patients with NOD, and attempted to correlate the relationships between oesophageal motor abnormalities and oesophageal sensitivity (Bohn, Bonaz, et al. 2002). These authors observed a lowered threshold volume (i.e., increased sensitivity) during balloon distension of the oesophagus in NOD patients when compared with healthy controls.

- **Aim:** To evaluate the relationships among the sensory awareness of bolus presence, oesophageal persistalic function, and bolus clearance during individual swallows.
- **Hypothesis:** Impairment of peristalsis, impaired bolus clearance and heightened awareness of bolus presence can all contribute to the symptom

of dysphagia in NOD.

Data from this work will is presented in **Chapter 8.**

#5.2.2 Identification of impaired bolus clearance by secondary peristalsis in patients with NOD.

The hypothesis of impaired secondary peristalsis rather than primary peristalsis in NOD patients has been suggested by Schoeman et al., who have demonstrated a noticeable defect in secondary peristalsis in NOD patients (Schoeman and Holloway 1994b). Their study has implicated that this defect may further lead to delayed bolus transit along the oesophagus, but the relationship between such changes and alterations in bolus transport, if any, has not been extensively examined. In **Chapter 3**, the clinical utility of oesophageal impedance has been discussed.

- **Aim:** Apply MII-EM to evaluate the integrity and characteristics of oesophageal bolus transit and clearance by secondary peristalsis in NOD patients.
- **Hypothesis:** That triggering of secondary peristalsis and its effectiveness in oesophageal bolus transport and clearance are impaired in patients with NOD.

Data from this study is presented in **Chapter 9.**

#5.2.3 Patients with post-fundoplication dysphagia demonstrate impaired bolus clearance in response to primary and secondary peristalsis

Laparoscopic fundoplication reduce GOR by changing the mechanical properties and action of the gastro-oesophageal junction that result in incomplete abolition of the high-pressure zone during lower oesophageal sphincter (LOS) relaxation and reduced triggering of transient sphincter relaxations (Ireland, Holloway, et al. 1993). A previous investigation has undertaken to determine whether oesophageal motor function changes postoperatively and whether oesophageal dysmotility affects clinical outcome after laparoscopic fundoplication (Fibbe, Layer, et al. 2001). Oesophageal motility was assessed twice before and 4 months after the operation in 200 patients. Preoperative oesophageal dysmotility was associated with more severe reflux symptoms compared with normal motility. It was concluded that oesophageal dysmotility is not corrected by fundoplication and may occur as a result of fundoplication. The other study has characterized oesophageal bolus transit and

clearance in a population with post-fundoplication dysphagia (Yigit, Quiroga, et al. 2006). They concluded that combined impedance and manometry can detect oesophageal dysmotility not detected by conventional manometry. The demonstration of abnormal oesophageal bolus clearance may contribute to post-fundoplication dysphagia.

- **Aim:** To evaluate the integrity and characteristics of oesophageal bolus transit and clearance by secondary peristalsis in patients after fundoplication.
- **Hypothesis:** That patients after fundoplication demonstrate functional abnormalities in the triggering of the secondary peristaltic reflex and are more likely to generate abnormal secondary peristaltic sequences and defective bolus clearance by this motor pattern.

Data from this study is presented in **Chapter 10.**

5.3 Peristaltic dysfunction, impaired bolus clearance, and symptom perception in GORD and globus patients

#5.3.1 Impaired bolus clearance by primary peristalsis exists in GORD.

Chapter 4 of the literature review discussed dysphagia in non-stricturing GORD. Hypotensive peristaltic contractions are low-amplitude (<30 mmHg) peristaltic contractions detected on oesophageal manometry during water swallowing. They are associated with ineffective oesophageal transit (Kahrilas, Dodds, et al. 1988b). In patients with dysphagia or GORD, the frequency of this disturbance increases. By combining manometry with either videofluoroscopy or impedance recording, defective oesophageal bolus transport as well as impaired clearance of the refluxate occurred with contraction amplitudes of <30 mmHg, i.e., hypotensive contractions. This type of esopahageal dysmotility has been called IEM which has been linked to impaired oesophageal bolus clearance in GORD patients by combined MII-EM (Tutuian and Castell 2004b)

- **Aim:** To investigate whether combined MII-EM is superior to EM in identifying patients with potentially significant oesophageal dysmotility.
- **Hypothesis:** That impaired bolus clearance can be demonstrated in reflux patients with potential oesophageal dysmotility in whom traditional manometry may provide limited information.

The Data from this study is presented in **Chapter 11**.

#5.3.2 Impaired bolus clearance by primary peristalsis is more marked in erosive *vs.* non-erosive reflux disease GORD.

As discussed earlier in this section, IEM characterized by an increased proportion of low amplitude peristalsis during traditional manometry is the most common pattern of peristaltic failure, and associated with delayed acid clearance in reflux disease (Leite, Johnston et al. 1997). Although acid reflux occurs greater in erosive than non-erosive reflux disease(NERD), the presence and severity of IEM dose not differ between the two groups of patients (Lemme, Abrahao-Junior et al. 2005). A recent study has reported subtle bolus transit abnormalities in patients with mild GORD with normal oesophageal peristalsis (Domingues, Winograd et al. 2005). To date, data are very few regarding bolus clearance characteristics in patients with erosive GORD compared with those in NERD.

- **Aim:** To determine any difference in oesophageal bolus clearance by primary peristalsis between patients with and without erosive GORD using combined MII-EM.
- **Hypothesis:** That patients with erosive GORD are characterized by delayed oesophageal bolus clearance, whereas their manometric results are similar to patients without erosive oesophagitis.

The data from this study is presented in **Chapter 12.**

#5.3.3 Oesophageal visceral hypersensitivity exists in globus

In Chapter 4 the plausible theories on the etiology of globus were discussed, including pharyngeal dysfunction (Watson and Sullivan 1974, Wilson, Pryde, et al. 1989) and GOR (Batch 1988a). Whilst much has been investigated regarding potential factors contributing to globus, few studies have focused on the neurophysiological role in globus sensation. In 1989, Cook et al. (Cook 1989) first noted that typical globus sensation was elicited by balloon distension of the oesophagus in a majority of patients with globus who appeared to be hypersensitive to oesophageal stretch rather than acidic exposure. Thus, oesophageal visceral hypersensitivity is implicated in the pathogenesis of globus.

- **Aim:** To determine whether visceral hypersensitivity plays a role in the pathogenesis of globus.
- **Hypotheses:** That globus patients have: 1) lower perception and pain thresholds for oesophageal mechanical and electrical stimuli; 2) increased

areas of viscerosomatic pain referrals; 3) aberrant referral of oesophageal stimuli to the neck.

Data from this work is presented in **Chapter 13.**

5.4 Genetic characterization of TRPV1 in oesophageal mucosa of patients with GORD

In **Chapter 1** our current understanding of the relationship between TRPV1 receptor and visceral pain was discussed. TPRV1 has been implicated in the mechanism of pain produced in GORD. In patients with oesophagitis, the proportion of papillae positive for these nerve fibres was elevated, suggesting that acid-induced inflammation may up-regulate expression of acid-sensitive receptors such as TRPV1. Therefore, enhanced expression of TRPV1 in human oesophagus may contribute to the visceral hypersensitivity often seen in patients with GORD (Matthews, Aziz, et al. 2004). An additional study in patients with NERD revealed an increase in TRPV1-expressing nerve fibres in the oesophageal mucosa but without inflammation, further supporting this hypothesis (Bhat and Bielefeldt 2006)).

- **Aim:** To indentify and compare TRPV1 gene expression differences in oesophageal mucosa between GORD patients and controls
- **Hypotheses:** That oesophageal inflammation up-regulates the expression of TRPV1 in oesophageal mucosa, and that increased TRPV1 gene expression is greater in erosive than non-erosive reflux disease

Data from this work is presented in **Chapter 14.**

SECTION B

EVALUATION OF IMPEDANCE AND ITS UTILITY IN DISTINGUISHING CLEARANCE CHARACTERISTICS BETWEEN PRIMARY AND SECONDARY PERISTALSIS

The work presented in this chapter has been published in J Gastroenterol Hepatol 2007:**22**:1039-1043.

Chapter 6

Assesment of oesophageal motor function using combined multichannel intraluminal impedance and manometry

6.1 Introduction

MII allows evaluation of bolus transit without radiation.(Silny 1991) The principles of impedance technique are based on measuring differences in resistance to alternating current of the intraluminal contents.(Srinivasan, Vela et al. 2001) Using multiple impedance measuring sites, it allows detection and quantification of bolus movement. Previous studies with combined video-fluoroscopy and MII have validated the ability of impedance to detect bolus movement.(Silny 1991, Simren, Silny, et al. 2003) Using MII and oesophageal manometry (MII-EM), Tutuian et al. have established normal values for this technique (Tutuian, Vela, et al. 2003) In addition, MII-EM has been used to clarify functional abnormalities in patients with abnormal manometric studies. (Tutuian and Castell 2004b)

Since potential differences may occur in oesophageal motility due to ethnic differences, it is important that normative values may be required for different populations. There has been no previous study on normal parameters for combined MII-EM in Chinese population. Our study aimed to establish normal values for combined MII-EM in healthy Taiwanese residents.

6.2Methods

#6.2.1 Subjects

Our subjects were 18 healthy volunteers (twelve men and six women; mean age 24 years, range 19-36 years) who were non-smokers and took no medication. They were totally asymptomatic and had no history of oesophageal, gastric or duodenal disease. They had not taken any medication in the week prior to the study. Informed written consent was obtained from each subject prior to the study.

#6.2.2 Oesophageal manometry and impedance recording

Each subject underwent oesophageal function testing using combined MII-EM with a Koenigsberg 9-channel probe (Sandhill EFT catheter; Sandhill Scientific, Inc.,

Highlands Ranch, CO). The 4.5 mm diameter catheter design has two circumferential solid-state pressure sensors at 5 cm and 10 cm from the tip and three unidirectional pressure sensors at 15, 20, and 25 cm. Impedance measuring segments including two rings placed 2 cm apart, were centered on 10, 15, 20, and 25 cm from the tip, thus across the four proximal pressure transducers. The EFT catheter was inserted transnasally into the oesophagus up to a depth of 60 cm. Lower oesophageal sphincter (LOS) was identified using stationary pull-through technique and the most distal sensor was placed in the high-pressure zone of the LOS. Intraoesophageal pressure sensors and impedance measuring segments were thus located at 5 cm, 10 cm, 15 cm, and 20 cm above the LOS (Figure 6.1). In the supine position, each subject was given 10 swallows of 5 cc normal saline and 10 swallows of 5 cc viscous (apple-sauce like consistency) (Sandhill Scientific) material each 20–30 sec apart. Normal saline was used instead of regular water since it provides better impedance change with a standardized ionic concentration.

#6.2.3 Analysis

Manometric parameters included: (1) contraction amplitude at 5 and 10 cm above the LOS, (2) distal oesophageal amplitude (DEA) as average of contraction amplitude at 5 and 10 cm above the LOS, and (3) onset velocity of oesophageal contractions in the distal part of the oesophagus (i.e., between 10 cm and 5 cm above the LOS). Mid-respiratory resting pressure and LOS residual pressure during swallowing were used to assess LOS function. MII parameters analyzed included bolus entry at each specific level obtained at the 50% point between 3-sec pre-swallow impedance baseline and impedance nadir during bolus presence and bolus exit determined as return to this 50% point on the impedance-recovery curve.

Impedance characteristics included (1) total bolus transit time (TBTT) as time elapsed between bolus entry at 20 cm above LOS and bolus exit at 5 cm above LOS, (2) bolus head advance time (BHAT) as time elapsed between bolus entry at 20 cm above LOS and bolus entry at 15, 10, and 5 cm above LOS, (3) bolus presence time (BPT) as time elapsed between bolus entry and bolus exit at each impedance measuring site (5, 10, 15, and 20 cm above LOS), and (4) segmental transit times as time elapsed between bolus entry at a given level above LOS and bolus exit at the next lower level (Figure 6.2).

Swallows were manometrically classified as: (1) normal, if contraction

amplitudes at 5 and 10 cm above the LOS were each greater than or equal to 30 mmHg and distal onset velocity was less than 8 cm/sec; (2) ineffective, if either of the contraction amplitudes at 5 and 10 cm above the LOS was less than 30 mmHg (this includes contractions defined as "poorly transmitted" or "not transmitted" described by other authors); (3) simultaneous, if contraction amplitudes at 5 and 10 cm above the LOS were each greater or equal to 30 mmHg and distal onset velocity was greater than 8 cm/sec. Swallows were classified by MII as showing: (1) complete bolus transit (CBT), if bolus entry occurred at the most proximal site (20 cm above LOS) and bolus exit points were recorded in all three distal impedance-measuring sites (i.e., 15 cm, 10 cm, and 5 cm above the LOS) and (2) incomplete bolus transit, if bolus exit was not identified at any one of the three distal impedance-measuring sites.

#6.2.4 Statistical analysis

The data of manometry and impedance were expressed as median (interquartile range, and 95 percentile) because they were not normally distributed. Statistical analysis was performed using non-parametric tests. Significance was determined at $p < 0.05$.

6.3 Results

The study included 18 healthy subjects with 360 swallows (180 liquid and 180 viscous). Impedance parameters for the 18 normal subjects are shown in Table 6.1. There was significantly faster advance of the head of liquid compared to viscous boluses ($p < 0.001$). The BPT progressively increased in each segment as the bolus traveled down the oesophagus for liquid but not viscous boluses. BPT for liquid were significantly longer than viscous ($p \leqq 0.001$) in the distal oesophagus. The TBTT for liquid was significantly shorter than for viscous boluses ($p < 0.001$). Complete transit through the entire oesophagus occurred in 95% of both liquid and viscous swallows in less than 10.7 seconds.

The prevalence of complete transit for both liquid and viscous bolus materials is presented in Table 6.2. CBT of at least 70 % liquid swallows was found in 14 of 18 (89%) of the normal subjects. CBT of at least 70% of the viscous swallows was seen in 17 of 18 (94%) of the normal subjects. None of the subjects had more than 40% incomplete liquid and 40% incomplete viscous swallow.

Manometric features for the 18 normal subjects are shown in Table 6.3. Some of

manometric parameters for liquid and viscous material were different. Contraction amplitude for the viscous swallows was slightly higher at 5 cm above the LOS (p = 0.052). Duration of contractions for the viscous swallows was longer at 20 cm and 5 cm above the LOS compared to liquid swallows. Distal oesophageal velocity of onset contractions did not differ for liquid and viscous swallows (p = 0.416). LOS residual pressure during viscous swallows was greater than the residual pressure during liquid swallow (p = 0.069). There was no difference in relation duration of LOS for liquid and viscous swallows (p = 0.157).

The percentage of normal and ineffective peristalsis on manometry was similar for both liquid and viscous swallows (p = 0.50). The percentage of swallows with complete and incomplete bolus transit by impedance was similar for both liquid and viscous swallows (p = 0.68). 98.4% of normal liquid swallows and 97.7% of normal viscous swallows had CBT on impedance. 56.3% of ineffective liquid swallows and 50% of ineffective viscous swallows had CBT on impedance. No simultaneous contraction was found in the study.

6.4 Conclusion

The results of this study using combined MII-EM as the standard approach to oesophageal testing help clarify oesophageal function, in particular, bolus transport and clearance. Previous studies have demonstrated normal values derived from recordings using MII with solid-state or perfusion manometry. (Nguyen, Rigda, et al. 2005, Tutuian, Vela, et al. 2003) The present study was the first to provide normal values for Chinese population using combined solid state manometry and MII. Our findings in the manometric features for liquid and viscous are lower than those reported by Tutuian et al. (Tutuian, Vela, et al. 2003) with regard to peristaltic amplitude and duration, but our median values for onset velocity of oesophageal contraction are greater than theirs. This discrepancy may be due to the fact that data from healthy subjects with ineffective motility have been excluded in their study. (Tutuian, Vela, et al. 2003) Furthermore, ethnic differences could play a role leading to these differences.

Our study has shown normal (median, inter-quartile range, and 95th percentile) values for TBTT of liquid and viscous boluses. It has been suggested that the value in less than 12.5 seconds (95th percentile) could be considered the upper limit of normal impedance bolus transit for oesophageal clearance. (Tutuian, Vela, et al. 2003)

Comparing with their results, we have found our upper limit of 95% of liquid and viscous boluses transit in less than 11.0 seconds. In addition, we suggest, based on the studies population, Chinese subjects with 70% or more CBT for liquid or with 70% or more CBT for viscous to be considered as having normal oesophageal transit.

The results of oesophageal bolus transit by using MII are similar to previous data which, with radiographic or scintigraphic method, have shown oesophageal clearance times of 8-10 seconds. (Kahrilas, Dodds et al. 1988a, Maddern, Slavotinek et al. 1985) In addition, we have demonstrated that viscous materials exhibit more bolus transit time but similar contraction amplitude compared with liquid swallows. Similar findings have been reported elsewhere. (Srinivasan, Vela, et al. 2001, Tutuian, Vela, et al. 2003) The reasons for this difference may relate to the fact that the presence of the viscous bolus with increased viscosity and cohesion leads to a denser bolus that does not travel through the oesophagus as smooth as a liquid bolus. Because of the difference in the transit pattern found with liquid and viscous boluses, the use of both test media should be valuable to detect oesophageal dysmotility. Further studies are needed to investigate the value of information obtained from viscous swallows.

The findings of impedance characteristics compared with manometric tracings showed that more than half of the liquid and half of viscous ineffective swallows had CBT. The results are in accordance with the previous observation in normal volunteers, (Tutuian, Vela, et al. 2003) although greater viscous ineffective peristalsis with CBT by impedance was observed in our study. On the other hand, above 50% of ineffective viscous and 44% of ineffective liquid swallows had also incomplete bolus transit, suggesting that impedance together with a test medium such as a viscous solution may be helpful in characterize further detailed information in oesophageal motility.

Since the aims of the present study were to investigate the impedance features of oesophageal motor function in healthy Chinese subjects, the results are descriptive. Comparing with the data in Caucasians, (Tutuian, Vela, et al. 2003) some differences in MII-EM arising in our study are: (I) lower oesophageal contraction amplitude and duration occur in our subjects; (II) our subjects have lower values for TBTT, i.e., rapid oesophageal transit; and (III) Chinese subjects with viscous ineffective peristalsis are more likely to have CBT.

In conclusion, the application of MII on a traditional solid-state manometric recording may enhance the diagnostic capability of oesophageal function testing.

Normal variables for impedance parameters in combination with manometry have been created in Chinese population, which will help standardization in such diagnostic technique for routine clinical use as well as for future research.

Table 6.1: Impedance features for Liquid and Viscous Swallows

	Liquid (N = 180)		Viscous (N = 180)		
	Median (interquartile range)	95 percentile	Median (interquartile range)	95 percentile	*p* value
Bolus head advance time (BHAT) (s)					
20-15 cm	0.1 (0.1-0.2)	0.5	0.8 (0.4-1.5)	2.8	<0.001
20-10 cm	0.3 (0.2-0.5)	1	3.3 (2.4-4.3)	4.8	<0.001
20-5 cm	0.6 (0.5-0.9)	2.6	5.0 (4.1-6.0)	7	< 0.001
BPT (s)					
at 20 cm	2.5 (1.9-3.4)	4.7	2.9 (2.0-3.8)	6.2	0.168
at 15 cm	3.9 (3.2-4.8)	6.3	3.9 (3.2-4.6)	5.8	0.73
at 10 cm	4.9 (4.2-5.7)	7	3.4 (2.5-4.4)	5.7	0.001
at 5 cm	5.7 (5.1-6.6)	8.1	3.4 (2.7-4.3)	5.8	<0.001
Segment transit time (s)					
20-15 cm	4.0 (3.4-4.9)	6.4	4.9 (4.2-5.6)	8	0.004
15-10 cm	5.2 (4.4-6.2)	7.6	5.6 (4.7-6.6)	8.8	0.588
10-5 cm	6.0 (5.5-7.0)	8.5	5.1 (4.3-6.1)	7.7	0.003
TBTT (s)	6.5 (5.9-7.5)	9.6	8.5 (7.6.3-9.4)	10.7	<0.001

Table 6.2

	Subjects (N = 18)	
% Swallows with CBT	%	Cumulative %
Liquid		
90	66.7	66.7
80	11.1	77.8
70	11.1	88.9
60	11.1	100
Viscous		
90	61.1	61.1
80	11.1	72.2
70	22.2	94.4
60	5.6	100

Table 6.3

	Liqiud (N =80)		Viscous (N = 80)		
	Median (interquartile range)	95 percentile	Median (interquartile range)	95 percentile	*p* value
Amplitude of constractions (*mm Hg*)					
at 20 cm	36 (31-49.5)	74	39 (29-48)	66.6	0.649
at 15 cm	52 (40.5-73.0)	88.6	55 (47.5-67)	83	0.859
at 10 cm	68 (54.5-88)	108.6	73 (57-83)	111.6	0.626
at 5 cm	105 (92.5-120.5)	145	115 (108.5-127.5)	151.8	0.052
DEA	91 (80.5-99)	121.6	93 (84-108.5)	121.4	0.367
Duration of contractions (*s*)					
at 20 cm	2.3 (1.7-2.6)	3.1	2.2 (2.6-2.8)	3.7	0.001
at 15 cm	2.5 (2.1-2.8)	3.2	2.6 (2.4-3.0)	3.4	0.117
at 10 cm	2.4 (2.1-2.7)	2.8	2.5 (2.2-2.8)	3.7	0.227
at 5 cm	2.9 (2.4-3.4)	3.9	3.2 (2.9-3.7)	4.5	0.015
Onset velocity of contractions (*cm/s*)					
10-5 cm	4.7 (3.9-5.8)	6.9	4.4 (3.3-7.8)	11.3	0.416
Low oesophageal sphincter					
Residual Pressure (*mm Hg*)	5.7 (1.3-8.8)	12.3	4.9 (1.1-6.9)	8.9	0.069
Relaxation duration (*s*)	6.1 (3.4-8.6)	11.5	9.4 (7.1-11.4)	12	0.157

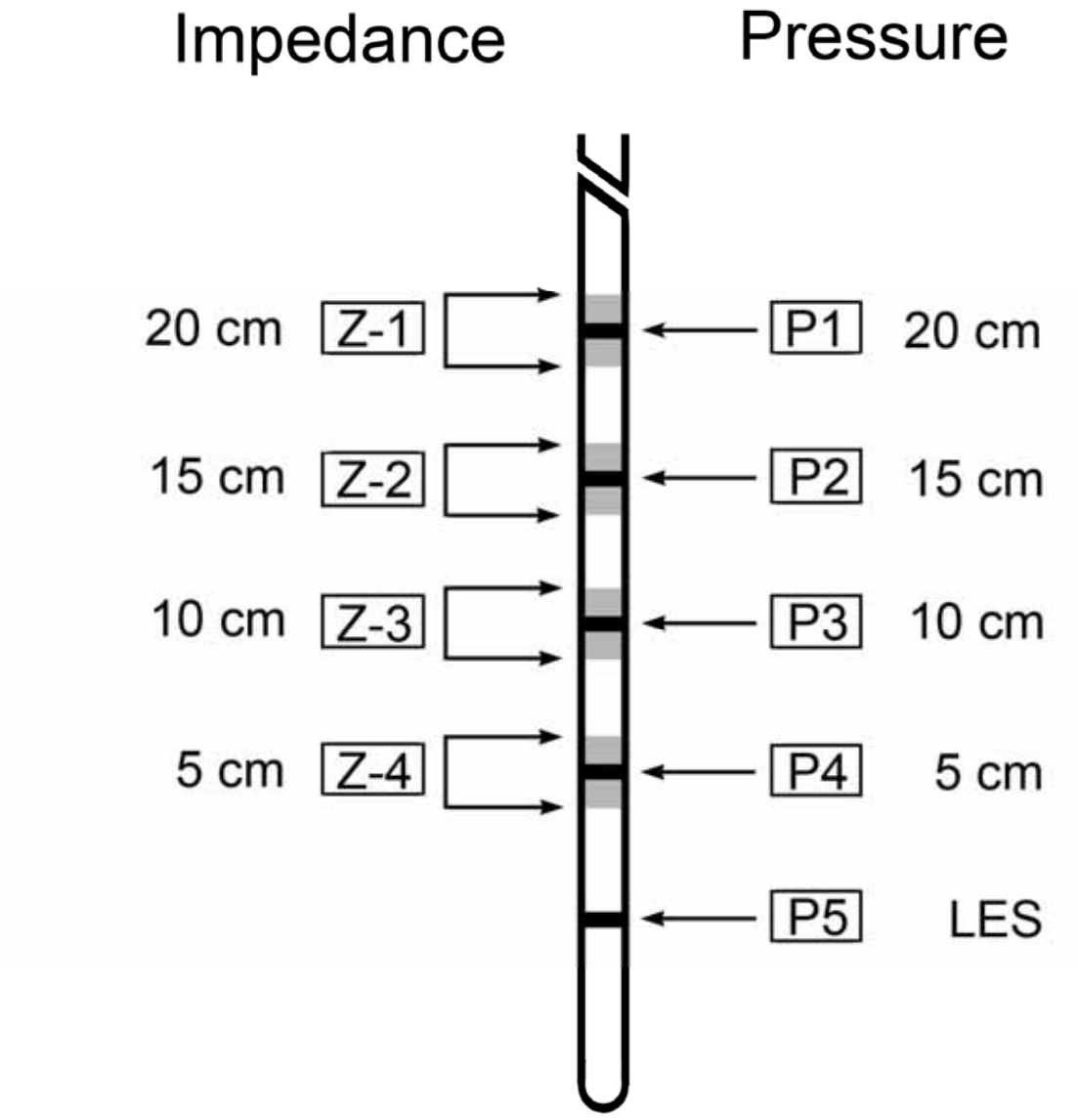


Figure 6.1: 9-channel combined MII/EM catheter. Circumferential solid-state pressure sensors located in LOS high-pressure zone (P5) and 5 cm above it (P4), unidirectional solid-state pressure sensors located 10 (P3), 15 (P2), and 20 cm (P1) above LOS. Impedance-measuring segments centered at 5 cm (Z4), 10 cm (Z3), 15 cm (Z2), and 20 cm (Z1) above LOS.

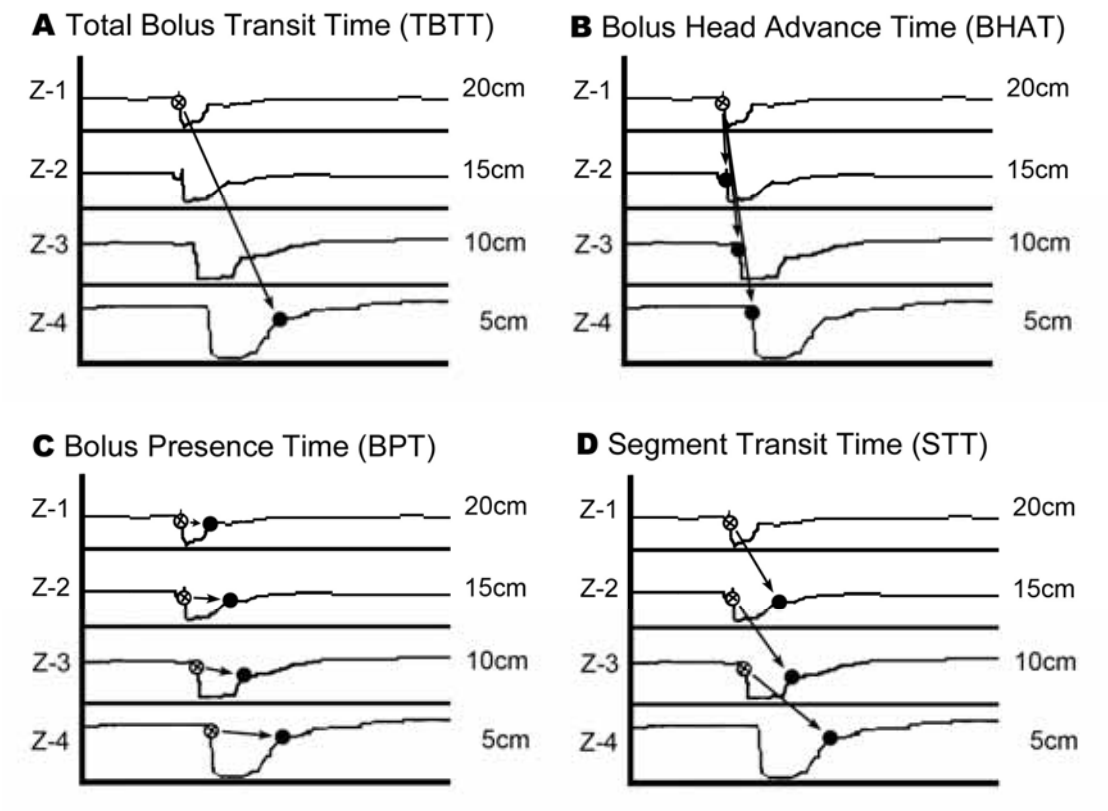


Figure 6.2: Four impedance parameters were defined: (1) TBTT as time elapsed between bolus entry at 20 cm above LOS and bolus exit at 5 cm above LOS, (2) BHAT as time elapsed between bolus entry at 20 cm above LOS and bolus entry at 15, 10, and 5 cm above LOS, (3) BPT as time elapsed between bolus entry and bolus exit at each impedance measuring site (5, 10, 15, and 20 cm above LOS), and (4) segmental transit times as time elapsed between bolus entry at a given level above LOS and bolus exit at the next lower level.

The work presented in this chapter has been published in Eur J Gastro Hepatol 2008 ;**20**(12):1129-35.

Chapter 7

Bolus transit and clearance by secondary peristalsis in the healthy oesophagus: studies using combined impedance-manometry

7.1 Introduction

Primary peristalsis transports oesophageal bolus after swallowing, whereas distension-induced or secondary peristalsis functions to maintain an empty oesophagus by clearing refluxed gastric contents (Helm, Dodds, et al. 1984) or residual food bolus. Secondary peristalsis may be important for acid clearance during sleep states when salivation and swallowing are suppressed (Orr, Johnson, et al. 1984). Secondary peristalsis can be reliably triggered by intra-oesophageal air or water infusion (Schoeman and Holloway 1994b) Whilst ample data are available in published works about the manometric characteristics and reproducibility of initiation of secondary peristalsis in health and a variety of disease states (Pandolfino, Shi, et al. 2005, Schoeman and Holloway 1994a, Schoeman and Holloway 1994b, Schoeman and Holloway 1995, Sifrim and Janssens 1996), there is a paucity of data on the efficiency of oesophageal bolus transport and clearance by secondary peristalsis in health or in disease.

Multichannel intraluminal impedance (MII) is a technique that allows detection of oesophageal bolus transport and real-time quantification of bolus movement without radiation (Silny 1991). Multichannel intraluminal impedance and oesophageal (MII-EM) is also able to enhance the diagnostic capability and clarify functional abnormalities in patients with disordered oesophageal clearance (Tutuian and Castell 2004b). By using MII-EM, our primary aim was to quantify the relationship between secondary peristalsis and bolus transport as well as effectiveness of oesophageal clearance by this motor pattern. Specifically, we hypothesized that secondary peristalsis may be equally effective as primary peristalsis regarding oesophageal bolus transport and clearance. A secondary aim of our study was to determine bolus transit times of primary peristalsis, as determined by impedance, for solids and liquid boluses and to confirm the hypothesis that total transit time for solid boluses is slower than that for liquid boluses in response to primary peristalsis.

7.2 Methods

#7.2.1 Subjects

We studied eleven healthy volunteers (4 female, mean age 22, range 19-26 yr) recruited from the community by advertisement. All subjects were free of oesophageal symptoms and had no evidence of acute or chronic illness. None of the subjects were taking medications known to influence oesophageal motor function. All subjects gave written informed consent prior to the study, and the protocol was approved by the Human Ethics Committee of the South-Eastern Sydney Area Health Service.

#7.2.2 Oesophageal manometry and impedance recording

Oesophageal motility and impedance were recorded with a custom-designed silicone rubber manometric catheter (outer diameter 2.5 mm) with 8 recording sideholes spaced at 3-cm intervals and 7 stainless steel electrode rings (4 mm long) spaced at 3-cm intervals. An additional polyvinyl catheter, 1.8 mm internal diameter was attached to the manometric catheter so that the injection port was located in the mid oesophagus (Figure 7.1). The injection was performed with a hand-held syringe which was connected to the proximal end of the catheter. The manometric assembly was perfused with degassed distilled water by a low-compliance pneumohydraulic perfusion pump (Dentsleeve; Wayville, South Australia, Australia) at 0.3 ml/min per channel. Pressures were recorded for each perfused channel by 9 external pressure transducers. Pressure and impedance signals were acquired simultaneously using computer-based data-acquisition system (Solar GI; MMS, The Netherlands). Swallowing was detected via the most proximal channel of the assembly, which was sited in the pharynx, thus enabling primary and secondary peristalsis to be distinguished.

#7.2.3 Study protocol

The subjects were fasted for at least 4h. The assembly was passed via the nose and positioned such that the most distal side hole was located on the upper margin of the lower oesophageal sphincter (LOS). The subjects were then positioned in supine position and allowed to accommodate for 10-15 minutes. Primary peristalsis was studied using 5 ml boluses of normal saline and solid agar, each tested 5 times. Each swallow was separated by a 30 second interval. The subject had to chew solid agar

into smaller particles as possible before swallowing. For the induction of secondary peristalsis, rapid injection of saline into mid-oesophagus was performed by hand. The 20 ml saline was injected over 3.0 seconds. We used five 20 ml boluses of normal saline to determine peristaltic response, bolus transit and clearance of the secondary peristalsis. An interval of 20 seconds was allowed after the stimulus for any response to occur, during which subjects were instructed not to swallow. At the end of 20 seconds, subjects were allowed to have a dry swallow to ensure clearance of any residual material before the next stimulus and to reduce the desire to swallow during the distension.

#7.2.4 Data analysis

7.2.4.1 Manometry

The peristaltic amplitude at each recording site and the latency of the wave onset between adjacent recording sites were measured for both primary and secondary peristalsis. Contraction velocity was measured and defined as the speed (cm/sec) of the contraction wave from the most proximal to most distal recording site. Primary peristalsis was considered to be complete if the pressure wave of 12 mmHg in the proximal oesophagus and 25 mmHg in the distal oesophagus propagated through all oesophageal recording channels (Schoeman and Holloway 1994b). The minimal latency of wave onset between two recording channels was 0.5 seconds. A failed peristalsis was either failure of a pressure wave, 12 mmHg in the proximal oesophagus and 25 mmHg in the distal oesophagus, to traverse each of the recording channels or synchronous pressure waves occurring at two or more recording channels. Secondary peristalsis in response to liquid bolus injection was analyzed in the same manner as primary peristalsis. No response to distension was judged to have occurred if a pressure wave 10 mmHg was seen in less than two recording sites (Schoeman and Holloway 1994b). Due to the fact that not all pressure waves of secondary peristalsis were propagated down the oesophagus, successful or complete peristalsis was recognized using the same criteria as for primary peristalsis. The amplitude and velocity of the successfully propagated waves were measured.

7.2.4.2 Impedance

The recordings were analyzed using the impedance analysis software (Solar GI; MMS, The Netherlands). Oesophageal bolus transit and clearance were evaluated by

measurement of two variables: total bolus transit time (TBTT) and bolus presence time (BPT). TBTT represents the time for the bolus to traverse the entire oesophagus and was measured as time when the bolus head entered at the most proximal recording segment (Z1) and the bolus tail cleared at the most distal recording segment (Z7). BPT represents the time for the bolus to completely **traverse** an individual recording segment from the time when the bolus head entered the segment, as indicated by a drop in impedance to 50% of the baseline value, until the bolus tail had cleared the segment, as determined by recovery of the impedance level to 50% of the baseline value for 5 seconds. For each swallow response there were one of TBTT and 7 individual measurements of BPT corresponding to the seven impedance segments (Z1-Z7).

Swallows were classified by MII as showing: 1) complete bolus transit (CBT) if bolus entry occurred at the most proximal site (Z1) and bolus exit points were recorded in all the distal impedance-measuring sites (i.e., Z2-Z7), and 2) incomplete bolus transit if bolus exit was not identified at any of the distal impedance-measuring sites (i.e., Z2-Z7). Impedance data for secondary peristalsis were analyzed in a similar way as in primary peristalsis, and were determined for all peristaltic responses. Distal bolus propagation time for saline injection was recorded, the time of clearance of the bolus from Z4 to clearance of the bolus from Z7, and was compared with that of primary peristalsis over the same segment of distal oesophagus. We used bolus propagation time (Z4-Z7) instead of bolus transit time for the following reasons. First, the location and pattern of oesophageal stimulation differ between primary and secondary peristalsis; second, bolus propagation time should be better than bolus transit time to reflect oesophageal bolus clearance by secondary peristalsis, since the former corresponds to the propagation time of the corresponding peristaltic wave (Srinivasan, Vela, et al. 2001) whereas the latter may be confounded and influenced by factors other than secondary peristalsis such bolus delivery and behavior generated by mid-oesophageal injection.

Secondary peristalsis with saline injection were classified by MII as showing: 1) CBT if bolus entry occurred at the injection site (Z4) and bolus exit points were recorded in other impedance-measuring sites, and 2) incomplete bolus transit if bolus exit was not identified at any of the impedance-measuring sites.

#7.2.5 Statistical analysis

All results were expressed as mean ± SEM. The normality of all data was examined by D'Agostino's K-squared test. Differences in peristaltic amplitude and velocity, rate of peristaltic responses, and BPT were compared using analysis of variance with Bonferroni post hoc correction factor. For each subject, the mean values of distal bolus propagation time for primary and secondary peristalsis as well as the mean TBTT for saline and solid swallows were determined. A paired *t*-test was used to assess differences in the rate of CBT (%) and distal bolus transit time (DBTT) between primary and secondary peristalsis, and differences in the rate of CBT (%) and TBTT between saline and solid swallows. The differences in the proportion rate of bolus transit of secondary peristalsis for different motor components were examined by a chi-square test. A *p*-value of < 0.05 was accepted as indicating statistical significance.

7.3 Results

#7.3.1 Secondary peristalsis

The amplitudes of secondary peristalsis stimulated by saline were less than those of primary peristalsis with saline and solid swallows in some manometric channels (Table 7.1). The response rate of complete peristalsis by secondary peristalsis was significantly less than that of primary peristalsis with either saline or solid swallows (Table 7.1).

As shown in Figure 7.2, the bolus movement of saline injection was antegrade distal to the injection site and retrograde proximal to the injection site, although it looked indistinguishable from primary peristalsis by manometry. The rate for CBT of secondary peristalsis with saline was less than that of saline swallows (69% *vs* 95%, $p = 0.02$) (Figure 7.3A). There was no statistical difference in distal bolus propagation time between primary and secondary peristalsis (3.64 *vs* 3.80 seconds, $p = 0.45$) (Figure 7.3B). The rate for CBT of secondary peristalsis was significantly greater with peristaltic responses [24/27 (89%)] than synchronous contractions [1/3 (33%), $p = 0.014$], failed responses [4/20 (20%), $p = 0.001$], or no response [0/5, (0%), $p = 0.001$] (Figure 7.4). BPT of secondary peristalsis was significantly longer than that of primary peristalsis with saline or solid swallows for all impedance measuring segments (all $p < 0.05$) (Figure 7.5).

 # 7.3.2 Primary peristalsis

In all volunteers, complete primary peristalsis occurred with at least four of 5 saline or 5 solid agar swallows. The mean success rate of complete peristalsis was 95% for saline swallows and 93% for solid agar swallows. The pressure wave amplitudes and velocities were similar between saline and solid agar swallows (Table 7.1).

The mean rate of CBT was greater for saline swallows compared with that for solid swallows (95% *vs* 85%, $p = 0.03$) (Figure 7.6A). TBTT of saline boluses was significantly shorter than that of the solid swallows (7.4 *vs* 10.11 seconds, $p = 0.001$) (Figure 7.6B). The values for BPT progressively increased in each segment as the bolus traveled down the oesophagus. This was true for both liquid and solid boluses. The values for BPT were significantly longer with solid agar swallows when compared with saline swallows ($p < 0.05$) (Figure 7.5).

7.4 Conclusion

This study utilized concurrent impedance and manometry to quantify the relationship between secondary peristalsis and bolus transport as well as effectiveness of oesophageal clearance by this motor pattern. We found that oesophageal transit time of secondary peristalsis was comparable to that of primary peristalsis, although CBT by secondary peristalsis was seen less frequently than that of primary peristalsis. Furthermore, bolus dwell in regional oesophageal segment was longer in secondary peristalsis than primary peristalsis.

We showed that solid swallows differed from saline swallows with lower rate of CBT and longer bolus transit time. The current study reconfirms earlier observations that the oesophageal clearance is less effective and longer with solid bolus than with liquid bolus (Pouderoux, Shi et al. 1999, Srinivasan, Vela, et al. 2001). When consistencies are changed with constant volume, Srinivasan et al. could not find any difference in contraction velocity among different consistencies (Srinivasan, Vela, et al. 2001). Similar results have been reported elsewhere (Frieling, Hermann, et al. 1996, Nguyen, Silny, et al. 1997). Along with the previous studies, we found that oesophageal velocities as measured by manometry were not significantly different for both liquid (saline) and solid (agar) swallows. However, we observed different transit of liquid and solid boluses as they advanced through the oesophagus, similar to the findings reported by Srinivasan et al. (Srinivasan, Vela, et al. 2001). It is likely that

increased cohesion of solid bolus may lead to a more compact bolus with more intraluminal resistance that does not disperse through the oesophagus as easily as a liquid bolus, although the volume of each solid swallow after chewing might be different and therefore difficult to compare with liquid swallows. Our results were consistent with a previous study done by Kim et al., who have shown that oesophageal emptying is associated with bolus viscosity (Kim, Hsu et al. 1994). Based on the finding that the rate of CBT was less for solid swallows compared with saline swallows, it is apparent that solid boluses are more likely to exhibit failed clearance than liquid boluses, but have similar rate of failed peristalsis. These finding support a previous notion that the use of solid bolus may be more sensitive and more discriminatory to detect abnormal oesophageal function (Allen, Orr, et al. 1988).

In the current study, liquid swallows with 5-ml saline were used for primary peristalsis while liquid ingestions with 20-ml saline were performed for secondary peristalsis. It has been reported that the frequency of secondary peristalsis increases significantly with bolus volumes (Schoeman and Holloway 1994b). However, the concern may arise for that different volumes used might influence the results of bolus clearance between primary and secondary peristalsis. Although this issue has not yet been addressed, a previous study using MII-EM has shown bolus clearance time remains constant for liquid boluses at varying volumes of 1-20 ml (Srinivasan, Vela, et al. 2001).

It has been demonstrated that the manometric characteristics of complete secondary peristalsis were comparable to those of primary peristalsis suggesting common neural mechanisms (Schoeman and Holloway 1994b), although other studies have shown secondary peristalsis differs from primary peristalsis using balloon distension technique (Paterson, Hynna-Liepert, et al. 1991). Despite a reduction in response rate of complete peristalsis in secondary peristalsis, we found similar results between primary and secondary peristalsis regarding some of wave amplitudes and velocity. The responses of secondary peristalsis reflect the behavior of the stimulus, and are not influenced by the site of injection (Schoeman and Holloway 1994b). Saline bolus disperses along the oesophagus and can be moved ahead of any induced propagated wave. The moving bolus may also help reinforce the response in a manner similar to that of primary peristalsis (Dodds, Hogan et al. 1973, Hollis and Castell 1975, Janssens, Valembois, et al. 1974).

Currently, analysis of impedance recordings is based on measurement of BPT

which indicates segmental bolus clearance at a particular level of the oesophagus, and TBTT which indicates total oesophageal bolus clearance (Nguyen, Silny, et al. 1997, Srinivasan, Vela, et al. 2001, Tutuian, Vela, et al. 2003, Wise, Murray et al. 2004). We are not aware of any data reported regarding oesophageal bolus transport by secondary peristalsis. It has been shown that mechanisms regulating the dynamics of bolus propulsion are complex in the oesophagus, and different parts of a bolus have different propulsion behavior (Nguyen, Silny, et al. 1997). For determining bolus clearance of secondary peristalsis, we measured the transit of a bolus tail (Z4-Z7) which is directly induced by a sequence of peristaltic contractions. (Kahrilas, Dodds, et al. 1988b, Ren, Massey et al. 1993) Because of technical difference in the bolus delivery into the oesophagus between primary and secondary peristalsis, it would be inappropriate to compare bolus transit time in which the time of bolus head entry needs to be taken into account. Our observation revealed that oesophageal bolus transit of secondary peristalsis was similar to that of primary peristalsis. The result can be explained by the evidence of nearly comparable motility results between primary and secondary peristalsis over distal oesophagus. Nevertheless, we found in every regional oesophageal segment that BPT was longer in secondary peristalsis than that of primary peristalsis. The reasons for these findings were unclear but may relate to several factors such as the integrative aspects of bolus transport resulting from the global traction force of the oesophageal wall, different peristaltic responses between primary and secondary peristalsis, the location of the stimulation, and the existence of pharyngeal pump during swallowing, etc. Furthermore, as BPT was determined from the time of bolus entry, the relationship between bolus entry and the start of peristalsis is likely to differ between primary and secondary peristalsis, which may potentially explain for this difference. Further studies should be performed to elucidate this finding.

BPT has been regarded as a function of the rate of entrance of bolus and the rate of its clearance or exit for a certain oesophageal region. The values for BPT progressively increased in each segment as the bolus traveled down the oesophagus. It is possible that the bolus is slowed down when it enters the more compliant muscular region (Patel and Rao 1998). Furthermore, as the BPT was measured from the entry of the bolus in the individual segment, the time relationship between the arrival of the bolus and the onset of the clearance wave in the respective recording segment may increase progressively along the oesophagus due to the nearly simultaneous arrival of

the injected bolus at all recording sites. Even if peristaltic velocity was constant along the oesophagus, the effect of bolus delivery may play a part in the finding we observed.

One may argue that without additional recordings on the transport dynamics across LOS, an important barrier in oesophageal clearance, it is somewhat difficult to determine whether the findings of oesophageal bolus clearance by secondary peristalsis would be influenced and possibly confounded by the competence of the LOS. Although the study was not aimed to assess the effect of oesophageal distension on LOS functioning, we did observe transit LOS relaxations could be induced in most of secondary peristalsis via the LOS sidehole. This finding was consistent with a previous study in that transit LOS relaxations could be induced by direct oesophageal stimuli (Orenstein, DiLorenzo et al. 1997). Therefore, it would be less likely that bolus clearance in secondary peristalsis could be influenced by LOS functioning. However, an improvement in manometric or impedance recordings across the oesophagogastric junction might conceivably overcome this limitation.

The mechanisms underlying impaired bolus transit in secondary peristalsis in response to saline injection remain to be defined, but inferences are possible from the pattern of the manometric responses. Our data suggest that the effectiveness of secondary peristaltic responses is an important determinant of oesophageal bolus clearance by secondary peristalsis. On the other hand, most of non-peristaltic responses come with incomplete bolus transit. The association of prolonged clearance and defective motor response to intraluminal distension has been noted in previous work done by Kendall et al., who have demonstrated that abnormality of secondary clearance mechanism occurs in oesophageal clearance disorders (Kendall, Thompson et al. 1987). Our results are substantiated by a recent study which has shown prolonged TBTT occur more with ineffective oesophageal motility (IEM) than with manometrically normal motility (Tutuian, Vela, et al. 2003).

In summary, the significance of our observation is that secondary peristalsis appears to be somewhat less effective than primary peristalsis when it is ready for propelling a liquid bolus out of the oesophagus. Effective secondary peristaltic responses are important for ensuring more efficient oesophageal clearance by secondary peristalsis in healthy volunteers. The data presented here may serve as a useful comparator for characterization of the efficiency of oesophageal bolus transport

by secondary peristalsis and other clinical conditions with disordered oesophageal clearance mechanisms.

Table 7.1: Manometric characteristics of primary and secondary peristalsis

Data are expressed as mean (SEM); [a] $p < 0.05$ solid *vs* secondary peristalsis; [b] $p < 0.05$ solid *vs* secondary peristalsis; [c] $p < 0.05$ primary peristalsis (saline and solid) *vs* secondary peristalsis; [d] $p < 0.05$, primary peristalsis (saline and solid) *vs* secondary peristalsis.

	Primary peristalsis		Secondary peristalsis
	Saline	Solid	Saline
Amplitude of contractions (*mm Hg*)			
P2 [a]	82.0 (8.9)	91.2 (10.2)	63.0 (9.9)
P3	53.3 (6.6)	60.0 (6.1)	42.0 (4.6)
P4 [b]	59.5 (7.7)	68.5 (7.6)	44.5 (7.7)
P5 [c]	76.6 (10.5)	85.4 (11.8)	44.4 (6.6)
P6	99.4 (16.1)	102.3 (16.3)	57.0 (13.7)
P7	68.7 (11.5)	65.5 (10.8)	49.6 (9.7)
Complete peristalsis (%)[d]	95 (3)	93 (4)	75 (5)
Onset velocity of contractions (*cm/sec*)			
P2-P7	2.7 (0.1)	2.4 (0.2)	2.2 (0.2)

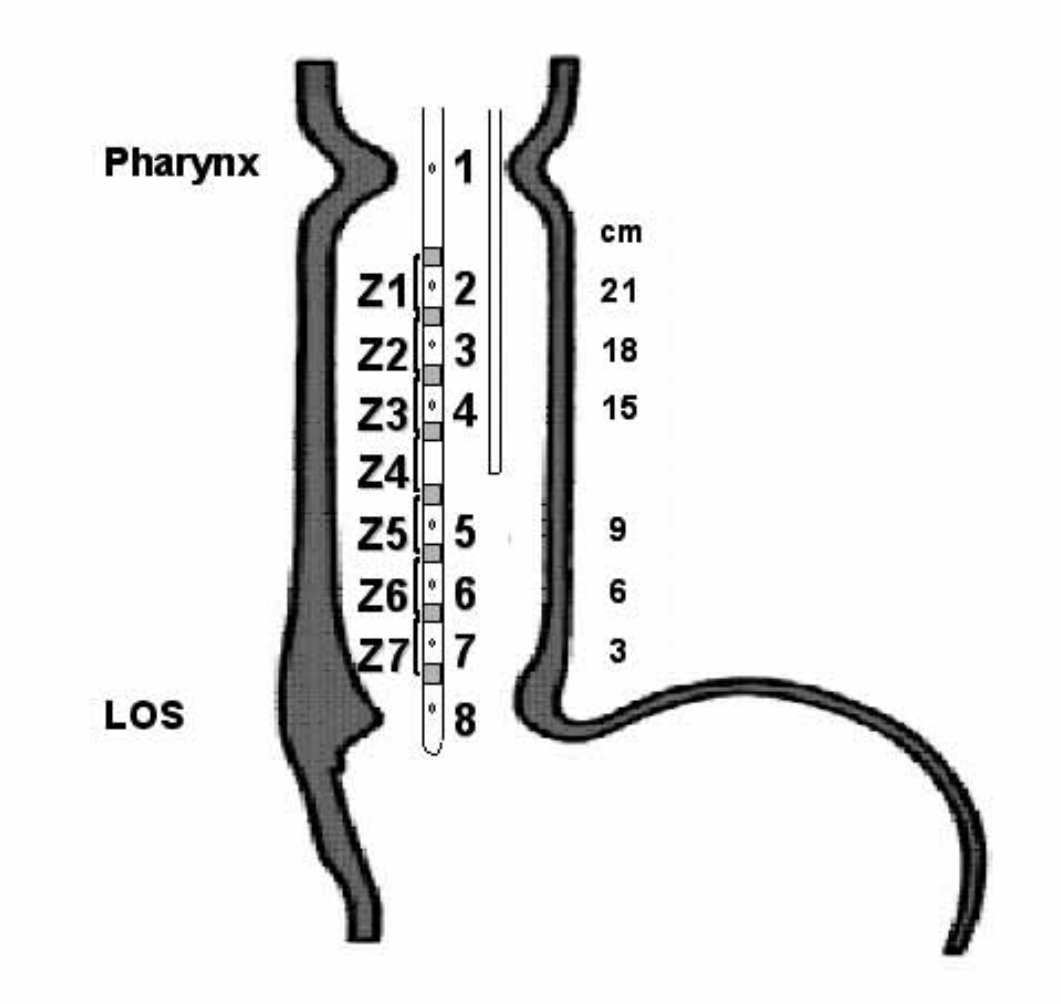


Figure 7.1: Schematic diagram of the combined manometric and impedance assembly with 8 manomteric sideholes and 8 impedance electrodes spaced at 3-cm intervals. An additional polyvinyl catheter with an orifice for injection over its distal end was assembled with the manometry-impedance catheter so that the injection pole was located in the mid-oesophagus. The reference distance of the manometric port is from LOS at P8.

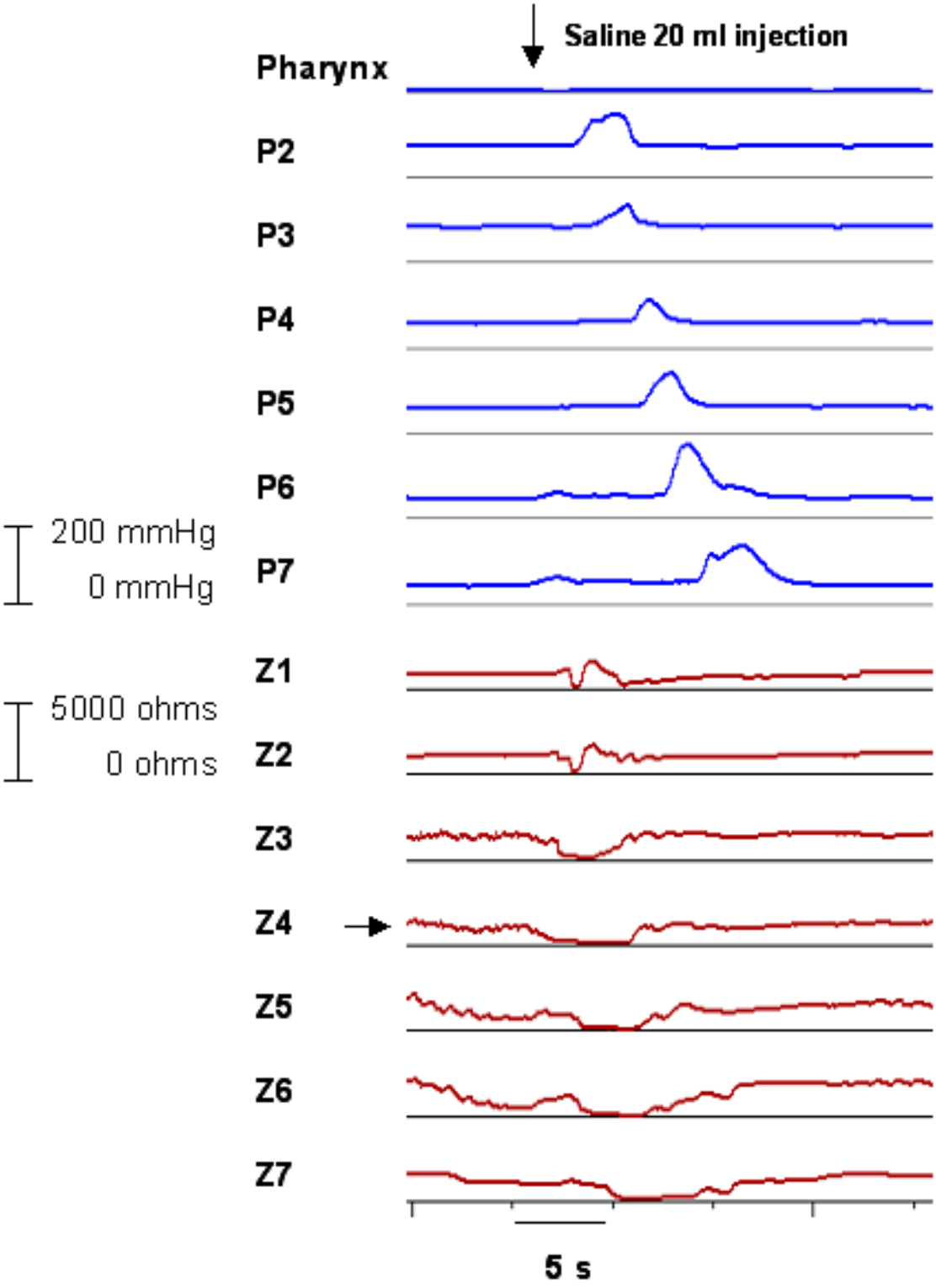
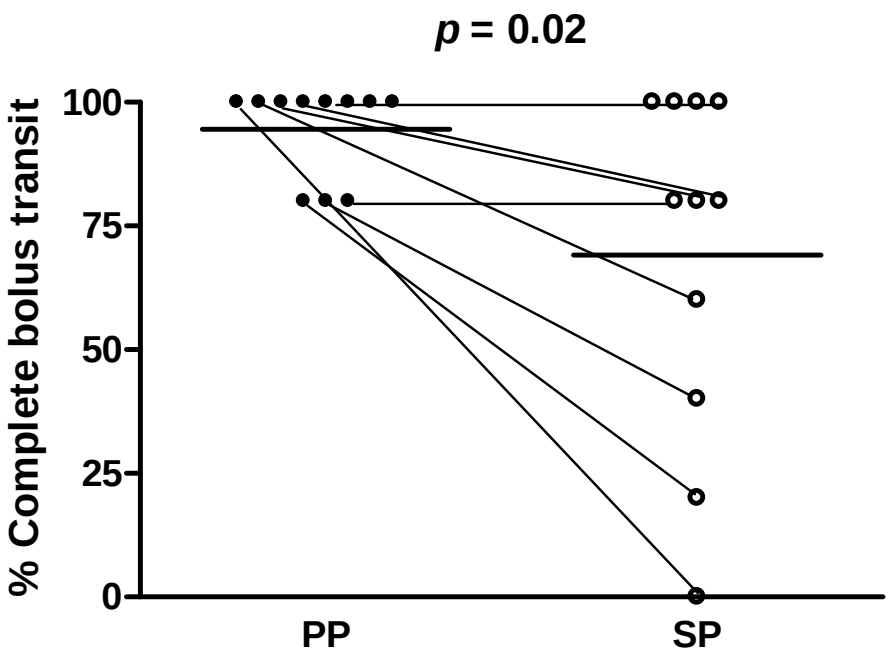


Figure 7.2: Sample manometric and impedance tracings from a representative subject to illustrate the pattern of oesophageal contractions and bolus transit during secondary peristalsis with saline injection. The long arrows indicate the timing of the stimulus (saline injection). The short arrow indicates the site of the stimulus.

A.



B.

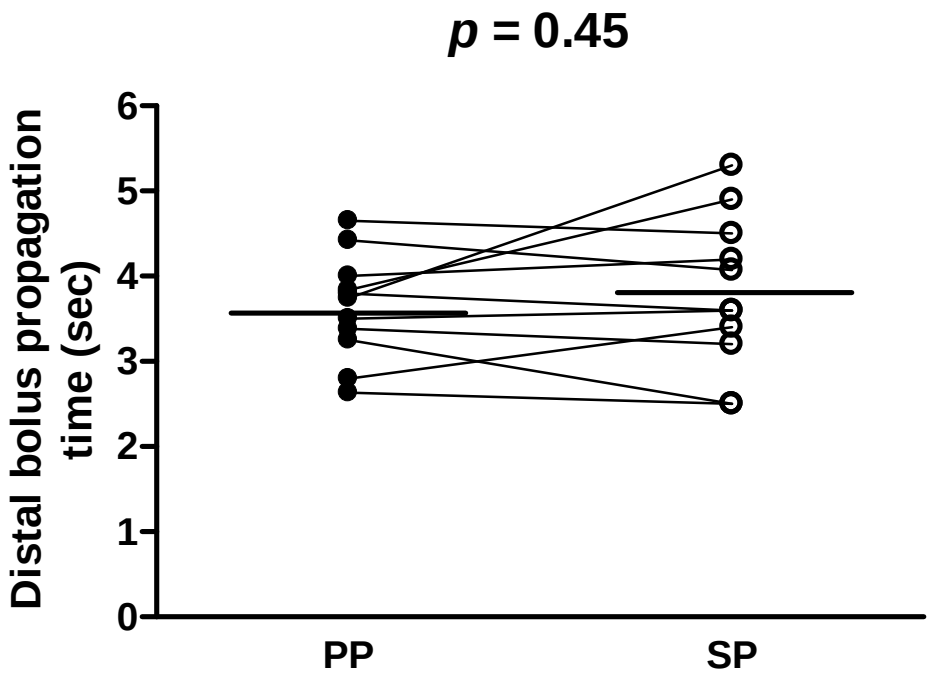


Figure 7.3: Comparisons of impedance characteristics between primary and secondary peristalsis. (A) The rate for CBT. The rate for CBT of secondary peristalsis with saline was less than that of saline swallows. (B) Distal bolus propagation time.

There was no difference in distal bolus propagation time between primary and secondary peristalsis. Each data point represents an individual subject and the mean values are shown by the bars. PP, primary peristalsis (●); SP, secondary peristalsis (o).

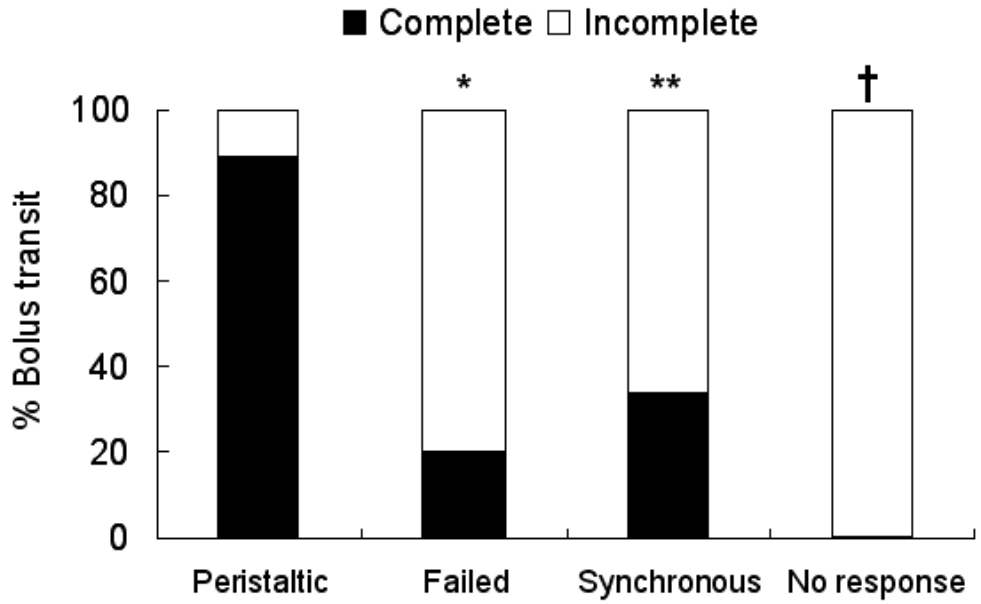


Figure. 7.4: Relationship between oesophageal bolus transit and manometric characteristics in secondary peristalsis as assessed by CBT (%). *p = 0.001, peristaltic *vs* failed responses; **p = 0.014, peristaltic *vs* synchronous responses; †p = 0.001, peristaltic *vs* no responses.

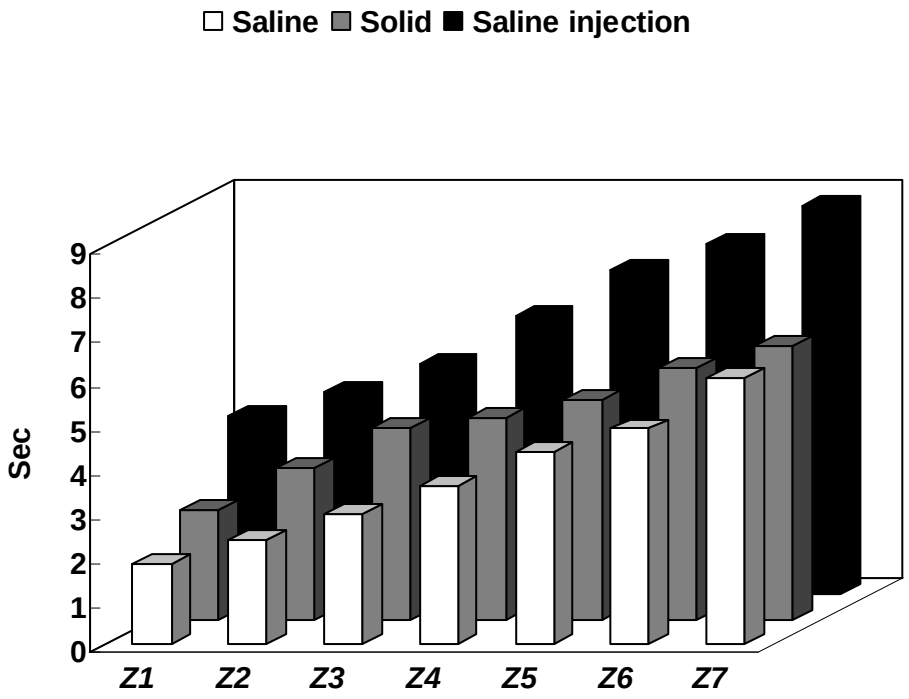
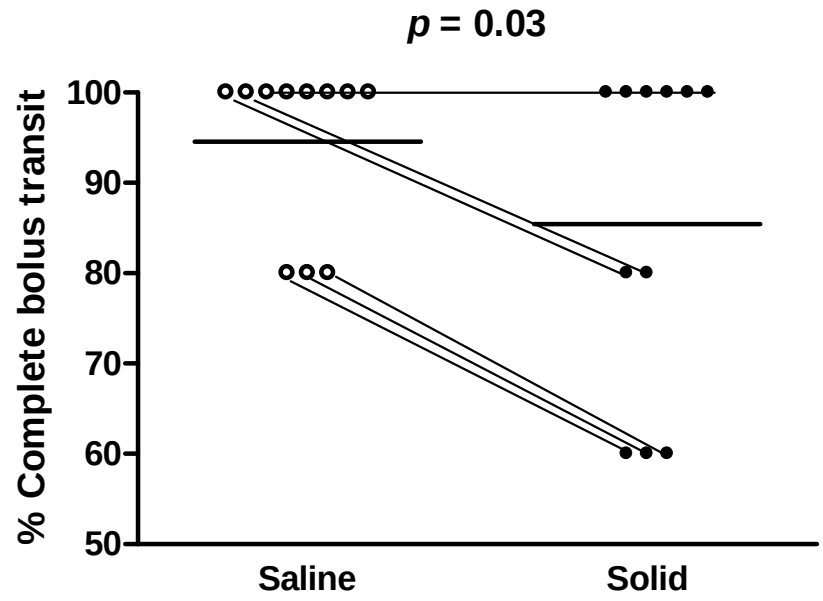


Figure. 7.5: Oesophageal BPT during primary and secondary peristalsis. BPT progressively increased in each segment as the bolus traveled down the oesophagus. BPT was longer in secondary peristalsis than that of primary peristalsis. BPT were significantly longer with solid agar swallows when compared with saline swallows.

A.



B.

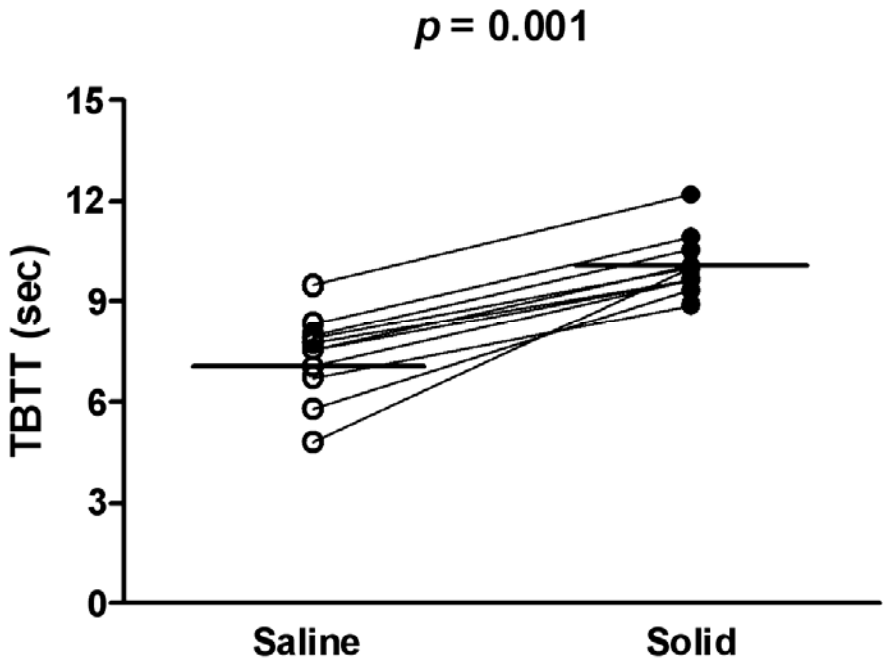


Figure. 7.**6:** Comparisons of impedance characteristics between liquid (o) and solid swallows (●). (A) The rate for CBT was greater for saline swallows compared with that of solid swallows. (B) TBTT. TBTT of saline boluses was shorter than that of solid swallows. Each data point represents an individual subject and the mean values are shown by the bars.

SECTION C

EVALUATION OF PERISTALTIC MOTOR CHARACTERISTICS, BOLUS CLEARANCE AND SYMPTOM PERCEPTION IN DYSPHAGIA SYNDROMES

The work presented in this chapter has been published in Neurogastroenterol Motil 2008 ;**20**:611-617.

Chapter 8

Evidence for poor correlations between perception of dysphagia and oesophageal dysmotility

8.1 Introduction

Dysphagia is an important symptom often leading to the finding of an anatomic or functional disorder of the oesophagus. The term “non-obstructive dysphagia” (NOD) is used to describe the presence of the sensation of difficulty in swallowing solids or liquids in the absence of endoscopically or radiologically demonstrable oesophageal lesions.(Parkman, Maurer, et al. 1996, Richter, Baldi, et al. 1992) Motility disorders may be found manometrically in NOD patients but do not necessarily temporally correlate or explain the symptom of dysphagia under usual conditions with water swallows.(Benjamin, Castell, et al. 1983, Benjamin, Gerhardt, et al. 1979, Clouse and Ferney 1986, Howard, Pryde, et al. 1989) Conflicting results have been published in NOD patients examined by oesophageal manometry with solid or viscous swallows(Aprile, de Oliveira et al. 2006, Cordier, Bohn, et al. 1999, Sears, Castell, et al. 1990) A recent study has noted both oesophageal motor and sensitivity impairments in some of NOD patients.(Bohn, Bonaz, et al. 2002)

Multichannel intraluminal impedance (MII) has been widely used to evaluate oesophageal bolus transport with the advantage of avoiding radiation exposure.(Silny 1991) Previous studies with combined video-fluoroscopy and MII have validated the accuracy of impedance to detect bolus transit in the oesophagus.(Silny 1991, Simren, Silny, et al. 2003) Using Multichannel intraluminal impedance and oesophageal (MII-EM), previous studies have reported normal values for impedance changes associated with liquid and viscous swallows.(Tutuian, Vela, et al. 2003)

In order to elucidate the pathogenesis of underlying dysphagia, we thought to determine the simultaneous relationships between subjective symptom of dysphagia and motility parameters such as oesophageal contractions and bolus transits during individual swallowing. The study was also undertaken to test the hypothesis that different pattern of swallowed boluses, liquid *versus* viscous, might influence subjective perception and the prevalence of oesophageal dysmotility.

8.2 Methods

#8.2.1 Subjects

All subjects complained of dysphagia as their predominant symptom for more than 3 months with at least one weekly episode. Patients' complaint was considered as dysphagia only when food got stuck on its way down. They did not have mechanical obstruction on barium oesophagogram or oesophagoscopy which was performed less than 3 months before the study. Patients taking medication possibly affecting oesophageal motility were asked to hold this from at least 48 hours before the study. Healthy subjects participating in this study were recruited from a community population by an advertisement. They were totally asymptomatic and had no history of oesophageal, gastric or duodenal disease. They did not take any drugs known to affect oesophageal function. All patients and controls gave written informed consents and the study was approved by the Human Ethics Committee of the Tzu Chi General Hospital.

#8.2.2 Study of oesophageal function

Subjects were studied after an overnight fast. Each subject underwent oesophageal function testing using combined MII-EM with a Koenigsberg 9-channel probe (Sandhill Scientific, Inc., Highlands Ranch, CO, US). The 4.5 mm diameter catheter design has two circumferential solid-state pressure sensors at 5 cm and 10 cm from the tip and three unidirectional pressure sensors at 15, 20, and 25 cm. Impedance measuring segments including two rings placed 2 cm apart, were centered on 10, 15, 20, and 25 cm from the tip, thus across the four proximal pressure transducers. The catheter was inserted transnasally into the oesophagus up to a depth of 60 cm. Lower oesophageal sphincter (LOS) was identified using stationary pull-through technique and the most distal sensor was placed in the high-pressure zone of the LOS. Intraoesophageal pressure sensors and impedance measuring segments were thus located at 5 cm, 10 cm, 15 cm, and 20 cm above the LOS. In the supine position, each subject was given 10 swallows of 5 cc normal saline and 10 swallows of 5 cc viscous (apple-sauce like consistency) (Sandhill Scientific, US) material each 20–30 second apart.

Subjective perception of each swallow was recorded and assessed using a five-point-scale (1-5, 1 = feeling full passage without difficulty and 5 = feeling complete blockade). Subjective response to an individual swallow was considered an

enhanced perception or dysphagia if the swallow score was > 1.(Aprile, de Oliveira, et al. 2006)

#8.2.3 Data analysis

Manometric parameters included: (1) distal oesophageal amplitude (DEA) as average of contraction amplitude at 5 and 10 cm above the LOS, and (2) onset velocity of oesophageal contractions in the distal part of the oesophagus (i.e., between 10 cm and 5 cm above the LOS). Mid-respiratory resting pressure and LOS residual pressure during swallowing were used to assess LOS function.

Swallows was considered normal if contraction amplitudes at 5 and 10 cm above LOS were each ≧ 30 mmHg and distal onset velocity was < 8 cm/s.(Tutuian, Vela, et al. 2003) Individual swallow with ineffective contraction was defined if either of the contraction amplitudes at 5 and 10 cm above LOS was less than 30 mmHg, while that with simultaneous contractions was identified if contraction amplitudes at 5 and 10 cm above LOS were each greater than or equal to 30 mmHg and distal onset velocity was greater than 8 cm/s. Overall diagnosis of motility abnormalities were based on the 10 saline swallows by a previous reference.(Spechler and Castell 2001)

MII parameters analyzed included bolus entry at each specific level obtained at the 50% point between 3-second pre-swallow impedance baseline and impedance nadir during bolus presence and bolus exit determined as return to this 50% point on the impedance-recovery curve. Swallows were classified by MII as showing: (1) complete bolus transit (CBT), if bolus entry occurred at the most proximal site (20 cm above LOS) and bolus exit points were recorded in all three distal impedance-measuring sites (i.e., 15 cm, 10 cm, and 5 cm above the LOS) and (2) incomplete bolus transit, if bolus exit was not identified at any one of the three distal impedance-measuring sites. Patients were judged to have normal transit if ≥80% liquid and ≥70% of viscous swallows showed normal transit.(Tutuian, Vela, et al. 2003)

#8.2.4 Statistical analysis

Data are given as mean ± SEM or percentage. The data for subjective perception, manometry, and impedance were determined for each swallow in each subject. Statistical comparisons were done by Student *t* test for continuous data and by chi-square test for frequencies. A *p* value of less than 0.05 was considered significant.

Kappa (κ) statistics was used to determine the relationships and agreements between enhanced perception (score >1) with oesophageal motility and bolus transport in NOD patients.(Byrt, Bishop et al. 1993) The strength of agreement was as follows: poor, < 0.20; fair, 0.21-0.40; moderate, 0.41-0.60; good, 0.61-0.80; and very good, 0.81-1.00.

#8.2.5 Sample size calculation

In this study, we calculated the power and sample size for κ agreement based on a Wald test.(Lin, Williamson et al. 2003) Assuming a moderate agreement of 0.41 between subjective perception and the oesophageal functional tests, we determined that 170 swallows would be necessary to detect statistically significant differences with a power of 80%.

8.3 Results

The control group consisted of 14 subjects (5 women, 9 men; mean age, 43 years; range, 25-60 years) with 280 swallows (140 liquid and 140 viscous). The NOD group included 18 subjects (7 women, 11 men; mean age, 47 years; range, 19-71 years) with 358 swallows (179 liquid and 179 viscous). There was no statistically significant difference between the two groups regarding age or gender.

#8.3.1 Oesophageal motility characteristics

8.3.1.1 Manometric characteristics

Table 8.1 summarizes the results of oesophageal manometry in all patients and healthy controls. Healthy controls had significantly greater distal oesophageal peristaltic amplitude than NOD patient during liquid ($p = 0.01$) and viscous swallows ($p = 0.03$). The duration for oesophageal contraction was longer in NOD patients than in healthy controls at 15 cm ($p < 0.05$) and 10 cm ($p = 0.02$) from LOS during viscous swallows. The relaxation of the LOS took longer in NOD patients than in healthy controls during viscous swallow ($p = 0.04$). Normal peristalsis was found more in healthy controls than NOD patients during liquid and viscous swallows (both $p = 0.01$).

Out of 18 patients receiving liquid swallows, ten (56%) had normal oesophageal manometry. Eight patients (44%) had ineffective oesophageal motility (IEM). Applying the same manometric criteria to those patients with viscous swallows, ten patients (56%) had IEM. In most patients the additional of viscous swallows did not

change the diagnosis, but some variations occurred when comparing results of liquid *versus* viscous as shown in Table 8.2.

8.3.1.2 Impedance findings

With regard to impedance parameters (Table 8.3), we found NOD patients were characterized with longer bolus presence time (BPT) than healthy controls during viscous swallows. NOD patients had slower advance time of the head of liquid boluses (between 20 and 5 cm above LOS) compared to healthy controls ($p = 0.01$). The segment transit time (between 15 and 10 cm above LOS) for viscous boluses was longer in NOD patients compared to healthy controls ($p = 0.03$). However, the total bolus transit time (TBTT) was similar between both groups for liquid and viscous boluses. The prevalence of complete bolus transit (CBT) was seen less in NOD patients compared to healthy controls for liquid swallows ($p = 0.001$) and viscous swallows ($p = 0.001$).

Abnormal bolus transit was found in 41% of NOD patients with liquid boluses while 59% of patients with viscous boluses. During liquid swallowing, abnormal bolus transit was found in 20% of patients with normal motility and 63% of patients with ineffective motility, whereas abnormal bolus transit was observed in 38% of patients with normal motility and 70% of patients with ineffective motility (Figure 8.1).

8.3.1.3 Analysis of individual swallows in NOD patients

The manometric analysis of 179 liquid swallows has shown 96 (53.65%) normal peristaltic and 83 (46.4%) ineffective swallows. CBT was identified in 78.1% of manometric normal swallows and 60.2% of manometric ineffective swallows. The analysis of 179 viscous swallows identified 97 (54.2%) normal peristaltic and 82 (45.8%) manometric ineffective swallows. Complete viscous bolus transit was identified in 75.3% of manometric normal swallows and 26.8% of manometric ineffective swallows. The majority of swallows with incomplete liquid bolus transit were found in the manometric ineffective swallows with either liquid boluses (61.1%) or viscous boluses (71.4%) (Table 8.4).

#8.3.2 Study protocol Subjective perception of swallow

None of healthy controls had experienced increased perception during either

liquid or viscous swallowing. At least one enhanced perception episode was reported by 12 of 18 patients with liquid swallows (67%) and 13 of 18 patients with viscous swallows (72%). Swallowing provoked enhanced perception in 108 of 179 liquid swallows (60%) and 131 of 179 viscous swallows (73%) (p = 0.01). More viscous swallows were identified with greater perception (grade 3) than liquid swallows (36[20%] *vs* 74[41%], p = 0.001) (Figure 8.2). None of liquid swallows had grading scale greater than 4 while three swallows (2%) were perceived as grade 4 during viscous swallowing (Figure 8.2). None of liquid or viscous swallows were perceived as grade 5 (Figure 8.2).

#8.3.3 Agreement between subjective perception of swallow and oesophageal motility

The per-swallow analysis revealed a poor agreement between impedance and enhanced perception (κ = 0.12, 95% CI: −0.003-0.233) during liquid swallowing and (κ = 0.12, 95% CI: −0.004- 0.244)(Table 8.5). The agreement was even poorer between manometry and enhanced perception during liquid swallowing (κ = −0.16, 95% CI: −0.302-0.022) and viscous swallowing (κ = −0.12, 95% CI: −0.25-0.002)(Table 8.5).

8.4 Conclusion

The main result of this study is the demonstration of a significantly poor agreement between dysphagia and oesophageal dysmotility in terms of poor contractility and impaired bolus transport, although NOD patients had more oesophageal dysmotility than healthy controls. In addition, our data re-confirmed the previous observation in NOD patients that the symptom of dysphagia can be provoked during standard manometry with swallowing.(Bohn, Bonaz, et al. 2002, Meshkinpour and Eckerling 1996) Therefore, our study could implicate that oesophageal hypocontractility as well as impaired bolus transport may play a limited role in the symptomatic generation of dysphagia during swallowing.

Recent studies have suggested that patients complaining of dysphagia have a high probability of demonstrated true oesophageal dysfunction.(Benjamin, Gerhardt, et al. 1979, Herrington, Burns et al. 1984, Jacob, Kahrilas, et al. 1990) We showed that near 50% of NOD had normal manometry with either liquid or viscous swallowing. The pathophysiological implication of dysphagia is that there is some

resistance or delay to the passage of a bolus, the final form of which is bolus impaction.(Conchillo, Nguyen, et al. 2005) Although motility disorders can be demonstrated in NOD patients, a significant minority of patients with dysphagia will not show any anatomic or motility abnormality.(Katz, Dalton, et al. 1987) Thus, in spite of some motility abnormalities associated with NOD, it is difficult to understand how actually the observed oesophageal dysmotility will provoke symptom such as dysphagia. To do so, it will be more appropriate to assess the oesophageal function and identify motor abnormalities associated with complaint rather than categorization of motor disorders based on morphology of oesophageal contractions. A recent study utilizing 24-h oesophageal manometry was able to detect and characterize abnormal oesophageal motor activity with NOD, and found that the prevalence of meal-related peristaltic contractions correlated well with the presence of dysphagia.(Stein, Singh, et al. 2004)

Deschner et al. noted that a reproduction of dysphagia sensation could occur with balloon distension in a majority of subjects with NOD.(Deschner, Maher, et al. 1989) They found that repeated simultaneous contractions occurred distal to the balloon in their patients with reproduced symptoms, and thus suggested that the development of abnormal distal motility is crucial to dysphagia. Other studies using intra-oesophageal balloon distention have indicated that oesophageal sensory dysfunction only partially overlaps with motor dysfunction and such dysfunction has a positive association with dysphagia that is independent of motor abnormality on baseline manometry.(Clouse, McCord, et al. 1991) It seems likely that this symptom can be another manifestation of oesophageal sensory dysfunction.

The present study was attempting to delineate whether additional use of viscous boluses to MII-EM could improve the diagnostic yield in NOD patients. This study clearly showed that viscous swallows not only provoked symptoms in most swallows, but also produced impaired bolus transit in a greater majority of them, despite a failure to show any significant difference regarding oesophageal manometry when comparing liquid with viscous swallows. The current findings are similar to a recent study with solid swallowing,(Bohn, Bonaz, et al. 2002) but are different to the results of a previous investigation, which has demonstrated a significant difference between the response rate of dysphagia provoked by viscous (89%) and liquid swallows (9%).(Meshkinpour and Eckerling 1996) This discrepancy may possibly be explained by different including criteria of patients' enrollment since their study included the

subjects only with normal standard oesophageal manometry. However, the findings presented here were in accordance with Allen et al., who observed higher incidence of motor abnormalities during ingesting of different varieties of solid meals.(Allen, Orr, et al. 1988)

We found that 40-60% of our patients had abnormal bolus transit, which was also associated with IEM. The findings are in the same line with a recent study in 350 patients with oesophageal symptoms referred to a motility laboratory. (Tutuian and Castell 2004b) These authors have shown that fifty-one percent of patients with IEM, and abnormal bolus transit occurred more frequent in patients presenting with dysphagia, 42% of whom had abnormal liquid bolus transit while 18-24% of patients with other oesophageal symptoms.(Tutuian and Castell 2004b) The clinical implication for such findings may suggest in NOD that oesophageal motility abnormalities is potentially associated with combined pressure defects (IEM) and abnormal bolus transit. However, some of NOD patients had normal bolus transit indicating the difficulty of identifying an objective cause for the symptom.

The application of observer agreement with κ statistics in this study is based on the fact that κ statistics can be used to assess the consistency of the diagnostic test for indicating the severity or extent of disease and determining the reliability of various signs of disease.(Baker, Kornguth et al. 1996, Markus, Somers et al. 1989) A previous work using MII-EM and κ statistics in a small group of healthy subjects demonstrated that sildenafil-related peristaltic dysfunction exhibited a poor agreement with subjective perception of dysphagia, and thus concluded that oesophageal hypocontractility as well as abnormal bolus transport may play a limited role in the symptomatic genesis of dysphagia.(Aprile, de Oliveira, et al. 2006) In accordance with these findings, we also demonstrated a poor agreement between the perception of dysphagia and oesophageal dysmotility in terms of poor contractility and impaired bolus transport. The current finding might potentially infer an evidence of impaired oesophageal sensitivity that has been reported in patients presenting with various oesophageal symptoms. (Deschner, Maher et al. 1990, Ghillebert, Janssens et al. 1990, Katz, Dalton, et al. 1987, Richter, Barish et al. 1986) Another study has further extended these findings by showing that such sensory impairment can be observed in NOD patients outside periods of food ingestion, and suggested a direct link between sensory impairment and dysphagia symptom. (Bohn, Bonaz, et al. 2002)

In summary, the evaluation of the simultaneous relationships between subjective

experience of dysphagia and motility parameters during individual swallowing seems to be helpful for further understanding the pathogenesis underlying dysphagia in NOD patients. The additional use of viscous swallows was likely to provoke greater severity of dysphagia, despite a lack in the simultaneous relationships between dysphagia and oesophageal contractions as well as bolus transits. The present study reinforces a previous notion which suggests factors other than oesophageal dysmotility, i.e. oesophageal sensitivity disorder might be potentially more relevant in symptom genesis. (Bohn, Bonaz, et al. 2002) Future works are warranted to determine the exact role of oesophageal sensitivity predisposing to NOD.

Table 8.1: Manometric features for liquid and viscous swallows in all subjects

	Liqiud			Viscous		
	NOD	healthy controls	*p* value	NOD	healthy controls	*p* value
Amplitude of constractions (*mm Hg*)						
at 20 cm	34.4 (3.4)	39.4 (3.4)	0.31	37.4 (4.6)	39.9 (3.0)	0.66
at 15 cm	46.3 (4.9)	58.6 (5.9)	0.12	38.9 (4.4)	57.4 (4.6)	0.01
at 10 cm	59.0 (7.4)	78.0 (6.7)	0.07	56.6 (7.5)	71.7 (5.0)	0.1
at 5 cm	78.3 (9.4)	109.7 (6.8)	0.01	87.0 (10.0)	115.4 (7.1)	0.03
Duration of contractions (*s*)						
at 20 cm	1.8 (0.2)	2.2 (0.2)	0.17	2.6 (0.2)	2.0 (0.2)	0.06
at 15 cm	2.7 (0.1)	2.5 (0.1)	0.26	3.2 (0.2)	2.6 (0.2)	0.05
at 10 cm	2.7 (0.2)	2.4 (0.1)	0.2	3.3 (0.2)	2.6 (0.2)	0.02
at 5 cm	3.3 (0.2)	2.9 (0.1)	0.07	3.3 (0.1)	3.2 (0.1)	0.76
Onset velocity of contractions (*cm/s*)						
10-5 cm	4.9 (0.4)	5.6 (0.8)	0.41	4.8 (0.6)	6.0 (0.8)	0.26
Low oesophageal sphincter						
Residual Pressure (*mm Hg*)	6.4 (1.5)	5.5 (1.0)	0.61	8.0 (2.2)	4.8 (0.9)	0.2
Relaxation duration (*s*)	6.2 (0.7)	5.1 (0.6)	0.22	6.7 (0.7)	4.7 (0.7)	0.04
Normal peristalsis	51 (5)	91 (6)	0.001	49 (4)	83 (5)	0.001

Values expressed as mean (SEM); NOD, non-obstructive dysphagia; HC, healthy controls.

Table 8.2: Number of patients with manometric diagosis based liquid *versus* viscous swallows

	Manometry diagnosis (viscous)	
	Normal	IEM
Normal	7	3
IEM	1	7

Table 8.3: Impedance features for liquid and viscous swallows in all subjects

	Liqiud			Viscous		
	NOD	healthy controls	*p* value	NOD	healthy controls	*p* value
BHAT (s)						
20-15 cm	0.3 (0.04)	0.2 (0.02)	0.34	1.2 (0.1)	0.9 (0.2)	0.12
20-10 cm	0.6 (0.07)	0.4 (0.03)	0.06	3.5 (0.2)	3.2 (0.3)	0.43
20-5 cm	1.1 (0.09)	0.8 (0.07)	0.01	5.1 (0.2)	4.9 (0.3)	0.65
BPT (s)						
at 20 cm	2.7 (0.3)	2.7 (0.2)	0.98	3.1 (0.3)	2.5 (0.2)	0.04
at 15 cm	4.0 (0.3)	3.7 (0.2)	0.34	4.0 (0.3)	3.1 (0.2)	0.02
at 10 cm	5.1 (0.4)	4.6 (0.2)	0.26	3.8 (0.3)	2.8 (0.2)	0.008
at 5 cm	6.1 (0.4)	5.4 (0.2)	0.12	3.7 (0.2)	2.8 (0.2)	0.003
Segment transit time (s)						
20-15 cm	4.2 (0.3)	3.9 (0.2)	0.43	4.9 (0.3)	4.3 (0.2)	0.08
15-10 cm	5.7 (0.4)	5.0 (0.2)	0.36	5.9 (0.3)	5.0 (0.2)	0.03
10-5 cm	6.3 (0.4)	5.9 (0.2)	0.19	5.4 (0.3)	4.3 (0.3)	0.1
TBTT (s)	6.5 (0.2)	6.4 (0.2)	0.79	8.2 (0.2)	7.6 (0.3)	0.12
CBT (%)	73 (5)	95 (2)	0.001	57 (5)	85 (4)	0.001

Values expressed as mean (SEM); NOD, non-obstructive dysphagia; HC, healthy controls; BHAT, bolus head advance time.

Table 8.4: Manometric and impedance evaluation of liquid and viscous swallows in NOD patients

			Manometric evaluation				
			Normal		Ineffective		Total
			N	r%	N	r%	N
Liquid	Impedance evaluation						
	Complete transit	N	75	60	50	40	125
		c%	78.1		60.2		69.8
	Incomplete transit	N	21	38.9	33	61.1	54
		c%	21.9		39.8		30.2
	Total	N	96	53.6	83	46.4	179
Viscous	Impedance evaluation						
	Complete transit	N	73	76.8	22	23.2	95
		c%	75.3		26.8		53.1
	Incomplete transit	N	24	28.6	60	71.4	84
		c%	24.7		73.2		46.9
	Total	N	97	54.2	82	45.8	179

r%, row percent (percent with given manometric evaluation)

c%, column percent (percent with given impedance evaluation)

Table 8.5: Agreement between enhanced perception (score > 1) and oesophageal motility

	kappa value	Agreement (%)	Standard error	95% CI
Liquid				
Impedance	0.12	52	0.06	-0.003-0.233
Manometry	-0.16	41	0.07	-0.302--0.022
Viscous				
Impedance	0.12	55	0.06	-0.004-0.244
Manometry	-0.12	42	0.06	-0.25-0.002

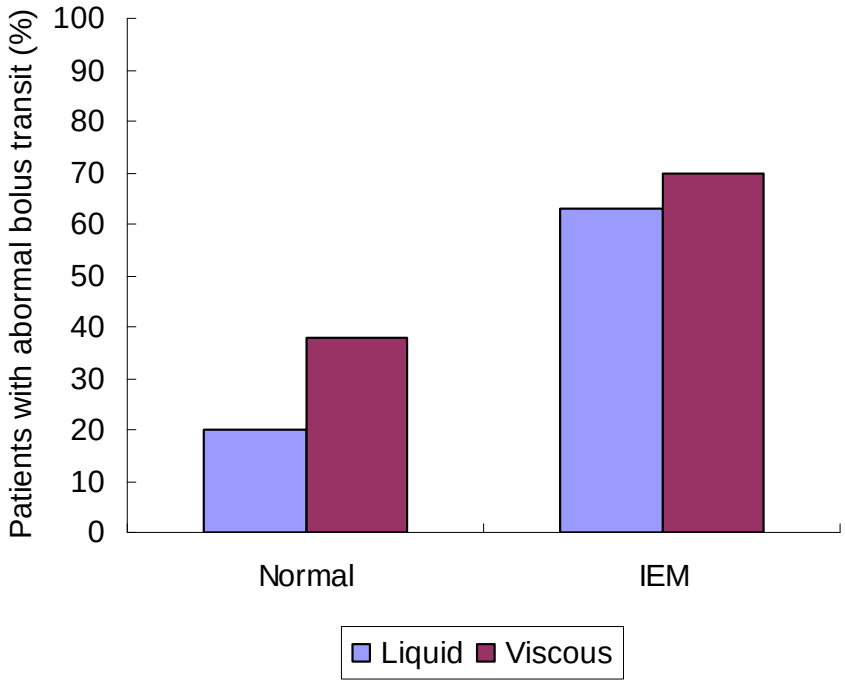


Figure 8.1: Percentage of patients with abnormal bolus transit according to manometric findings.

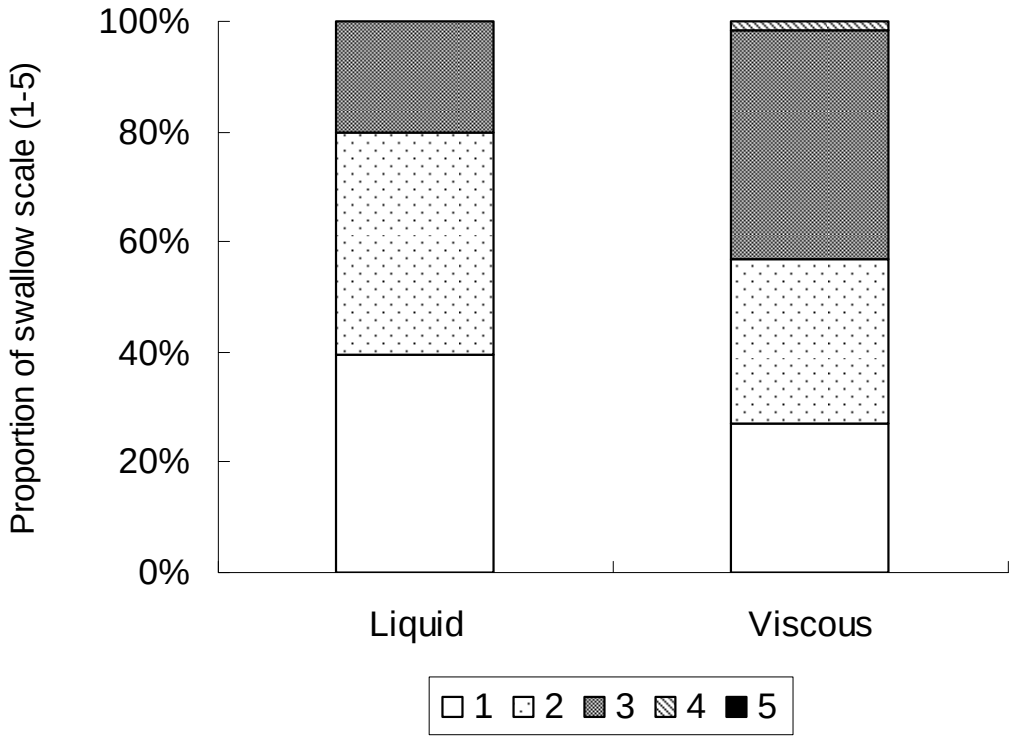


Figure 8.2: Proportion of swallow according to the swallow scale during liquid and viscous swallowing. None of liquid or viscous swallows were perceived as grade 5.

The work presented in this chapter is published in Neurogastroenterol Motil 2008 2008;**20**:980-988.

Chapter 9

Identification of impaired bolus transit and clearance by secondary peristalsis in non-obstructive dysphagia

9.1 Introduction

Oesophageal dysphagia is an important symptom often leading to the finding of an anatomical or functional disorder of the oesophagus. The term “non-obstructive dysphagia” (NOD) is used to describe the presence of the sensation of difficulty in swallowing solids or liquids in the absence of endoscopically or radiologically emonstrable oesophageal lesion or a significant motility disorder. (Parkman, Maurer, et al. 1996, Richter, Baldi, et al. 1992) With the exception of defined dysmotility syndromes such as achalasia, diffuse oesophageal spasm and scleroderma, the relevance of non-specific oesophageal motor disorder to NOD remains controversial.(Barish, Castell, et al. 1986, Jacob, Kahrilas, et al. 1990, Katz, Dalton, et al. 1987, Kjellen, Svedberg et al. 1984)

Multichannel intraluminal impedance (MII) permits detection of oesophageal bolus transport and real-time quantification of bolus movement without radiation.(Silny 1991) and may enhance the diagnostic capability, and clarify functional abnormalities, in patients with disordered oesophageal clearance.(Tutuian and Castell 2004b) Using MII, Conchillo et al. found that impedance yielded the results that can’t be predicted on the basis of oesophageal motility by identifying impaired oesophageal bolus transport in NOD patients with and without normal standard manometry.(Conchillo, Nguyen, et al. 2005)

The possibility exists that impaired secondary peristalsis, rather than primary peristalsis, may account for altered bolus transport and symptoms in NOD. For example, Schoeman et al., found secondary peristalsis was triggered significantly less often in NOD patients when compared with controls.(Schoeman and Holloway 1994b) They speculated that this finding might translate to disordered bolus transport in patients with NOD, but this notion has never been extensively examined.

Utilizing Multichannel intraluminal impedance and oesophageal (MII-EM), we recently systematically evaluated the relationships among primary peristalsis, secondary peristalsis, and bolus clearance in healthy subjects.(Chen, Cook et al. 2007)

The objective of this study was to evaluate the integrity and characteristics of oesophageal bolus transit and clearance by secondary peristalsis in patients with NOD. Specifically, we tested the hypothesis that triggering of secondary peristalsis and its effectiveness in oesophageal bolus clearance are impaired in patients with NOD.

9.2 Methods

#9.2.1 Subjects

We studied 10 patients (5 men and 5 women; mean age, 64 years; age range, 46-76 years) with NOD, and 11 asymptomatic healthy subjects (7 men and 4 women; mean age, 25 years; age range, 19-54 years). All patients had dysphagia for more than 3 months with at least one weekly episode. Dysphagia was defined as a sensation of food sticking, either in the neck or retrosternal, which was experienced immediately after the act of deglutition and occurring with solids, liquids, or both. Mechanical obstruction was excluded in all patients by barium radiology and endoscopy within 3 months of the study. Whether or not the endoscopic appearances were abnormal, routine empiric oesophageal dilatation, with repeat inspection of the oesophageal mucosa after dilatation, was performed to confidently exclude structural abnormalities such as mucosal ring or stricture. Where clinically indicated, oesophageal biopsies were taken from the oesophagus to exclude disorders such as eosinophilic oesophagitis. All patients underwent standard manometry with water swallows to exclude significant dysmotility. Gastro-oesophageal reflux (GOR) was also excluded in all patients by a 24-hour ambulatory oesophageal pH monitoring. Healthy controls were recruited by a community advertisement. Controls were excluded if they had any oesophageal symptoms, prior oesophageal or abdominal surgery except appendectomy or hysterectomy, or were taking any medications. Patients taking medication that might affect oesophageal motility were asked not to take this from at least 48 hours before the study. All subjects gave written informed consent prior to the study, and the study was approved by the Human Ethics Committee of the South-Eastern Sydney Area Health Service.

#9.2.2 Oesophageal Manometry and Impedance Recording

Oesophageal motility and impedance were recorded with a custom-designed silicone rubber manometric catheter (outer diameter 2.5 mm) with 9 recording sideholes spaced at 3-cm intervals and 7 stainless steel electrode rings (4 mm long)

spaced at 3-cm intervals. An additional polyvinyl catheter, 1.8 mm internal diameter was attached to the manometric catheter so that the injection port was located in the mid oesophagus. The injection was performed with a hand-held syringe which was connected to the proximal end of the catheter. The manometric assembly was perfused with degassed distilled water by a low-compliance pneumohydraulic perfusion pump (Dentsleeve; Wayville, South Australia, Australia) at 0.3 ml/min per channel. Pressures were recorded for each perfused channel by 9 external pressure transducers. Pressure and impedance signals were acquired simultaneously using computer-based data-acquisition system (Solar GI; MMS, The Netherlands). Swallowing was detected via the most proximal channel of the assembly, which was sited in the pharynx, thus enabling primary and secondary peristalsis to be distinguished.

#9.2.3 Study Protocol

After a minimum of a 4hr fast, the cathter assembly was passed via the nose and positioned such that the most distal side hole was located on the upper margin of the LOS identified by pull-through. The subjects were then positioned in supine position and allowed to accommodate for 10-15 minutes.

Primary peristalsis was studied using 5 ml boluses of normal saline and solid agar, each tested 5 times. Each swallow was separated by a 30 second interval. The subject had to chew solid agar into smaller particles as possible before swallowing. For the induction of secondary peristalsis, rapid injection of air or saline into mid-oesophagus was performed by hand. To determine the threshold volumes for saline or air necessary to trigger secondary peristalsis we administered graded volumes, commencing at 1 ml and increasing stepwise in 1 ml increments until either a secondary peristaltic response was generated or the volume injected reached 20 ml. The threshold volume was determined as the lower injection volume that triggered the secondary peristaltic pressure wave. The rate of the injection was determined by the amount and content of stimulus used. The 20 ml saline was injected over 3.0 seconds while the injection of air 20 ml was within 0.5 seconds. We used five 20 ml boluses of normal saline to determine bolus transit and clearance of the secondary peristalsis. In addition, five 20 ml boluses of air were used to determine peristaltic response. An interval of 20 seconds was allowed after the stimulus for any response to occur, during which subjects were instructed not to swallow. At the end of 20 seconds, subjects were allowed to have a dry swallow to ensure clearance of any residual air or

water before the next stimulus and to reduce the desire to swallow during the distension.

#9.2.4 Data Analysis

9.2.4.1Manometry

The peristaltic amplitude at each recording site and the latency of the wave onset between adjacent recording sites were measured for both primary and secondary peristalsis. Contraction velocity was measured and defined as the speed (cm/s) of the contraction wave from the most proximal to most distal recording site. Primary peristalsis was considered to be complete if the pressure wave of $\geqq$ 12 mmHg in the proximal oesophagus and $\geqq$ 25 mmHg in the distal oesophagus propagated through all oesophageal recording channels.(Schoeman and Holloway 1994b) The minimal latency of wave onset between two recording channels was 0.5 seconds. An ineffective peristalsis was either failure of a pressure wave, $\geqq$ 12 mmHg in the proximal oesophagus and $\geqq$ 25 mmHg in the distal oesophagus, to traverse each of the recording channels or nontransmitted when wave amplitudes were $\leq$10 mmHg at any site. Responses were classified as simultaneous when wave amplitudes were > 10 mmHg and wave velocity > 8 cm/second. Secondary peristalsis in response to air and liquid bolus injection was analyzed in the same manner as primary peristalsis. No response to distension was judged to have occurred if a pressure wave $\geqq$ 10 mmHg was seen in less than two recording sites.(Schoeman and Holloway 1994b) Due to the fact that not all pressure waves of secondary peristalsis were propagated down the oesophagus, successful or complete peristalsis was recognized using the same criteria as for primary peristalsis. The amplitude and velocity the successfully propagated waves were measured.

9.2.4.2 Impedance

The recordings were analyzed using the impedance analysis software (Solar GI; MMS, The Netherlands). Oesophageal bolus transit and clearance were evaluated by measurement of two variables: total bolus transit time (TBTT) and bolus presence time (BPT). TBTT represents the time for the bolus to traverse the entire oesophagus and was measured as time the bolus head entered at the most proximal recording segment (Z1) and the bolus tail cleared at the most distal recording segment (Z7). BPT represents the time for the bolus to completely transverse an individual recording

segment from the time the bolus head entered the segment, as indicated by a drop in impedance to 50% of the baseline value, until the bolus tail had cleared the segment, as determined by recovery of the impedance level to 50% of the baseline value for ≧ 5 seconds. For each swallow response there were one of TBTT and 7 individual measurements of BPT corresponding to the seven impedance segments (Z1-Z7).

Swallows were classified by MII as showing: 1) complete bolus transit (CBT) if bolus entry occurred at the most proximal site (Z1) and bolus exit points were recorded in all the distal impedance-measuring sites (i.e., Z2-Z7), and 2) incomplete bolus transit if bolus exit was not identified at any of the distal impedance-measuring sites (i.e., Z2-Z7). Impedance data for secondary peristalsis were analyzed in a similar way as in primary peristalsis, and were determined for all peristaltic responses. Due to the mid-oesophageal stimulation for producing secondary peristalsis, distal bolus transit time (DBTT) (Z4-Z7) for secondary peristalsis with saline injection was measured. Secondary peristalsis with saline injection were classified by MII as showing: 1) CBT if bolus entry occurred at the injection site (Z4) and bolus exit points were recorded in other impedance-measuring sites, and 2) incomplete bolus transit if bolus exit was not identified at any of the impedance-measuring sites. The percentage of CBT for primary and secondary peristalsis, reflecting the efficiency of bolus clearance, was measured for each subject.

#9.2.5 Statistical Analysis

The normality of all data was examined by D'Agostino's K-squared test. All results were expressed as mean ± SEM. Differences in peristaltic amplitude and velocity, rate of peristaltic responses, rate of CBT, and BPT were compared using analysis of variance with Bonferroni post hoc correction factor. For each subject, the mean DBTT for secondary peristalsis as well as the mean TBTT for saline and solid swallows were determined. The student *t*-test was used to assess differences in TBTT of primary peristalsis, and DBTT of secondary peristalsis between the two groups. The group differences in the relation between abnormal bolus transit and secondary peristalsis for different motor components were examined by a chi-square test. A *p*-value of < 0.05 was accepted as indicating statistical significance.

9.3 Results

#9.3.1 Secondary Peristalsis

All the healthy subjects had exhibited secondary peristalsis with lower threshold volume for saline at 13 ml and air at 13 ml. In contrast, secondary peristalsis could not be elicited with injection at any tested volume for air and saline in 5 NOD patients ($p < 0.001$, both saline and air) (Figure 9.1). Fifty percent of control subjects had secondary peristalsis at a volume of ≤ 8 ml of saline or air, whereas NOD patients did not have secondary peristalsis until at least 10 ml of saline or 9 ml of air (Figure 9.1). The response rate of complete peristaltic responses was lower in NOD patients when compared with controls for both air and saline injection ($p < 0.001$) (Figure 9.2). The amplitudes of secondary peristalsis stimulated by air were lower in NOD patients than controls at P3 ($p = 0.04$) and P5 ($p = 0.02$), but the wave velocities were similar in the two groups (Table 9.1). The pattern of the motor responses for air and saline are shown in Figure 9.3. In controls, most of air boluses triggered a peristaltic response while a small proportion of air boluses triggered ineffective, synchronous, or no response. In NOD patients, however, only 34% of air boluses and 16% of saline boluses triggered secondary peristalsis while 34% of air boluses and 47% of saline boluses produced no response. The proportions of ineffective and synchronous responses were similar for saline injection between the two groups. Air boluses resulted in more ineffective response in NOD patients than controls ($p < 0.05$).

The prevalence of CBT by of secondary peristalsis was significantly lower in NOD patients than controls ($p < 0.001$) (Figure 9.4). DBTT of secondary peristalsis was longer in NOD patients than controls (11.3 *vs* 8.1 seconds, $p = 0.005$) (Figure 9.5A). Similarly, BPT of secondary peristalsis was significantly longer in NOD patients when compared with controls for all impedance segments (all $p < 0.05$) (Figure 9.5B). The mean number of sites with abnormal BPT per secondary peristalsis was significantly higher in NOD patients (4.4) than controls (0.8) ($p < 0.001$). Figure 9.6 shows percentages of incomplete bolus transit for secondary peristalsis according to manometric responses to 20 ml boluses of saline. Patients with NOD had more incomplete bolus transit than controls ($p < 0.001$) for complete peristalsis, and such difference was also noted for ineffective or synchronous response. In both groups, the proportion of incomplete bolus transit was significantly lower with complete peristaltic responses than ineffective or synchronous responses ($p < 0.001$).

#9.3.2 Primary Peristalsis

The mean frequency of complete peristalsis was significantly lower in NOD

patients than controls for solid agar swallows ($p < 0.05$), but was similar for saline swallows (p = NS) (Figure 9.2). The mean pressure wave amplitudes and velocities are summarized in Table 9.1. The pressure wave amplitudes were greater in NOD patients than controls at distal channel (P7) for both saline ($p = 0.05$) and solid swallows ($p = 0.04$), but were lower in NOD patients than controls at proximal channel (P1) for both saline ($p = 0.001$) and solid swallows ($p = 0.004$). The wave velocities were similar in the two groups.

The prevalence of CBT was significantly lower in NOD patients than controls for both saline ($p < 0.05$) and solid swallows ($p < 0.001$) (Figure 9.4). TBTT was significantly longer in NOD patients than controls for solid swallows ($p < 0.01$), but not for saline swallows (p = NS). The values for BPT were significantly longer in NOD patients than controls for solid agar swallows ($p < 0.05$), but not for saline swallow (p = NS). The mean number of sites with abnormal BPT per swallow was significantly higher in NOD patients (2.1) than controls (0.7) ($p < 0.001$) for solid swallows, but was similar for saline swallows.

9.4 Conclusion

In this study we investigated whether the pattern and characteristics of oesophageal transport and clearance by secondary peristalsis would differ between NOD patients and healthy subjects. We found in NOD patients that the triggering of secondary peristaltic reflex and its effectiveness in oesophageal bolus clearance were significantly different than those observed in healthy subjects. By using MII-EM, secondary peristalsis in NOD patients was characterized by a longer oesophageal clearance time when compared with healthy subjects. Furthermore, we found that, during complete secondary peristaltic responses, patients with NOD still had greater incomplete bolus clearance than healthy subjects.

NOD is a common indication for referral for oesophageal manometry as it is often supposed to be due to some oesophageal motility disorder,(Jacob, Kahrilas, et al. 1990) although manometric assessment of primary peristalsis can be normal in NOD patients.(Barish, Castell, et al. 1986, Katz, Dalton, et al. 1987, Kjellen, Svedberg, et al. 1984) Sometimes dysphagia has been reported in patients with GOR in whom results have been demonstrated including acid-induced dysmotility, reduced oesophageal contractile amplitudes, or failed peristalsis of the distal oesophagus.(Singh, Stein, et al. 1992, Triadafilopoulos 1989) Therefore, it appears that NOD patients comprise a

heterogeneous group including those with normal primary peristalsis, those with oesophageal peristaltic dysfunction, and reflux patients with oesophageal dymotility. In this study, we enrolled NOD patients with normal endoscopy/barium swallows as well as normal manometry and negative reflux tests to obtain a more homogeneous patient population and reduce the potential confounding factors. Also, this group of patients may present a diagnostic challenge in clinical practice.

Although secondary peristalsis has not been routinely examined in NOD patients, recent studies have shown that these patients have defective secondary peristalsis in response to oesophageal distension with boluses of air and water.(Schoeman and Holloway 1994b) The finding of the failure in NOD patients to respond to acute intra-oesophageal distension was inferred to the notion that defective secondary peristalsis might be an important mechanism in the development of dysphagia.(Schoeman and Holloway 1994b) Consistent with these findings, we noted a significantly lower response rate of secondary peristalsis induced by mid-oesophageal air and saline injections, even though standard manometric assessment did not disclose any abnormality in these patients. In addition, we revealed that secondary peristalsis could not be elicited with injection of any of tested volumes in some of NOD patients. Our findings may reinforce the fact that testing primary peristalsis alone is not sufficiently sensitive to detect subtle oesophageal motor abnormalities, and a normal peristaltic pattern does not necessarily exclude a defective transport. This notion has been supported by other studies using radiological or scintigraphic techniques in patients with dysphagia and normal peristalsis.(Blackwell, Hannan et al. 1983, Howard, Pryde, et al. 1989, Keren, Argaman, et al. 1992, Russell, Hill et al. 1981)

The finding of increased incidence of abnormal contractions after solid swallowing in NOD patients was in agreement with previous investigations in patients with dysphagia, whose standard manometric study is normal or nearly normal. (Allen, Orr, et al. 1988, Keren, Argaman, et al. 1992) Our study has extended these findings by showing defective solid bolus transit and clearance in NOD patients. Pharyngeal pump,(Fisher, Hendrix, et al. 1978) peristaltic forces, and gravity are factors promoting bolus transit downward along the oesophagus. Initially, pharyngeal pump is the major factor, but as the bolus traverses distally, the oesophageal muscular activity becomes the major factor in transport. (Russell, Hill, et al. 1981) The effect of gravity was eliminated in this study by examining the patient in the supine position.

Evaluation of the manometric characteristics of the secondary peristaltic responses revealed lower mean peristaltic amplitudes in mid-oesophagus of NOD patients than those of healthy subjects, while higher distal primary peristaltic amplitudes were demonstrated in NOD patients than in healthy subjects. The importance of this finding is not clear but it can be explained by a previous study by Paterson et al., who have demonstrated secondary peristalsis differs significantly from primary peristalsis (Paterson, Hynna-Liepert, et al. 1991) and have suggested that primary and secondary peristalsis may involve different neuromuscular mechanisms.

Analysis of impedance recordings is based on measurement of BPT which indicates segmental bolus clearance at a particular level of the oesophagus, and TBTT which indicates total oesophageal bolus clearance. (Nguyen, Silny, et al. 1997, Srinivasan, Vela, et al. 2001, Tutuian, Vela, et al. 2003, Wise, Murray, et al. 2004)The study was the first assessment of oesophageal bolus transit by secondary peristalsis in NOD patients and revealed that oesophageal liquid transit as induced by direct mid-oesophageal saline injection was prolonged in NOD patients, who were also demonstrated with longer dwell time in each oesophageal segment during secondary peristalsis. The reasons for these findings are unclear but may partially related to the fact that NOD patients, when stimulated by mid-oesophageal saline injections, had significantly lower response rate of effective peristalsis which is crucial for effective bolus clearance.(Kahrilas, Dodds, et al. 1988b) This notion is further supported by the observation that secondary peristalsis with ineffective responses was more often associated with abnormal oesophageal bolus transit. The pathophysiological mechanisms of reduced capability in NOD patients to generate secondary peristalsis are unknown. Nevertheless, because our patients had normal primary peristalsis, this may implicate that a defect in the afferent pathway, and possibly decreased or absent tension-sensitive receptors of the oesophagus or their defective function may be responsible for the diminished response of secondary peristalsis in NOD patients. Similar results have been reported elsewhere. (Schoeman and Holloway 1994b)

The clinical relevance of defective triggering and bolus clearance of secondary peristalsis to the pathogenesis of dysphagia remains to be determined. Secondary peristalsis is generally believed to play a role in facilitating the volume clearance of the oesophagus from the ingested material left behind after a swallow or from the refluxate after reflux episodes. Defective bolus clearance by secondary peristalsis found in NOD patients may conceptually lead to a failure to expel the retained bolus

downward the oesophagus, and might therefore contribute to the feeling of dysphagia. (Castell and Donner 1987) However, further work will be necessary to elucidate the interrelationship between the defective clearance in secondary peristalsis and dysphagia.

The limitation of this study is that it is somewhat difficult to enroll patients under 50 years old due to the fact that the prevalence of dysphagia has been reported to be greater over 50 year of age. (Shaker and Staff 2001) Thus, it is not surprising that the age differs significantly between the two groups studied, which might potentially confound the current results. The effect of aging on secondary peristalsis has been studied previously by Ren J et al., who indicated in the elderly that secondary peristalsis is either absent or its stimulation is significantly less frequent compared with young volunteers.(Ren, Shaker, et al. 1995) Other studies have further extended these findings and revealed that aging is associated with decreased sensory perception and altered biomechanical properties of the oesophagus, and suggested that older individuals have a larger lumen but somewhat stiffer oesophageal wall, (Rao, Mudipalli et al. 2003) which might lead to age related impairment of secondary oesophageal peristalsis. However, some other studies have reported that changes in oesophageal motor function with aging remain minimal for subjects older than 75 years.(Ferriolli, Dantas et al. 1996, Grande, Lacima et al. 1999) Therefore, to demonstrate better convincible results from the current work, more age matched controls would be required, and this needs to be confirmed in a larger study.

Other potential limitations of this study include patient selection and small sample size. With regard to the small sample size, we recognize that insignificant differences may be due to a lack of statistical power but current sample size was sufficient to detect a difference in oesophageal bolus clearance by secondary peristalsis between NOD patients and controls. Whilst it is unlikely in human physiological studies, particularly in those which include invasive tests, that the selected subjects are representative of all patients with the same disease, a process of self-selection is difficult to avoid, even if larger samples are studied.

In summary, impedance measures allow identification of substantial defects in oesophageal bolus transit and clearance by secondary peristalsis in NOD patients. Patients with NOD demonstrate abnormalities in the triggering of the secondary peristaltic reflex and are more likely to generate aberrant secondary peristaltic sequences. The current work corroborates the previous speculation regarding

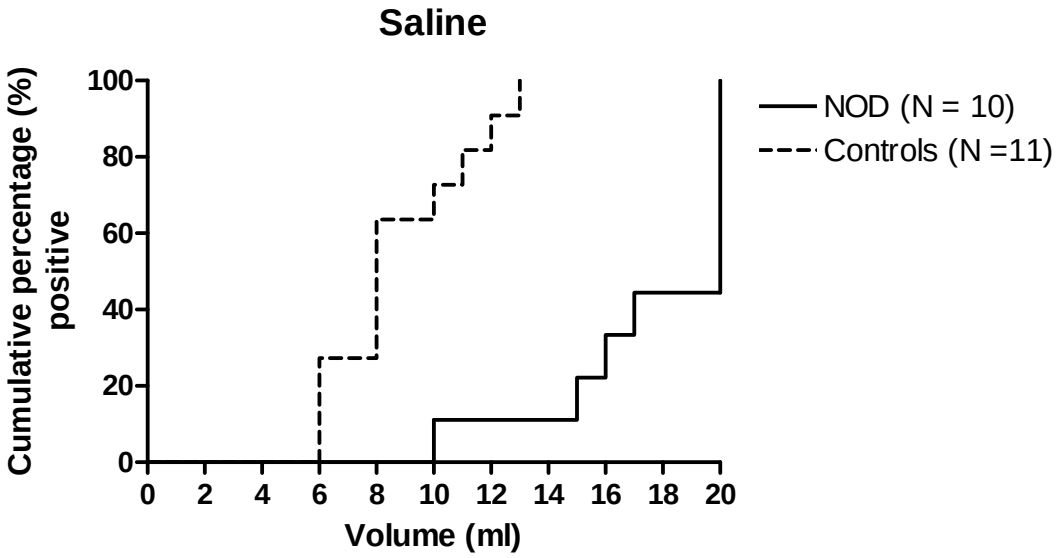
defective bolus clearance in secondary peristalsis of NOD patients (Schoeman and Holloway 1994b) by showing these abnormalities translate into diminished efficiency of bolus transport as characterised by a longer oesophageal clearance time. Therefore, abnormal secondary peristaltic reflex with defective bolus clearance may explain, in part, the dysphagia in NOD. Therapeutic implications of our findings are yet to be investigated.

Table 9.1:

	Primary peristalsis			**Secondary peristalsis**		
	Controls	**NOD**	***p*-value**	**Controls**	**NOD**	***p*-value**
Amplitude (mm Hg)						
	Saline 5ml			**Saline 20 ml**		
P2	82 (8.9)	30.7 (8.5)	0.001	63 (9.9)	71(10.1)	0.87
P3	53.3 (6.6)	35.9 (8.2)	0.18	42 (4.6)	38.7 (10.1)	0.67
P4	59.5 (7.7)	66.7 (16.9)	0.94	44.5 (7.7)	27.3 (10.5)	0.19
P5	76.6 (10.5)	70.4 (19.6)	0.79	44.4 (6.6)	48.4 (13.7)	0.8
P6	99.4 (16.1)	76.5 (24.3)	0.46	57 (13.7)	48.6 (10.0)	0.9
P7	68.7 (11.5)	118 (23.8)	0.08	49.6 (9.7)	36.8 (10.5)	0.59
	Solid 5ml			**Air 20ml**		
P2	91.2 (10.2)	40.7 (10.1)	0.004	57.2 (8.0)	36.4 (14.8)	0.31
P3	59.9 (6.1)	47.7 (9.4)	0.38	47.3 (5.6)	23.2 (7.6)	0.04
P4	68.5 (7.6)	53 (9.5)	0.15	53.6 (6.6)	50.6 (8.9)	0.95
P5	85.4 (11.8)	63.6 (6.2)	0.22	73.7 (11.4)	37.5 (9.7)	0.02
P6	102.3 (16.3)	65.8 (21.2)	0.27	84.9 (15.2)	58.2 (12.3)	0.37
P7	65.5 (10.8)	102.8 (13.3)	0.04	47.3 (11.4)	52.3 (14.9)	0.73
Velocity (cm/s)						
	Saline 5ml			**Saline 20 ml**		
	2.73 (0.13)	2.67 (0.11)	0.93	2.17 (0.24)	2.19 (0.28)	0.91
	Solid 5ml			**Air 20ml**		
	2.43 (0.21)	2.26 (0.15)	0.66	2.79 (0.17)	2.78 (0.41)	0.95

NOD, non-obstructive dysphagia; Data expressed as mean (SEM)

A



B

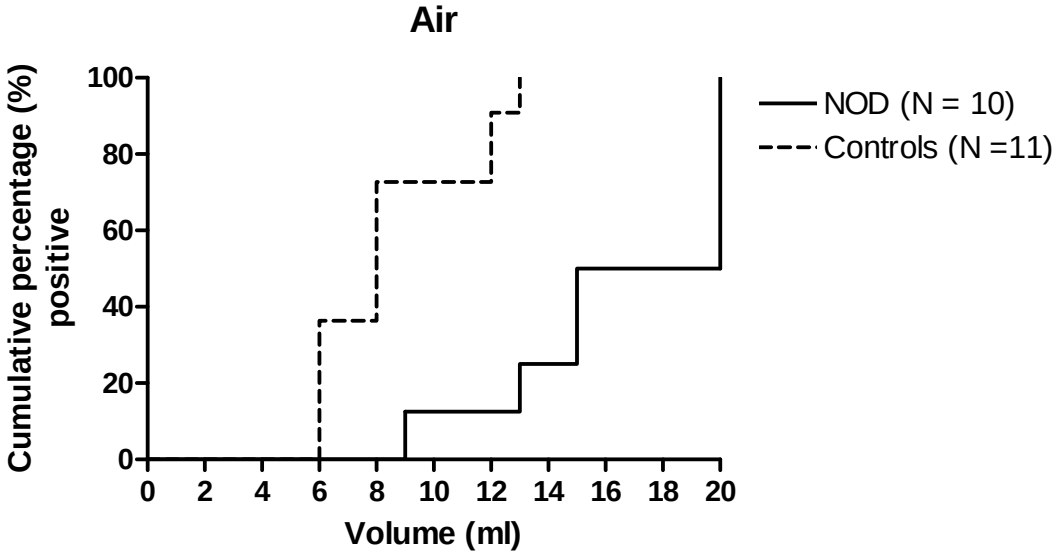


Figure 9.1: Secondary peristalsis with saline (A) and air injection (B). The difference is highly significant ($p < 0.001$ in saline and air) between patients with NOD and controls. Not only do controls more frequently develop secondary peristalsis with saline and air injections, but their secondary peristalsis occurs at smaller volumes.

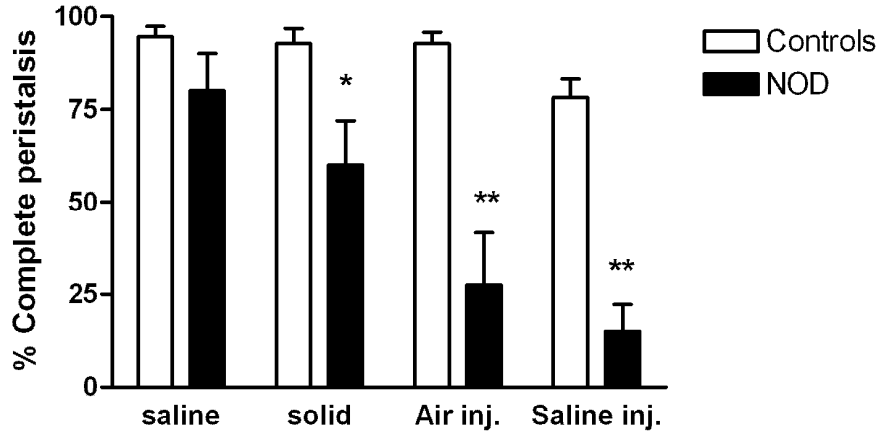


Figure 9.2: Comparisons of complete peristaltic responses (%) in primary and secondary peristalsis between NOD patients and controls. *$p < 0.05$, **$p < 0.001$; sal inj., saline injection; air inj., air injection.

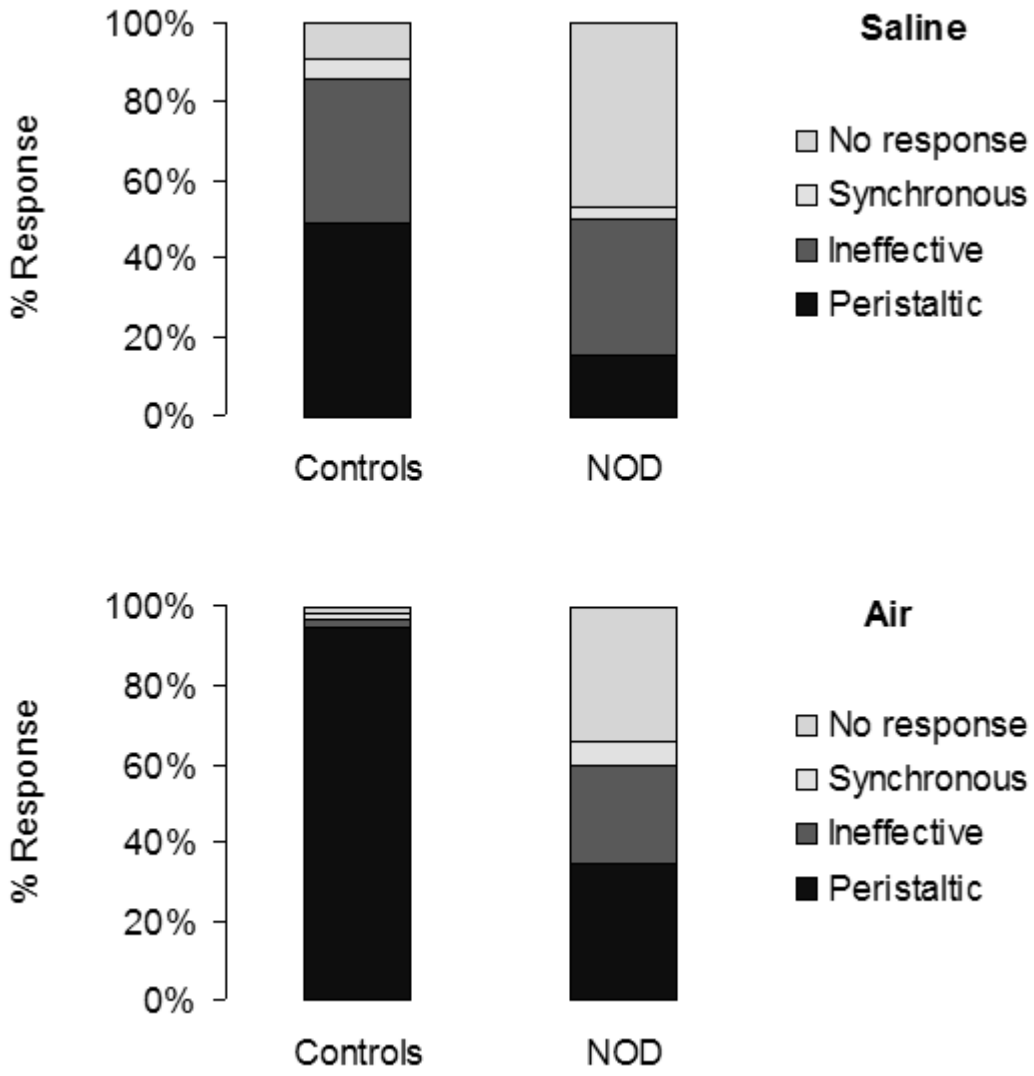


Figure 9.3: Patterns of the manometric responses to air and saline injections. The proportions of ineffective and synchronous responses were similar for saline injections between the two groups. Air injections resulted in more ineffective response in NOD patients than controls ($p < 0.05$).

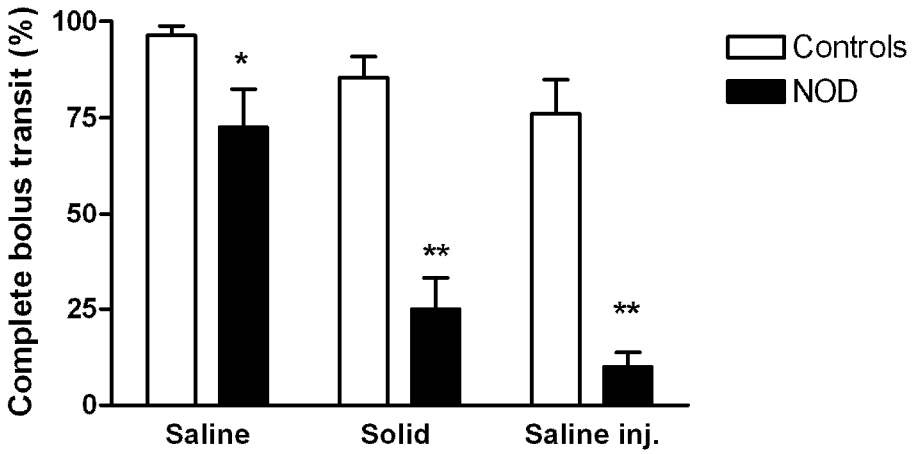
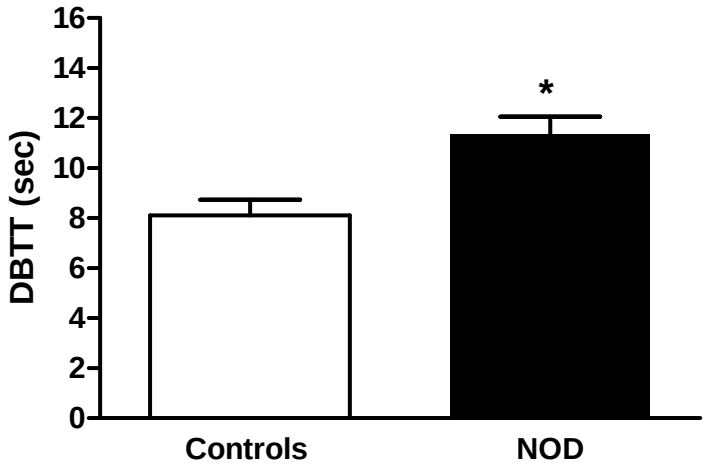


Figure 9.4: Comparisons of CBT (%) in primary and secondary peristalsis between NOD patients and controls. *$p < 0.05$, **$p < 0.001$; sal inj., saline injection; air inj., air injection.

A



B

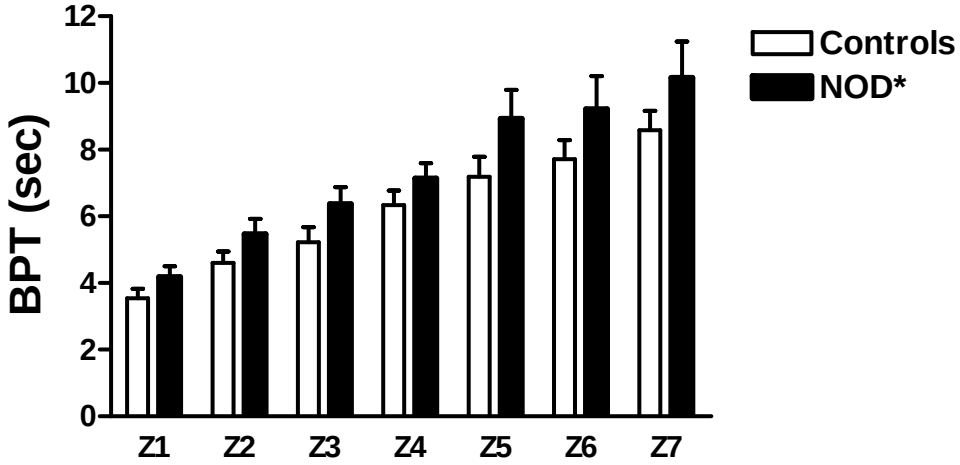


Figure 9.5: Comparisons of oesophageal bolus transit by secondary peristalsis between NOD patients and controls. (A) DBTT of secondary peristalsis with 20 ml saline injection, *p = 0.005. (B) BPT of secondary peristalsis with 20 ml saline injection, * $p < 0.05$ in all impedance channels.

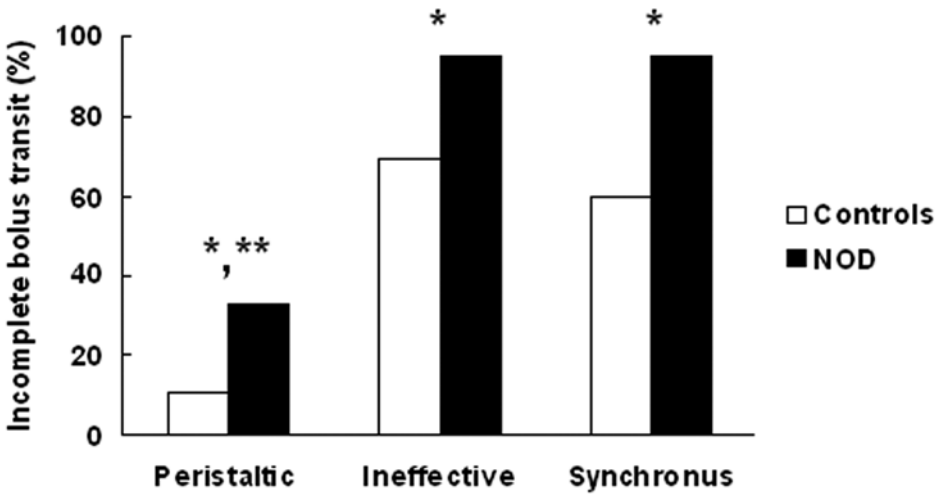


Figure 9.6: Relationships between oesophageal bolus transit and secondary peristaltic responses with 20 ml saline injection. *$p < 0.001$, NOD patients *vs* controls, **$p < 0.001$, peristaltic *vs* ineffective or synchronous responses in both groups.

Chapter 10

Sensory and motor responses in post-fundoplication dysphagia: a pilot study

10.1 Introduction

Multichannel intraluminal impedance (MII) is a technique that allows detection of oesophageal bolus transport and real-time quantification of bolus movement without radiation (Silny 1991). MII-EM is also able to enhance the diagnostic capability and clarify functional abnormalities in patients with disordered oesophageal clearance (Tutuian and Castell 2004b). Utilizing combined multichannel intraluminal impedance and oesophageal (MII-EM), we have recently successfully shown that secondary peristalsis behaves differently from primary peristalsis with longer oesophageal dwell and clearance time (Chen, Szczesniak et al. 2008). While secondary peristalsis is triggered less frequently after fundoplication (Rydberg, Ruth, et al. 2000), the functional consequences of this phenomenon for discrete bolus transport and clearance remain unclear.

Therefore, by using MII-EM, we aimed to evaluate the integrity and characteristics of oesophageal bolus transit and clearance by secondary peristalsis in patients after fundoplication, and to examine the hypothesis that the triggering of secondary peristalsis and its effectiveness in oesophageal bolus clearance may be impaired in patients after fundoplication.

10.2 Method

#10.2.1 Subjects

We studied 4 patients (2 men and 2 women; mean age, 50 years; age range, 46-60 years) after fundoplication and 11 asymptomatic healthy subjects (7 men and 4 women; mean age, 25 years; age range, 19-54 years). Between April 2006 and December 2007, 4 patients who previously had a laparoscopic fundoplication were prospectively enrolled into this study due to dysphagia. Dysphagia was defined as a sensation of food sticking experienced immediately after the act of deglutition and occurring with solids, liquids, or both. The sensation of the bolus lodging could occur at any part on its downward course through the oesophagus. All healthy controls had no oesophageal symptoms and no history of oesophageal or gastric surgery. All medications known to influence oesophageal motor function were hold in the week prior to the study. All subjects gave written informed consent prior to the study, and

the study was approved by the Human Ethics Committee of the South-Eastern Sydney Area Health Service.

 # 10.2.2 Oesophageal Manometry and Impedance Recording

Oesophageal motility and impedance were recorded with a custom-designed silicone rubber manometric catheter (outer diameter 2.5 mm) with 9 recording sideholes spaced at 3-cm intervals and 7 stainless steel electrode rings (4 mm long) spaced at 3-cm intervals. An additional polyvinyl catheter, 1.8 mm internal diameter was attached to the manometric catheter so that the injection port was located in the mid oesophagus (see **Chapter 7 and 9**). The injection was performed with a hand-held syringe which was connected to the proximal end of the catheter. The manometric assembly was perfused with degassed distilled water by a low-compliance pneumohydraulic perfusion pump (Dentsleeve; Wayville, South Australia, Australia) at 0.3 ml/min per channel. Pressures were recorded for each perfused channel by 9 external pressure transducers. Pressure and impedance signals were acquired simultaneously using computer-based data-acquisition system (Solar GI; MMS, The Netherlands). Swallowing was detected via the most proximal channel of the assembly, which was sited in the pharynx, thus enabling primary and secondary peristalsis to be distinguished.

#10.2.3 Study Protocol

The subjects were fasted for at least 4h. The assembly was passed via the nose and positioned such that the most distal side hole was located on the upper margin of the lower oesophageal sphincter (LOS). The subjects were then positioned in supine position and allowed to accommodate for 10-15 minutes. Primary peristalsis was studied using 5 ml boluses of normal saline. Each swallow was separated by a 30 second interval. For the induction of secondary peristalsis, rapid injection of saline into mid-oesophagus was performed by hand. First, we determined the threshold volume for saline that triggered the secondary peristalsis. We began at 1-ml volume and progressively increased the volume by 1-ml increments until a secondary peristalsis was generated or the volume of the injection reached 20 ml. The threshold volume was determined as the lower injection volume that triggered the secondary peristaltic pressure wave. The rate of the injection was determined by the amount and content of stimulus used. The 20 ml saline was injected over 3.0 seconds. We used

five 20 ml boluses of normal saline to determine bolus transit and clearance of the secondary peristalsis. An interval of 20 second was allowed after the stimulus for any response to occur, during which subjects were instructed not to swallow. At the end of 20 s, subjects were allowed to have a dry swallow to ensure clearance of any residual air or water before the next stimulus and to reduce the desire to swallow during the distension.

 # 10.2.4 Data Analysis

10.2.4.1 Manometry.

The peristaltic amplitude at each recording site and the latency of the wave onset between adjacent recording sites were measured for both primary and secondary peristalsis. Contraction velocity was measured and defined as the speed (cm/s) of the contraction wave from the most proximal to most distal recording site. Primary peristalsis was considered to be complete if the pressure wave of 12 mmHg in the proximal oesophagus and 25 mmHg in the distal oesophagus propagated through all oesophageal recording channels (Schoeman and Holloway 1994b). The minimal latency of wave onset between two recording channels was 0.5 seconds. An ineffective peristalsis was either failure of a pressure wave, 12 mmHg in the proximal oesophagus and 25 mmHg in the distal oesophagus, to traverse each of the recording channels or nontransmitted when wave amplitudes were ≤10 mmHg at any site. Responses were classified as simultaneous when wave amplitudes were > 10 mmHg and wave velocity > 8 cm/second. Secondary peristalsis in response to liquid bolus injection was analyzed in the same manner as primary peristalsis. No response to distension was judged to have occurred if a pressure wave 10 mmHg was seen in less than two recording sites (Schoeman and Holloway 1994b). Due to the fact that not all pressure waves of secondary peristalsis were propagated down the oesophagus, successful or complete peristalsis was recognized using the same criteria as for primary peristalsis. The amplitude and velocity the successfully propagated waves were measured.

10.2.4.2 Impedance.

The recordings were analyzed using the impedance analysis software (Solar GI; MMS, The Netherlands). Oesophageal bolus transit and clearance were evaluated by measurement of two variables: total bolus transit time (TBTT) and bolus presence

time (BPT). TBTT represents the time for the bolus to traverse the entire oesophagus and was measured as time the bolus head entered at the most proximal recording segment (Z1) and the bolus tail cleared at the most distal recording segment (Z7). BPT represents the time for the bolus to completely transverse an individual recording segment from the time the bolus head entered the segment, as indicated by a drop in impedance to 50% of the baseline value, until the bolus tail had cleared the segment, as determined by recovery of the impedance level to 50% of the baseline value for 5 seconds. For each swallow response there were one of TBTT and 7 individual measurements of BPT corresponding to the seven impedance segments (Z1-Z7).

Swallows were classified by MII as showing: 1) complete bolus transit (CBT) if bolus entry occurred at the most proximal site (Z1) and bolus exit points were recorded in all the distal impedance-measuring sites (i.e., Z2-Z7), and 2) incomplete bolus transit if bolus exit was not identified at any of the distal impedance-measuring sites (i.e., Z2-Z7). Impedance data for secondary peristalsis were analyzed in a similar way as in primary peristalsis, and were determined for all peristaltic responses. Due to the mid-oesophageal stimulation for producing secondary peristalsis, distal bolus transit time (DBTT) (Z4-Z7) for secondary peristalsis with saline injection was measured. Secondary peristalsis with saline injection were classified by MII as showing: 1) CBT if bolus entry occurred at the injection site (Z4) and bolus exit points were recorded in other impedance-measuring sites, and 2) incomplete bolus transit if bolus exit was not identified at any of the impedance-measuring sites. The percentage of CBT for primary and secondary peristalsis, reflecting the efficiency of bolus clearance, was measured for each subject.

#10.2.5 Statistical analysis

All results were expressed as mean ± SEM. Statistical differences were assessed using Mann-Whitney *U* test. The group differences in the relation between abnormal bolus transit and secondary peristalsis for different motor components were examined by a chi-square test. A *p*-value of < 0.05 was accepted as indicating statistical significance.

10.3 Results

#10.3.1 Primary peristalsis

The mean frequency of complete peristalsis was similar for saline swallows

between patients and healthy controls (p = NS). The manometric results were similar in the two groups. The mean frequency of CBT was significantly lower in patients than in the healthy subjects ($p < 0.05$) (Figure 10.1). TBTT was significantly longer in post-non-obstructive dysphagia (NOD) patients than in the healthy subjects ($p < 0.01$). The values for BPT were significantly longer in patient group than in the healthy subjects for all impedance measurement segments ($p < 0.05$).

#10.3.2 Secondary peristalsis

No significant difference was found in any of manometric parameters between the two groups. All the healthy subjects had exhibited secondary peristalsis with the threshold volume for air at 8 ml and saline at 9 ml, whereas secondary peristalsis could not be elicited with injection any tested volume upto 20 ml for air and saline in 2 of 4 patients. The response rate of complete peristaltic responses was lower in post-fundoplication patients when compared with the healthy subjects ($p < 0.001$).

The frequency for CBT of secondary peristalsis with saline was lower in post-fundoplication patients than in the healthy subjects ($p < 0.001$) (Figure 10.1). DBTT for secondary peristalsis was longer in post-fundoplication patients than in the healthy subjects (12.5 *vs.* 8.1 seconds, $p = 0.001$) (Figure 10.2). BPT of secondary peristalsis was significantly longer in post-fundoplication patients when compared with the healthy subjects for all impedance segments (all $p < 0.05$).

10.4 Conclusion

In this study we examined the efficiency of oesophageal bolus transport and clearance using MII-EM, and investigated whether the pattern and characteristics of oesophageal transport and clearance by secondary peristalsis differ between post-fundoplication patients and healthy subjects. We have found in the patient group that the triggering of secondary peristalsis and its effectiveness in oesophageal bolus clearance were significantly less efficient than those observed in healthy subjects. By using combined MII-EM, secondary peristalsis in post-fundoplication patients was characterized by longer oesophageal dwell and clearance time when compared with healthy subjects.

Although secondary peristalsis has not been routinely examined in post-fundoplication patients, recent studies have shown that these patients have defective secondary peristalsis in response to acute oesophageal distension by air

(Rydberg, Ruth, et al. 2000). Their finding of the failure in post-fundoplication patients to respond to acute intra-oesophageal distension was inferred to the notion that defective oesophageal motility might be a primary event leading to the development of gastro-oesophageal reflux disease (GORD) (Rydberg, Ruth et al. 1997). Similarly, we noted a significant lower response rate of secondary peristalsis induced by mid-oesophageal saline injections. In addition, we also revealed that secondary peristalsis could not be elicited with injection of any of tested volumes in some of the patients.

Analysis of impedance recordings is based on measurement of BPT which indicates segmental bolus clearance at a particular level of the oesophagus, and TBTT which indicates total oesophageal bolus clearance (Nguyen, Silny, et al. 1997, Srinivasan, Vela, et al. 2001, Tutuian, Vela, et al. 2003, Wise, Murray, et al. 2004). The study was the first assessment of oesophageal bolus transit by secondary peristalsis in post-fundoplication patients and revealed that oesophageal liquid transit as induced by direct mid-oesophageal saline injection is prolonged in patients after fundoplication, who were also demonstrated with longer dwell time in each oesophageal segment during secondary peristalsis. The reasons for these findings are unclear but may potentially related to the fact that post-fundoplication patients, when stimulated by mid-oesophageal saline injections, had significantly lower response rate of effective peristalsis which is crucial for effective bolus clearance (Kahrilas, Dodds, et al. 1988b). This notion is further supported by our previous observation in NOD that secondary peristalsis with ineffective responses was more often associated with abnormal oesophageal bolus transit (Chen and Yi 2008). The pathophysiological mechanisms of reduced capability in post-fundoplication patients to generate secondary peristalsis are unknown. Nevertheless, because our patients had competent primary peristalsis, this may implicate that a defect in the afferent pathway, and possibly decreased or absent tension-sensitive receptors of the oesophagus or their defective function may be responsible for the diminished response of secondary peristalsis in patients after fundoplication. Similar results have been reported elsewhere (Rydberg, Ruth, et al. 2000).

The clinical importance of defective triggering and bolus clearance of secondary peristalsis remains to be determined in patients after fundoplication. Secondary peristalsis is generally believed to play a role in facilitating the volume clearance of the oesophagus from the ingested material left behind after a swallow or from the

refluxate after reflux episodes. The findings of defective bolus clearance by secondary peristalsis found here may indicate a lack in efficient oesophageal clearance generated by secondary peristalsis in order to successfully expel the retained bolus downward the oesophagus which might potentially contribute to the feeling of dysphagia (Castell and Donner 1987). However, further work will be necessary to elucidate the interrelationship between this type of defective clearance and dysphagia after fundoplication.

In patents after fundoplication, our preliminary data support the hypothesis that oesophageal bolus transit and clearance of secondary peristalsis are prolonged and characterized by longer oesophageal dwell as well as prolonged clearance time. Impedance examination of secondary peristalsis may be complementary in the diagnostic evaluation of oesophageal dysmotility in patients after fundoplication, but more patients will be enrolled to achieve better and convincing results.

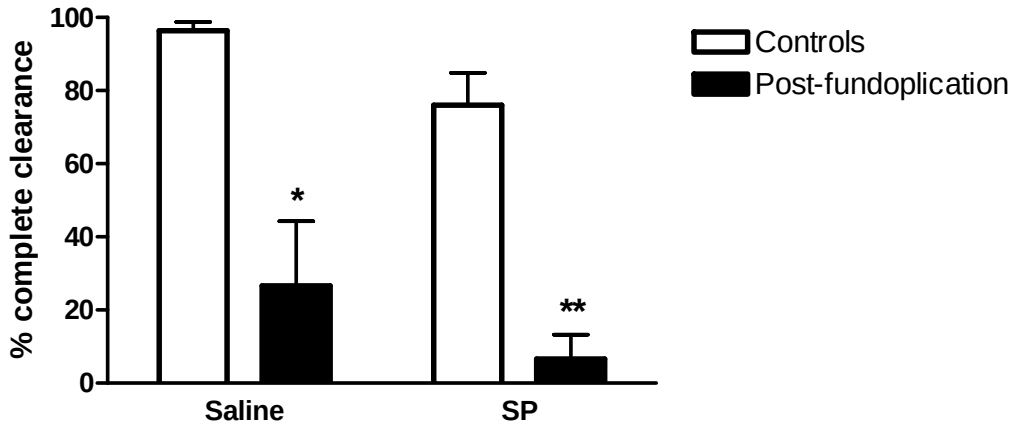


Figure 10.1: The mean frequency of CBT was significantly lower in patients than in the healthy subjects (*$p < 0.05$); the frequency for complete clearancet of secondary peristalsis with 20-ml saline injection was significantly lower in post-fundoplication patients ($p < 0.001$). SP = secondary peristalsis.

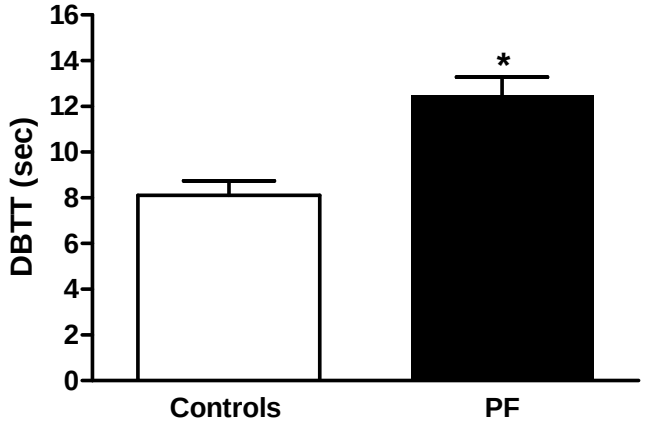


Figure 10.2: DBTT of patients after funplication (PF) was longer than healthy controls (*$p < 0.001$).

SECTION D

EVALUATION OF PERISTALSIS, BOLUS CLEARANCE AND SYMPTOM PERCEPTION IN GORD AND GLOBUS

The work presented in this chapter is accepted in Diseases of the Oesophagus 2008

Chapter 11

Evidence for impaired bolus clearance by primary peristalsis in erosive GORD

11.1 Introduction

The pathogenesis of gastro-oesophageal reflux disease (GORD) may variably involve incompetence of the lower oesophageal sphincter (LOS), increased transient relaxations of the LOS, defects in oesophageal body peristalsis, poor oesophageal clearance and impaired gastric emptying. (Dodds, Dent et al. 1982, Kahrilas, Dodds, et al. 1986, McCallum, Berkowitz et al. 1981) Normal *vs*. abnormal oesophageal function or oesophageal motility are normally defined based on previously published criteria of oesophageal motility using conventional manometry.(Kahrilas, Dodds, et al. 1988b, Richter, Wu et al. 1987) Multichannel intraluminal impedance (MII) allows evaluation of oesophageal bolus transport without involving exposure to radiation.(Silny 1991) The principles of the oesophageal impedance technique are based on measuring differences in resistance to alternating current due to the nature and progression of intraluminal contents. (Srinivasan, Vela, et al. 2001) Previous studies with combined video-fluoroscopy and MII have validated the ability of impedance to detect bolus movement.(Silny 1991, Simren, Silny, et al. 2003) Using multichannel intraluminal impedance and oesophageal (MII-EM), we have previously established normal values for this technique in a Taiwanese population.(Chen and Yi 2007)

MII-EM has been demonstrated to clarify functional abnormalities in patients with abnormal manometric studies.(Tutuian and Castell 2004b) More recently, this technique has been used to assess the oesophageal motility in patients with mild oesophagitis.(Domingues, Winograd, et al. 2005) However, the results revealed subtle bolus transit in a small proportion of patients with normal oesophageal peristalsis. The aim of the current study was to evaluate whether combined MII-EM is superior to EM in evaluating patients with GORD and in identifying patients with potentially significant oesophageal dysmotility.

11.2 Methods

#11.2.1 Subjects

Our subjects were 20 patients with GORD (ten men and ten women, mean age

45, 29-60) and 13 age-matched controls (seven men and six women; mean age 43 years, range 19-59 years) who were non-smokers and took no medication. The diagnosis of GORD was based on their symptoms (heartburn and/or acid regurgitation) lasting for more than 6 months along with endoscopic evidence of erosive oesophagitis.(Lundell, Dent et al. 1999) All patients had esophagogastroduodenoscope within 2 weeks prior to the study. Patients were excluded if they had the following conditions: (1) oesophageal strictures, (2) previous gastrointestinal surgery, (3) presence of systemic diseases that might interfere with oesophageal motility, and (4) use of medications known to affect oesophageal motility. Antisecretory agents and medications affecting oesophageal motility were discontinued within 2 weeks before enrolment and at the time of the study. All normal volunteers participating in this study were totally asymptomatic without histories of oesophageal, gastric or duodenal disease. The study was approved by the ethical committee of the Tzu Chi Medical Center. Informed written consent was obtained from each subject prior to the study.

#11.2.2 Oesophageal manometry and impedance recording

Each subject underwent oesophageal function testing using combined MII-EM with a Koenigsberg 9-channel probe (Sandhill EFT catheter; Sandhill Scientific, Inc., Highlands Ranch, CO). The 4.5 mm diameter catheter design has two circumferential solid-state pressure sensors at 5 cm and 10 cm from the tip and three unidirectional pressure sensors at 15, 20, and 25 cm. Impedance measuring segments including two rings placed 2 cm apart, were centered at 10, 15, 20, and 25 cm from the tip, thus across the four proximal pressure transducers. The EFT catheter was inserted transnasally into the oesophagus up to a depth of 60 cm. The LOS was identified using the stationary pull-through technique and the most distal sensor was placed in the high-pressure zone of the LOS. Intraoesophageal pressure sensors and impedance measuring segments were thus located at 5 cm, 10 cm, 15 cm, and 20 cm above the LOS. In the supine position, each subject was given 10 swallows of 5 cc normal saline and 10 swallows of 5 cc viscous material (apple-sauce like consistency) (Sandhill Scientific) material each 20–30 seconds apart. Normal saline was used instead of regular water for the non-viscous liquid bolus since it provides better impedance change with a standardized ionic concentration.

#11.2.3 Data analysis

Manometric parameters included: (1) contraction amplitudes at 5 and 10 cm above the LOS, (2) distal oesophageal amplitude (DEA) as average of contraction amplitude at 5 and 10 cm above the LOS, and (3) onset velocity of oesophageal contractions in the distal part of the oesophagus (i.e., between 10 cm and 5 cm above the LOS). Mid-respiratory resting pressure and LOS residual pressure during swallowing were used to assess LOS function. Swallows were manometrically classified as: (1) normal, if contraction amplitudes at 5 and 10 cm above the LOS were each greater than or equal to 30 mmHg and distal onset velocity was less than 8 cm/sec; (2) ineffective, if either of the contraction amplitudes at 5 and 10 cm above the LOS was less than 30 mmHg (this includes contractions defined as "poorly transmitted" or "not transmitted" as described by other authors;(Leite, Johnston, et al. 1997) (3) simultaneous, if contraction amplitudes at 5 and 10 cm above the LOS were each greater or equal to 30 mmHg and distal onset velocity was greater than 8 cm/sec. Diagnoses of manometric motility abnormalities were established according to previous criteria.(Spechler and Castell 2001) Subjects with 30% or more ineffective or 20% or more simultaneous contractions were considered to have abnormal EM.

MII parameters analyzed included bolus entry at each specific level obtained at the 50% point between the 3-second pre-swallow impedance baseline and the impedance nadir during bolus presence. Bolus exit was determined as the return to this 50% point on the impedance-recovery curve. Total bolus transit time (TBTT) was assessed as the time elapsed between bolus entry at 20 cm above LOS and bolus exit at 5 cm above LOS. Swallows were classified by MII as showing: (1) Complete bolus transit (CBT) if bolus entry occurred at the most proximal site (20 cm above LOS) and bolus exit points were recorded in all three distal impedance-measuring sites (i.e., 15 cm, 10 cm, and 5 cm above the LOS) and (2) incomplete bolus transit, if bolus exit was not identified at any one of the three distal impedance-measuring sites.

The diagnosis of oesophageal transit abnormalities is defined as normal liquid transit if at least 70% of liquid swallows had CBT and normal viscous transit if at least 70% of viscous swallows had CBT. These values are based on our previously published data on Taiwanese subjects. (Chen and Yi 2007)

#11.2.4 Statistical analysis

All results were expressed as mean ± SEM. Statistical comparisons were

assessed using student's *t* test or nonparametric testing as appropriate. Chi-square analysis was utilized to statistically analyze frequency variables. Receiver operative characteristic analysis was performed to assess the predictive power of various manometry and impedance variables. The receiver operative characteristic curves were calculated along with 95% CI. The α level was set at 0.05 for all statistical analysis. Statistical analyses were conducted with SPSS for Windows 11.0 (SPSS, Inc, IL, USA).

11.3 Results

None of our patients had hiatus hernia. In accordance with the LA classification, there were 5 patients with grade A oesophagitis and 15 patients with grade B oesophagitis. The symptom for which patients have experienced was heartburn (90%), regurgitation (75%), dysphagia (30%), chest pain (25%), and globus (8%).

According to the manometric results, the LOS pressure was lower in GORD patients compared to controls (13.0 ± 1.4 *vs*. 21.7 ± 2.1 mmHg, $p = 0.01$). Distal oesophageal contraction amplitude was significantly lower in GORD patients than controls for viscous swallows (58.3 ± 7.3 mmHg *vs*. 82.4 ± 4.1 mmHg, $p = 0.005$). There was no statistical significance regarding velocity or duration of oesophageal contraction between GORD and controls. Abnormal EM was found in 8 of 20 patients (40%) but not in controls ($p = 0.025$).

Considering impedance features (Figure 11.1), TBTT was significantly slower in GORD patients than controls for liquid swallows (8.7 ± 0.8 *vs*. 7.3 ± 0.3, $p = 0.035$) but not for viscous swallows (9.4 ± 0.6 *vs*. 8.8 ± 0.2, $p = 0.31$). The percentages of CBT were significantly lower in GORD patients compared with controls for both liquid (48% *vs*. 83%, $p = 0.005$) and viscous swallows (41% *vs*. 75%, $p = 0.005$). None of controls had abnormal bolus transit. Half of GORD patients with normal EM still had abnormal bolus transit (50%, 6 of 12 patients) while three-quarters of those with abnormal EM had abnormal bolus transit (75%, 6 of 8 patients) (Figure 11.2). Thus, 12 of 20 GORD patients (60%) had abnormal bolus transit by impedance.

Receiver operative characteristic analyses revealed good discriminating capability for both CBT (%) with liquid (0.77, $p = 0.01$, 95% CI = 0.59-0.94) and viscous swallows (0.75, $p = 0.04$, 95% CI = 0.54-0.95) compared with other EM or MII parameters (Figure 11.3).

11.4 Conclusion

The study was intended to determine whether MII-EM could detect more subtle defects underlying functional impairment in patients with GORD. We found that 50% of GORD patients with normal EM still had abnormal bolus transit for either liquid or viscous swallows, whereas 75% of GORD patients with abnormal EM also exhibited abnormal bolus transit. In addition, a significant portion of our GORD patients (60%) had abnormal bolus transit compared with none of healthy controls. Furthermore, we have demonstrated excellent capability for MII in distinguishing patients from controls with regard to oesophageal bolus transit.

It has been shown that oesophageal peristaltic dysfunction is associated with increasing severity of GORD,(Gill, Bowes et al. 1986, Kahrilas, Dodds, et al. 1986) and our patients also showed significantly lower distal oesophageal peristaltic wave amplitude as well as diminished LOS pressure when compared to healthy controls. Nevertheless, these results were still within the normal range and comparable to previous findings.(Kahrilas, Dodds, et al. 1986) In contrast, the finding of no difference in the propagation velocity of peristaltic between GORD patients and control was consistent with previous investigations,(Dodds, Hogan, et al. 1973, Ingelfinger 1958) although faster propagation velocity has been reported in GORD in other studies.(Gill, Bowes, et al. 1986, Kahrilas, Dodds, et al. 1986) Therefore, our manometric results support by inference the notion that other factors than the peristaltic profile may be involved in oesophageal propulsion mechanisms and bolus transport.(Clouse, Staiano et al. 1996, Russell, Bright et al. 1992)

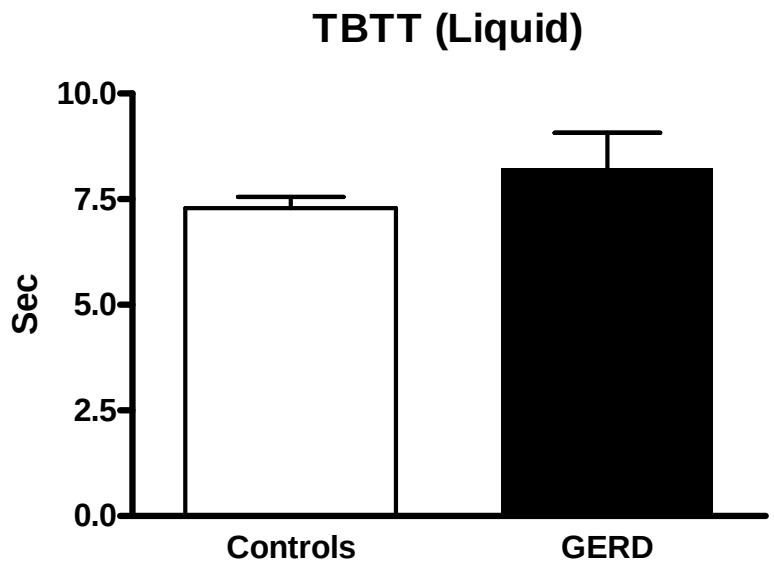
Domingues et al. has reported that concurrent use of impedance and manometry enhances sensitivity for the detection of motility disturbances in patients with mild oesophagitis.(Domingues, Winograd, et al. 2005) Although the swallows have been considered to have failed transport (4% liquid and 12% viscous), delayed transport (3% liquid and 8% viscous), and complex transport (2% liquid and 1 % viscous) in total swallows monitored in their GORD patients, the vast majority of all swallows were normal in their study. Conversely, we found above half of GORD patients have impaired bolus transport shown by impedance. This discrepancy may be explained by the fact that the subjects in their study had only mild GORD, whereas most subjects in our study had great GORD severity. In addition, the impedance and pressure electrode systems as well as their data analysis were different from those employed in our study. Further studies with MII-EM will be necessary to characterize this discrepancy.

The analysis of our study suggests that oesophageal testing using combined MII-EM provides more information regarding oesophageal functional abnormalities than EM alone. Abnormal bolus transit was found in GORD patients with or without functional defects detected by EM. Abnormal bolus transit occurring in 50% of GORD patients with abnormal EM suggests that the functional defect in these patients may predispose them to prolonged acid contact in the oesophagus. This finding was similar to a previous study done by Tutuian et al. which has shown abnormal bolus transit in 44% of patients with ineffective motility presenting with heartburn.(Tutuian and Castell 2004b)

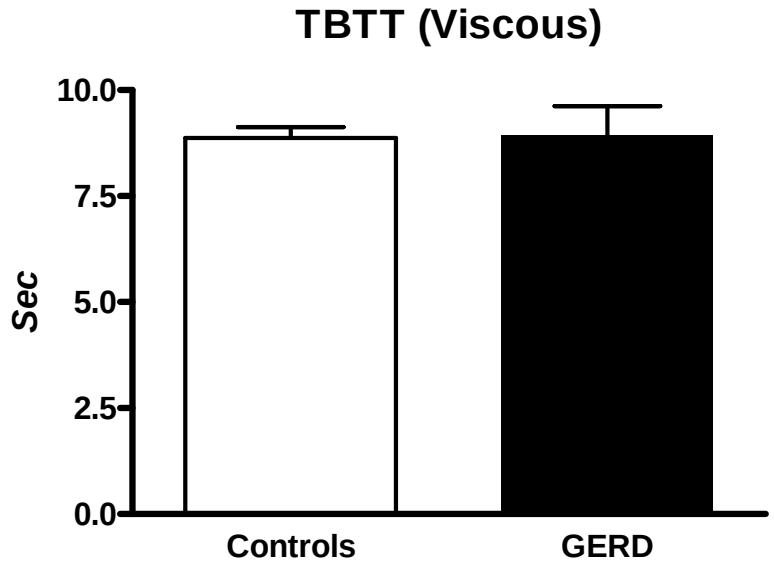
The results of current study using combined MII-EM as a standard test in clinical evaluation may provide additional benefits in that impedance variables such as CBT (%) for liquid and viscous swallows may better differentiate GORD patients from controls. Our studies raise the possibility that combined MII-EM will provide greater utility for the eventual identification of specific GORD-related manometric abnormalities that define pathophysiology in specific oesophageal disease states. Such information could eventually have additional value in the matching of therapeutic interventions with specific oesophageal motor abnormalities.

In summary, this paper demonstrates that the measurement of oesophageal impedance along with standard oesophageal manometry (combined EM-MII) enhances diagnostic capability. Our results illustrate that impedance can provide physiologically and clinically relevant information in GORD patients with possible oesophageal dysmotility in whom traditional manometry would have provided less definitive results. Further studies will be needed to investigate the utility of combined MII-EM for studying oesophageal motility in patients after anti-reflux surgery as well as patients in a wide variety of clinical settings.

A


TBTT (Liquid)
10.0
7.5
5.0
2.5
0.0
Sec
Controls
GERD

B


TBTT (Viscous)
10.0
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5.0
2.5
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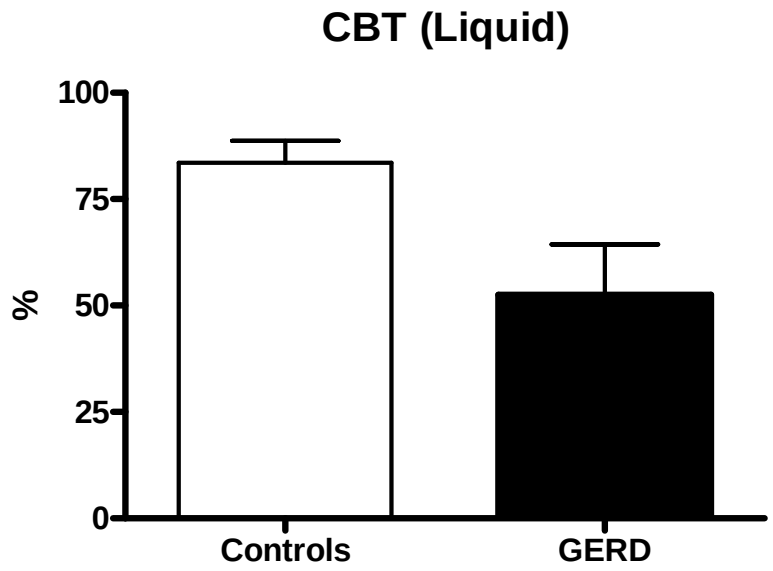
Controls
GERD

C



D

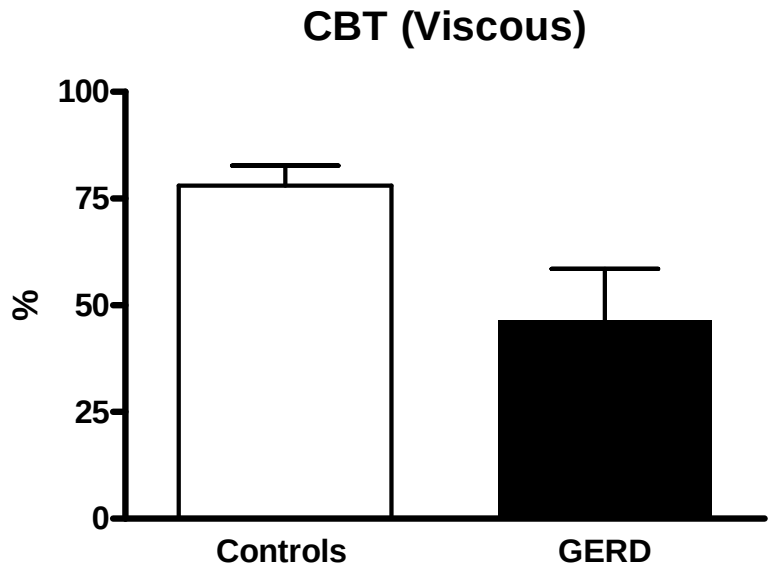


Figure 11.1: The TBTT was significantly slower in GORD patients than controls for liquid swallows (8.7 ± 0.8 *vs.* 7.3 ± 0.3, $p = 0.035$) (A) but not for viscous swallows (9.4 ± 0.6 *vs.* 8.8 ± 0.2, $p = 0.31$) (B). The percentages of CBT were significantly lower in GORD patients compared with controls for both liquid (48% *vs.* 83%, $p = 0.005$) (C) and viscous swallows (41% *vs.* 75%, $p = 0.005$) (D).

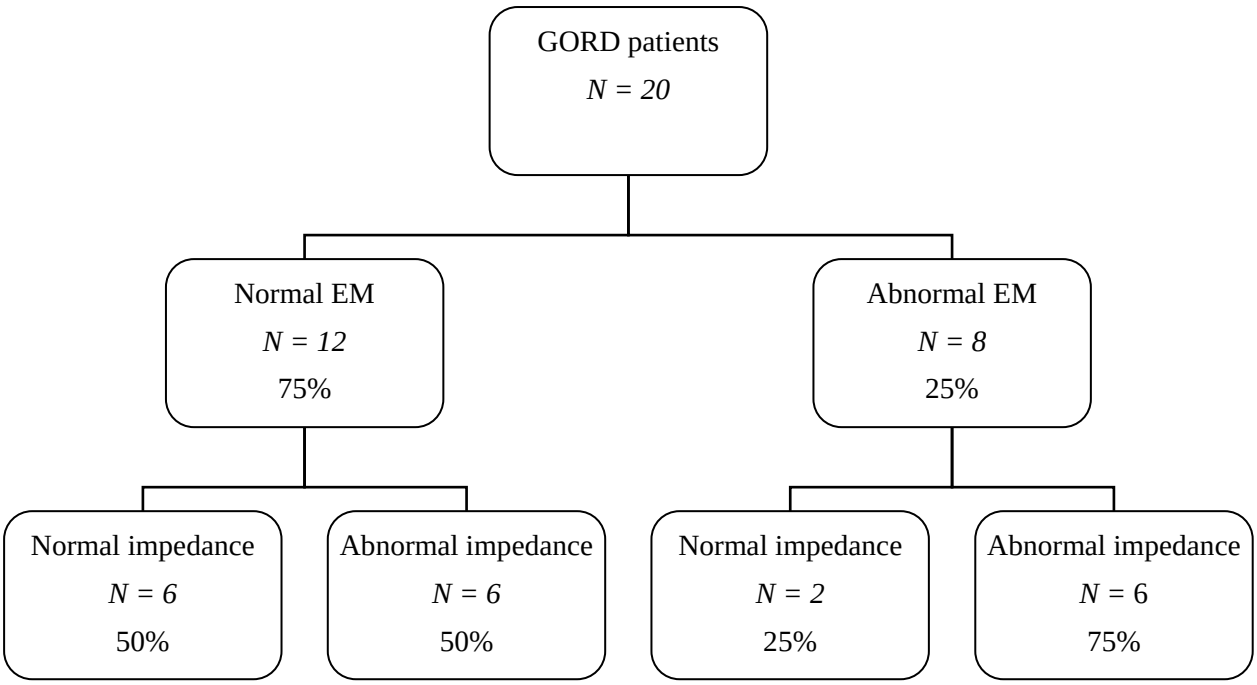
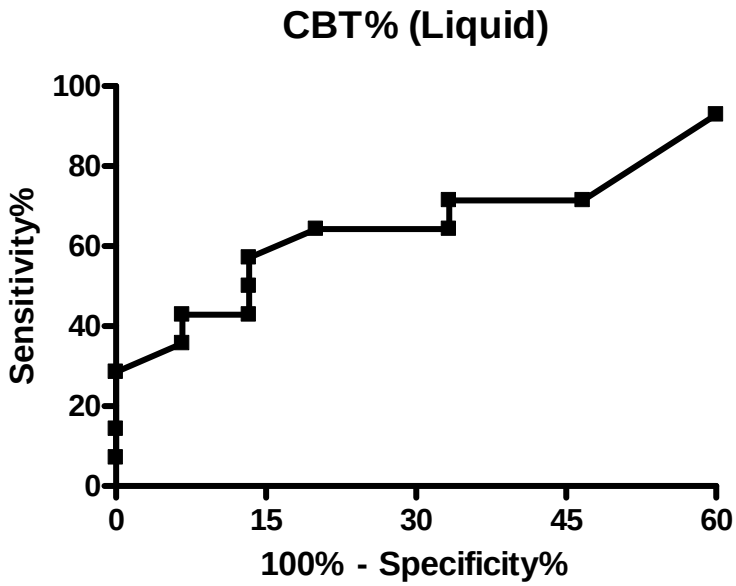


Figure 11.2: Classification of GORD patients according to the results of EM-MII.

A



B

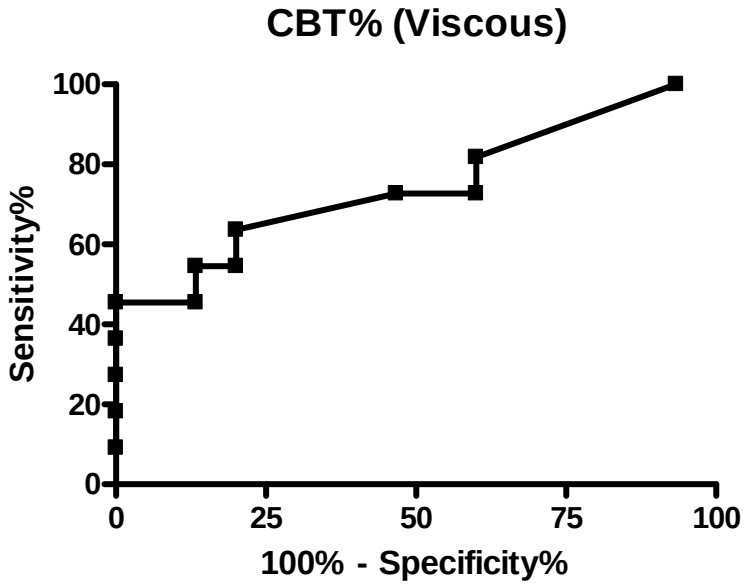


Figure 11.3: Receiving operative characteristic analyses of revealed good discriminating capability for both CBT (%) with liquid (0.77, p = 0.01, 95% CI = 0.59-0.94) (A) and viscous swallows (0.75, p = 0.04, 95% CI = 0.54-0.95) (B)

The work presented in this chapter is accepted in Digestive and Liver Disease 2008:**40** (5):348-54.

Chapter 12

Evidence for greater impairment of bolus clearance by primary peristalsis in erosive *vs.* non-erosive GORD

12.1 Introduction

The pathogenesis of gastro-oesophageal reflux disease (GORD) involves incompetence of the lower oesophageal sphincter (LOS), transient relaxations of the LOS, defects in oesophageal body peristalsis, poor oesophageal clearance and impaired gastric emptying (Dodds, Dent, et al. 1982, Kahrilas, Dodds, et al. 1986, McCallum, Berkowitz, et al. 1981). Patients with severe GORD are characterized by frequent supine GOR and increased prevalence of abnormal peristalsis (Kahrilas, Dodds, et al. 1986, Orr, Allen et al. 1994). In patients with Non-erosive reflux disease (NERD), however, most reflux episodes occur in the upright position during the day, and only 14% of those patients may have peristaltic failure (Kahrilas, Dodds, et al. 1986). In patients with reflux disease, the term ineffective oesophageal motility (IEM) characterized by an increased proportion of low amplitude peristalsis during traditional manometry is the most common pattern of peristaltic failure. This manometric pattern is commonly associated with delayed acid clearance (Leite, Johnston, et al. 1997). Although reflux is greater in erosive GORD than NERD, the presence and severity of IEM does not differ between the two groups of patients (Lemme, Abrahao-Junior, et al. 2005). In addition, the presence of IEM does not correlate well with dysphagia, nor does it predict the outcome of GORD surgery (Csendes, Maluenda et al. 1996, Fibbe, Layer, et al. 2001).

Multichannel intraluminal impedance (MII) is a new technique which allows a more sophisticated assessment oesophageal function. The principles of impedance technique are based on measuring differences in resistance to alternating current of the intraluminal contents (Srinivasan, Vela, et al. 2001). Using multiple impedance measuring sites, it allows detection and quantification of bolus movement. Previous studies with combined video-fluoroscopy and MII have validated the accuracy of impedance to detect bolus transit in the oesophagus (Silny 1991, Simren, Silny, et al. 2003). We have previously established normal values for Multichannel intraluminal impedance and oesophageal (MII-EM) in a Taiwanese population (Chen and Yi 2007). In patients with abnormal manometric studies, MII-EM is able to enhance the

diagnostic capability and clarify functional abnormalities with and without defective transit (Tutuian and Castell 2004a).

A recent study has reported subtle bolus transit abnormalities in patients with mild GORD (Domingues, Winograd, et al. 2005). We are unaware of any published study which compares impedance characteristics between erosive oesophagitis and NERD. Therefore, our study was designed to evaluate oesophageal function via MII-EM among patients with erosive oesophagitis, NERD and healthy controls to determine if the pattern of oesophageal function would differ between the two groups of patients.

12.2 Methods

#12.2.1 Subjects

All subjects agreeing to participate signed an informed consent which was approved by the ethical committee of the Tzu Chi Hospital. Patients with typical symptoms, heartburn and/or acid regurgitation at least 3 times per week, lasting for more than 6 months were enrolled in this study. Patients were excluded if they had the following conditions: (1) oesophageal strictures, (2) previous gastrointestinal surgery, (3) presence of systemic diseases that might interfere with oesophageal motility, and (4) chronic use of medications known to affect oesophageal motility. Antisecretory agents and medications affecting oesophageal motility were discontinued within 2 weeks before enrolment and at the time of the study. All healthy controls participating in this study were recruited from a university school population, who were totally asymptomatic and had no endoscopic evidence of oesophageal, gastric or duodenal disease.

#12.2.2 Symptom assessment

Before the study, each subject completed a reflux symptom scale. The patients were questioned with regard to the presence and severity of heartburn and regurgitation. All patients were asked to grade the severity (Visual analogue scale: 0–10; 0 = absent, and 10 = maximal) of the symptoms over the last 4 weeks (Chen, Orr et al. 2006).

#12.2.3 Endoscopic evaluation

We evaluated the distal portion of the oesophagus during standard upper

endoscopy to determine the presence of mucosal injury within 2 weeks prior to the study. The extent of mucosal damage was assessed using the Los Angeles grading system (Lundell, Dent, et al. 1999). Erosive oesophagitis was defined by the presence of endoscopically detectable mucosal breaks (erosions or ulcer), while the diagnosis of NERD was based on no endoscopically detectable mucosal lesions such as erosions or ulcers. Hiatal hernia was considered present if gastric folds were assessed as extending ≥ 2 cm above the diaphragmatic hiatus during quiet respiration (Wright and Hurwitz 1979).

#12.2.4 Oesophageal manometry and impedance recording

Each subject underwent oesophageal function testing using combined MII-EM with a Koenigsberg 9-channel probe (Sandhill EFT catheter; Sandhill Scientific, Inc., Highlands Ranch, CO). The 4.5 mm diameter catheter design has two circumferential solid-state pressure sensors at 5 cm and 10 cm from the tip and three unidirectional pressure sensors at 15, 20, and 25 cm. Impedance measuring segments including two rings placed 2 cm apart, were centered on 10, 15, 20, and 25 cm from the tip, thus across the four proximal pressure transducers. The EFT catheter was inserted transnasally into the oesophagus up to a depth of 60 cm. LOS was identified using stationary pull-through technique and the most distal sensor was placed in the high-pressure zone of the LOS. Intra-oesophageal pressure sensors and impedance measuring segments were thus located at 5 cm, 10 cm, 15 cm, and 20 cm above the LOS. In the supine position, each subject was given 10 swallows of 5 cc normal saline and 10 swallows of 5 cc viscous (apple-sauce like consistency) (Sandhill Scientific) material each 20–30 seconds apart. Normal saline was used instead of regular water since it provides better impedance change with a standardized ionic concentration.

#12.2.5 Data analysis

Manometric parameters included: (1) distal oesophageal amplitude (DEA) as average of contraction amplitude at 5 and 10 cm above the LOS, and (2) onset velocity of oesophageal contractions in the distal part of the oesophagus (i.e., between 10 cm and 5 cm above the LOS). Mid-respiratory resting pressure and LOS residual pressure during swallowing were used to assess LOS function. Swallows was considered normal if contraction amplitudes at 5 and 10 cm above LOS were each 30 mmHg and distal onset velocity was < 8 cm/s (Tutuian, Vela, et al. 2003). The

abnormal contractions included simultaneous contractions, repetitive contractions, and nonconductive peristalsis along the distal oesophagus. Individual swallow with ineffective contraction was defined if either of the contraction amplitudes at 5 and 10 cm above LOS was less than 30 mmHg, while that with simultaneous contractions was identified if contraction amplitudes at 5 and 10 cm above LOS were each greater than or equal to 30 mmHg and distal onset velocity was greater than 8 cm/s. Subjects with 30% or more ineffective or 20% or more simultaneous contractions were considered to have abnormal EM.

MII parameters analyzed included bolus entry at each specific level obtained at the 50% point between 3-second pre-swallow impedance baseline and impedance nadir during bolus presence and bolus exit determined as return to this 50% point on the impedance-recovery curve. Total bolus transit time (TBTT) was calculated as time elapsed between bolus entry at 20 cm above LOS and bolus exit at 5 cm above LOS. Swallows were classified by MII as showing: (1) complete bolus transit (CBT), if bolus entry occurred at the most proximal site (20 cm above LOS) and bolus exit points were recorded in all three distal impedance-measuring sites (i.e., 15 cm, 10 cm, and 5 cm above the LOS) and (2) incomplete bolus transit, if bolus exit was not identified at any one of the three distal impedance-measuring sites. Abnormal oesophageal transit was defined as the presence of less than 70% of liquid swallows with CBT and/or less than 70% of viscous swallows with CBT. These values are based on our previous study on Taiwanese subjects (Chen and Yi 2007).

#12.2.6 Statistical analysis

All results were expressed as mean ± S.E.M. Statistical comparisons were assessed using analysis of variance (ANOVA) with post-hoc tests. Chi-square analysis was utilized to statistically analyze frequency variables. The α level was set at 0.05 for all statistical analysis. Statistical analyses were conducted with SPSS for Windows 11.0 (SPSS, Inc, IL, USA).

12.3 Results

Between August 2005 and July 2006, twenty patients with erosive oesophagitis (ten women, mean age 49 years, range 39-60 years) (Grade A in sixteen, Grade B in two, Grade C in two) and 20 NERD patients (8 women, mean 50 years, range 41-65 years) met the enrollment criteria and entered the study. Fifteen healthy controls (7

women, mean age 24, range 22-30 years) were also included in this study. No age difference was found between the patient groups, but there was a significant age difference when compared healthy controls with any patient group (both $p < 0.05$). There were no significant differences in body mass index, current tobacco use, reflux symptoms, or presence of hiatal hernia between the patient groups (Table 12.1). Healthy controls differed from either of the patient groups regarding reflux symptoms ($p < 0.05$).

Table 12.2 summarizes the results of oesophageal manometry in all patients and healthy controls. Healthy controls had significantly greater distal oesophageal peristaltic amplitude than both patient groups with liquid and viscous swallows ($p < 0.05$). Normal peristalsis was found more in healthy controls than either of the patient groups ($p < 0.05$), but there was no difference between the patient groups. There was no difference in the other oesophageal functional characteristics (onset velocity of contractions, LOS residual/resting pressure, LOS relaxation duration, and UOS pressure) among the patients and healthy controls. Figure 12.1 has shown the results for manometric classification of individual swallows in all groups. There was no difference between the patient groups in terms of the percentage of manometrically normal, ineffective and simultaneous peristalsis with both liquid and viscous swallows. With regard to the impedance parameters, we found patients with erosive oesophagitis had a significantly lower percentage of CBT compared to healthy controls ($p < 0.05$) and NERD patients ($p < 0.05$) with liquid and viscous swallows (Figure 12.2A and B). Twelve patients with erosive oesophagitis (12/20) and one NERD patients (1/20) had abnormal bolus transit with liquid swallows ($p < 0.05$), while twelve patients with erosive oesophagitis (12/20) and two NERD patients had abnormal bolus transit with viscous swallows ($p < 0.05$) (Figure 12.2A and B). In addition, patients with erosive oesophagitis had a significant increase in TBTT compared to healthy controls ($p < 0.05$) and NERD patients ($p < 0.05$) with both liquid and viscous swallows (Figure 12.3).

12.4 Conclusion

The results of this study using combined MII-EM as the standard approach to oesophageal testing help clarify oesophageal function, and differentiate groups of patients with reflux disease and controls. We found patients with erosive oesophagitis had slower bolus transit as indicated by a significantly greater TBTT compared to

NERD patients and controls. In addition, there was a significant decrease in the percentage of CBT in patients with erosive oesophagitis compared with any other group.

Impedance data in NERD patients are sparse. A previous study has investigated the characteristics of bolus transport in patients with mild oesophagitis. Although delayed bolus transport was observed in the patient group, normal bolus transport was observed in the majority of the swallows (91% of liquid and 81% of viscous swallows) (Domingues, Winograd, et al. 2005). It appears that patients with mild oesophagitis present with low frequency of abnormal bolus transport, but overall most swallows in this group could be considered normal (Domingues, Winograd, et al. 2005). Similarly, we have noted no difference in the prevalence of complete bolus transport in NERD patients compared with healthy controls. However, patients with erosive oesophagitis exhibit an increase in the frequency of incomplete bolus transit which is also accompanied by more prolonged bolus transit.

Different patterns of oesophageal clearance between erosive oesophagitis and NERD may be explained by the difference in oesophageal tone noted in previous studies, which have found patients with severe GORD could have a very compliant oesophagus, whereas those with less severity may have normal oesophageal tone (Jenkinson, Scott et al. 2001, Mearin, Vasconez et al. 2000). In addition, a previous study done by Eriksen et al., using the radiolabeled solid bolus transit technique, has shown that almost 50% of reflux patients with negative pH and endoscopy have abnormal bolus transit (Eriksen, Cullen et al. 1991). Furthermore, a recent study utilizing MII-EM have shown abnormal bolus transport in almost 70% of patients with ineffective motility some of whom also experienced reflux symptoms (Tutuian and Castell 2004a). Therefore, the clinical significance of the current study is that our findings re-emphasize the utility of impedance for monitoring bolus transit during comprehensive oesophageal motility testing.

In this study, both oesophageal peristaltic amplitude and percentage of effective peristalsis were lower in GORD patients than the control group. Despite a trend toward more effective peristalsis in NERD, there was no difference between patients with erosive oesophagitis and NERD. Our findings are similar to previous studies that have shown no statistical differences between manometric findings in patients with erosive oesophagitis and NERD (Lemme, Abrahao-Junior, et al. 2005, Wong, Lai et al. 2004). However, other studies have disclosed lower DEA in erosive oesophagitis

when compared with NERD (Frazzoni, De Micheli et al. 2003). One possible reason for this discrepancy could be the difference in patient selection and ethnicity of the subjects studied. These aspects may merit further study. A recent study analyzing the relationship between peristaltic amplitude and bolus transit, has suggested incomplete bolus transit is strongly associated with the number of sites with decreased contraction amplitude (Tutuian and Castell 2004a). In addition, the effect of peristaltic dysfunction on oesophageal volume clearance was studied by Kahrilas et al. who found that normal bolus transit could occur within a wide range of manometric pressure values, and suggested that a manometric pressure amplitude greater than 30mmHg is necessary for normal transit in the distal oesophagus (Kahrilas, Dodds, et al. 1988b). In our study, however, all patients had contractions greater than 30 mmHg, so it appears that the peristaltic amplitude does not explain the rate of incomplete bolus transit.

In this study, we found both patient groups were significantly older than healthy controls. The issue of aging effect on oesophageal motility may influence the results of our study, although no age difference was observed between the patient groups. Previous studies have demonstrated a significantly increased occurrence of oesophageal dysmotility associated with aging (Adamek, Wegener et al. 1994, Khan, Shragge et al. 1977, Meshkinpour, Haghighat et al. 1994). However, studies focusing on the impact of aging on oesophageal contractile amplitudes failed to show a significant change (Adamek, Wegener, et al. 1994, Khan, Shragge, et al. 1977, Meshkinpour, Haghighat, et al. 1994, Richter, Wu, et al. 1987). Moreover, elderly patients tend to have an increase in abnormal oesophageal transit, despite a lack in a clear correlation between the status of oesophageal motility and oesophageal symptoms (Grishaw, Ott et al. 1996).

Despite similar occurrence in hiatal hernia between the patient groups, the presence of hiatal hernia can impact the clearance function of the body of the oesophagus and may aggravate the effects of GOR due to an incompetent cardia (DeMeester, Lafontaine et al. 1981). Hiatal hernia has been considered to be one of the pathological mechanisms contributing the development of GORD, promoting refluxate access and impairing acid clearance (Dent 1999). It has been demonstrated that the hiatal emptying is distinct from oesophageal bolus transport, since hernia emptying is driven by a hydrostatic pressure difference between the ampulla and stomach rather than by a peristaltic contraction (Lin, Ke et al. 1994).

In summary, we have shown that erosive oesophagitis was characterized by delayed oesophageal bolus clearance, whereas its manometry was comparable to NERD. However, since both groups showed significantly more motor dysfunction than normal controls, it would appear that the noted motor abnormalities may reflect a continuum of dysfunction secondary to increasing oesophageal mucosal damage.

Table 12.1: Baseline characteristics of the enrolled subjects

	Controls	Erosive Oesophagitis	NERD
Mean Age, yr, (range)	24 (22-30)[1]	49 (39-60)	50 (41-65)
Females (no. of cases)	7	10	8
Body mass index	21.0 ± 1.0	23.4 ± 1.0	21.6 ± 0.8
Smoker	2 (13 %)	3 (15 %)	3 (15 %)
Hiatal hernia	0 (0.0 %)[2]	5 (25 %)	4 (20 %)
Heartburn	0 (0.0%)[1]	20 (100 %)	18 (90 %)
Regurgitation	0 (0.0%)[1]	16 (80 %)	14 (70 %)
Heartburn (0-9)	0[1]	6.5 ± 0.9	6.3 ± 0.8
Regurgitation (0-9)	0[1]	5.8 ± 0.7	5.4 ± 0.9

Data are shown as mean ± S.E.M. or percentage. EE, erosive oesophagitis; NERD, non-erosive reflux disease.

[1] $p < 0.05$, controls vs. both patient groups

[2] $p < 0.05$, controls vs. Erosive Oesophagitis

Table 12.2: Oesophageal manometric results in all individuals

	Liquid			Viscous		
	Controls	Erosive Oesophagitis	NERD	Controls	Erosive Oesophagitis	NERD
DEA (*mm Hg*)	91 ± 7*	66 ± 8	62 ± 9	95 ± 5*	65 ± 6	64 ± 9
Normal peristalsis (%)	90 ± 5*	66 ± 8	72 ± 8	85 ± 4*	64 ± 5	74 ± 8
Onset velocity of contractions (*cm/s*)						
10-5 cm	5.1 ± 0.4	5.6 ± 1.1	6.2 ± 1.5	5.6 ± 0.7	4.0 ± 0.8	6.0 ± 1.3
Low oesophageal sphincter						
Residual Pressure (*mm Hg*)	5.5 ± 1.8	2.7 ± 1.4	3.2 ± 2.0	4.0 ± 0.9	3.1 ± 1.7	3.5 ± 1.6
Relaxation duration (*s*)	7.0 ± 0.8	6.1± 1.0	5.7 ± 0.9	7.1 ± 0.8	6.2 ± 1.5	5.8 ± 1.2

Data are shown as mean ± S.E.M. or percentage. EE, erosive oesophagitis; NERD, non-erosive reflux disease; $^{*}p < 0.05$, controls vs. both patient groups.

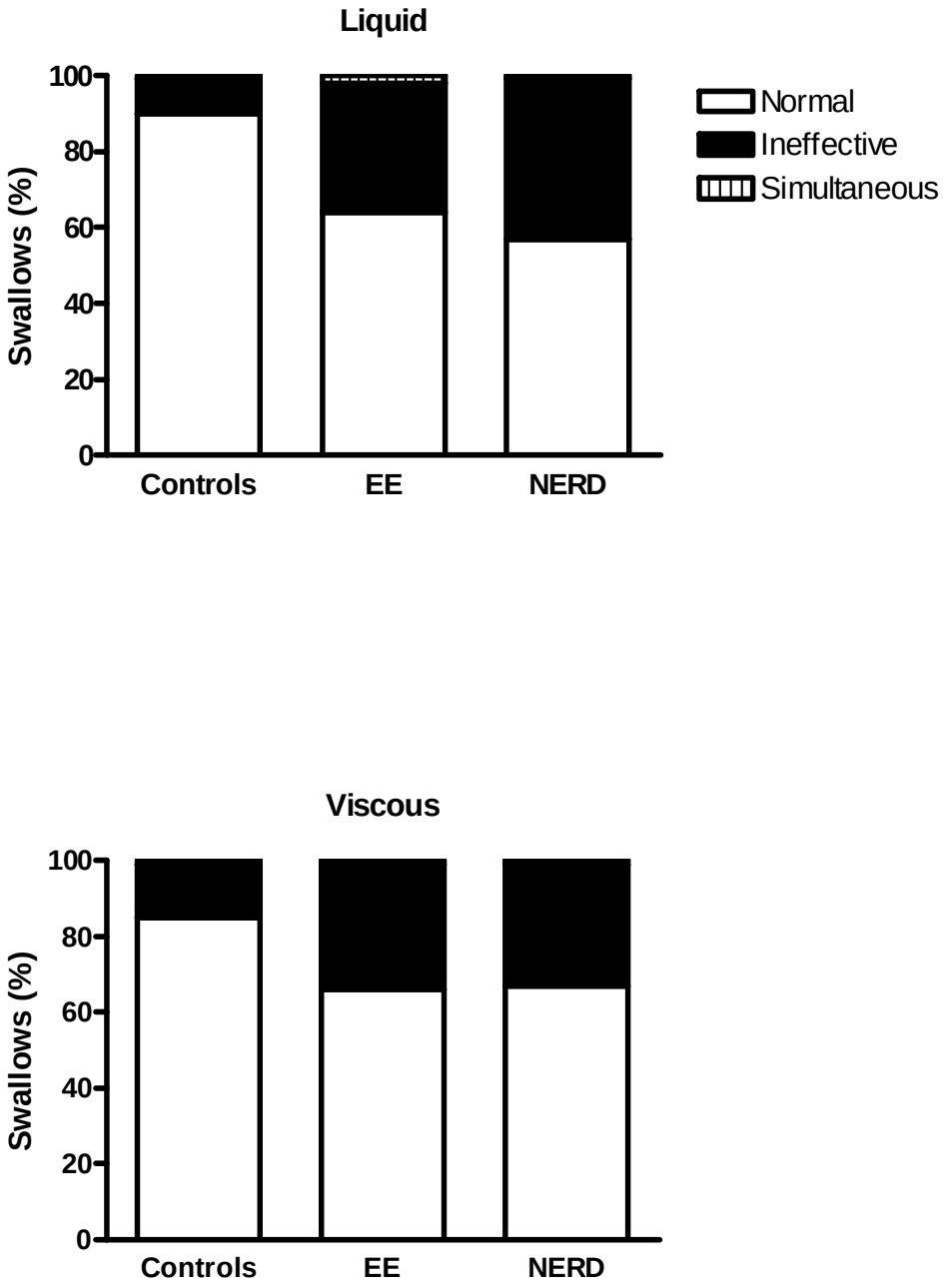
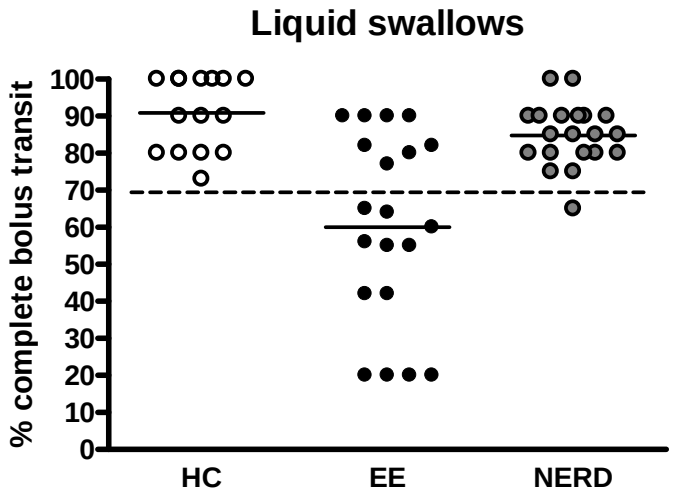


Figure 12.1: Classification of individual swallows based on manometry. Data are expressed as the percentage of the total number of swallows. Healthy controls had significantly more manomterically normal peristalsis compared to Erosive Oesophagitis ($p < 0.05$) and NERD patients ($p < 0.05$) with both liquid and viscous swallows. No difference was seen between Erosive Oesophagitis and NERD regarding manometrically normal, ineffective, or simultaneous peristalsis with either liquid or solid swallows. EE, erosive oesophagitis; NERD, non-erosive reflux disease.

A



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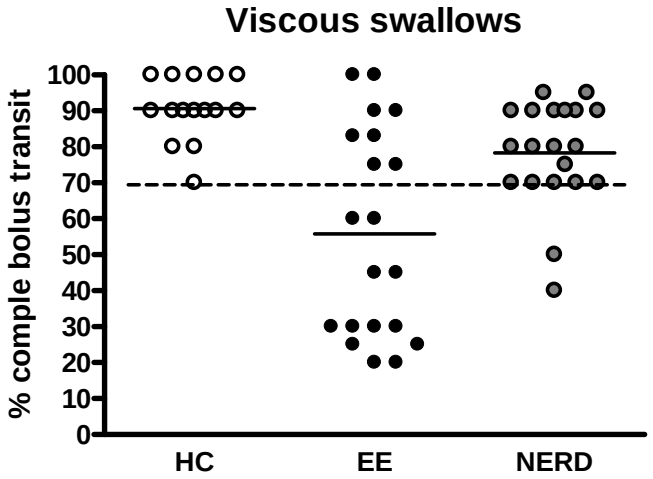


Figure 12.2: Patients with erosive oesophagitis had significantly lower frequency of CBT (%) compared to healthy controls ($p < 0.05$) and NERD patients ($p < 0.05$) with both liquid and viscous swallows (Figure 12.2A and B). Abnormal oesophageal transit occurred in six patients with erosive oesophagitis (6/10) and one NERD patients (1/9) with liquid swallows (2A), while six patients with erosive oesophagitis (6/10) and two NERD patients had abnormal oesophageal transit with viscous swallows (2B). Interrupted line indicates normal values of CBT (%) (Chen and Yi 2007). EE, erosive oesophagitis; NERD, non-erosive reflux disease.

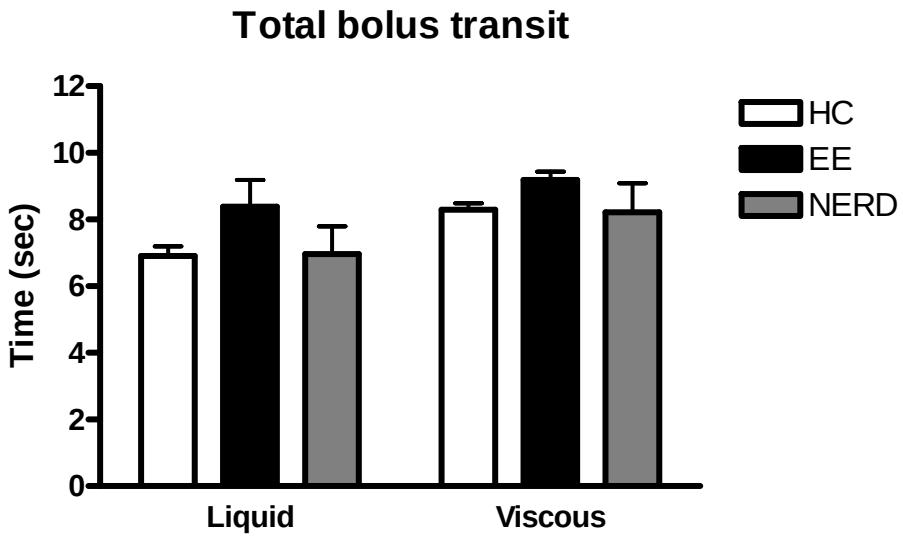


Figure 12.3: Patients with erosive oesophagitis had a significant increase in TBTT compared to healthy controls ($p < 0.05$) and NERD patients ($p < 0.05$) with both liquid and viscous swallows. EE, erosive oesophagitis; NERD, non-erosive reflux disease.

Chapter 13

Evidence of oesophageal hypersensitivity and aberrant viscerosomatic referral in patients with globus

13.1 Introduction

Globus is a commonly encountered clinical symptom (Moloy and Charter 1982). Typically the symptom is defined as a sensation of a lump or retained food bolus or tightness in the throat. A range of other foreign body-like descriptors are reported by patients including a sense of retained particulate matter, mucus accumulation, or a restrictive and choking sensation (Cook, Shaker, et al. 1991). The sensation is usually perceived in the midline between the thyroid cartilage and the manubriosternal notch. Globus is not a disease but rather than a symptom. As there is no current diagnostic or pathological marker for the condition, the diagnosis is based solely on history and the exclusion of structural abnormalities in the region.

There are many plausible theories reported on the etiology of globus. These include cricopharyngeal spasm (Watson and Sullivan 1974), temporomandibular joint dysfunction (Puhakka and Kirveskari 1988), pharyngeal dysmotility (Wilson, Pryde, et al. 1989), and gastro-oesophageal reflux (GOR) (Batch 1988a). A relationship has been established with GOR which was detected in a significant proportion of globus patients (Batch 1988a, Batch 1988b, Hill, Stuart, et al. 1997), but such evidence is conflicting (Harris, Deary, et al. 1996, Wilson, Heading, et al. 1987, Wilson, Pryde, et al. 1989). Moreover, it has been suggested that life stress might has a role in symptom genesis or exacerbation (Harris, Deary, et al. 1996). Therefore, the cause of this sensation remains intangible and the disorder is probably multifactorial in origin (Wilson, Deary, et al. 1991).

Whilst much evidence has been reported regarding the etiology of globus, few studies have focused on the neurophysiological role underlying globus sensation. In 1989, Cook et al. first noted that typical globus sensation was reproduced by balloon distension of the oesophagus, and speculated that globus is primarily sensory disorder and possibly associated with visceral hypersensitivity (Cook 1989). Visceral hypersensitivity with aberrant somatic referral of intestinal pain is common in all functional bowel disorders (Mertz 2003), but it is yet unclear whether similar finding could be observed in globus patients.

The aim of this study was to test the hypotheses that globus patients may have

diminished thresholds for stimulatory oesophageal afferent nerve with balloon distension and electrical stimulation, and that the characteristics of viscerosomatic referral of oesophageal stimuli is abnormal in globus patients. Specifically, we postulated that the somatic referral of oesophageal stimuli is larger and more likely to be perceived in the neck in globus patients.

13.2 Methods

#13.2.1 Subjects

Seventeen patients were referred from Gastroenterology and Ear, Nose and Throat Clinics at The St George Hospital with a history of persistent or intermittent non-painful sensation of a lump or foreign body in the throat without dysphagia or odynodysphagia for more than 3 months. The Exclusion criteria included a history of oesophageal motility disorder, nasolaryngeal tumor or surgery, autonomic or peripheral neuropathy, diabetes mellitus, or any other disease or medications that may affect perception of symptoms. Thirteen healthy subjects recruited from campus and paid for their services were similarly studied and served as normal controls. All healthy controls had no oesophageal symptoms and no history of oesophageal or gastric surgery. All subjects gave written informed consent prior to the study, and the study was approved by the Human Ethics Committee of the South-Eastern Sydney Area Health Service.

#13.2.2 Study Design

A perfused multilumen manometry catheter (diameter 5 mm) was adapted so that the latex balloon was incorporated and placed around one infusion sidehole at mid-oesophagus. The balloon was 3 cm in length and had a 3.5-cm maximum diameter at 20-ml air inflation. Figure 13.1 shows the volume-diameter characteristics of the balloon used with stepwise air inflation outside subjects. The same balloon was used for all subjects studied. The physical characteristics of the balloon assembly were tested at intervals during the studies and did not change with time and repeated use. Although in vivo assessment of possible effects of oesophageal resistance deforming the balloon was not performed, previous studies have found little deforming of similar balloons inflated in the oesophagus (Andreollo, Thompson et al. 1988). The bipolar oesophageal pacing electrodes were mounted on the same catheter positioned adjacent to upper and lower surfaces of the balloon around

mid-oesophagus.

The subjects were fasted for at least 4h. The assembly was passed via the nose and positioned such that the most distal side hole was located on the upper margin of the lower oesophageal sphincter (LOS). The position of the LOS in each subject was determined by a station pull through technique and in each subject the center of the balloon was positioned 12 cm proximal to the upper border of the LOS. Subjects were positioned in such a way as to ensure they were unaware of the occurrence or timing of any oesophageal stimulation. The participant sat upright in a comfortable chair throughout the study. The sequence of the different stimulation protocols was randomized.

Stepwise oesophageal balloon distension was performed with 1-ml increments of air. The balloon was sustained for 5 sec (s) and rapidly deflated (< 0.5s). The interval between balloon inflations was varied from time to time to avoid anticipatory effect. The volume at first occurrence of any new sensation in chest (perception threshold) and first occurrence of defined discomfort or pain (pain threshold) was measured. Studies were stopped when the subject reported discomfort. Subjects were asked to quantify the referred somatic pain evoked at pain threshold on a paper with a body map.

Electrical stimulation of the mid-oesophageal mucosa was performed by using one pair of 5-mm stainless steel ring electrodes incorporated into the manometric catheter assembly. The electrodes were connected to an external electrical stimulator and stimulus (duration 200 ms at 0.2 Hz) gradually increased in a step-wise manner in 1-mA increments from 0 - 100 mA. Subjects were asked to report the first perception of any new sensation in the chest (perception threshold) and any definite unpleasant sensation (pain threshold). The electrical stimulation was stopped when the subject reported discomfort. The pacing currents were recorded at these two thresholds. Subjects were asked to quantify the referred somatic pain evoked at pain threshold on a paper with a body map.

#13.2.3 Measurements and Analysis

The drawings were digitized for measurements of the referred pain areas and sites (signal processing toolbox for MALTLAB; The Math Works, Natick, MA, USA). The referred pain areas were normalized with chest area to obtain % of total chest area in all subjects. The group differences in the referred pain areas were assessed by

the Mann-Whitney test. Chi-squared 2 x 2 contingency tables were used to analyze proportional data between groups. A statistically significant difference was considered to exist when p was < 0.05. Mean (SEM) values are shown throughout.

13.3 Results

Nine patients (5 men and 4 women; mean age 35 years; age range, 20- 60 years) and 11 controls (7 men and 4 women; mean age, 32 years; age range, 19-54 years) completed the study protocol. There was no statistically significant difference in age or gender between patients and healthy subjects.

#13.3.1 Perception and Pain Thresholds during Balloon Distension and Electrical Stimulation

Intraoesophageal balloon volume at the onset of perception and pain distinguished the patients and normal subjects, as noted in Figure 13.2. All the patients reported their first sensation (perception) between 2 and 6 ml whereas controls reported their first sensation at a volume between 3 and 14 ml (p = 0.03) (Figure 13.2A). All the patients reported their pain at a volume between 5 and 12 ml whereas controls experienced their pain at a volume between 8 and 20 ml (p = 0.001)(Figure 13.2B). There was no statistical difference in the response to electrical stimulation between globus patients and controls for either perception (p = 0.4) (Figure 13.3A) or pain sensation (p = 0.3) (Figure 13.3B).

#13.3.2 Viscerosomatic Referral during Balloon Distension and electrical Stimulation

Figure 13.4 illustrates the site of viscerosomatic referral during balloon distension and electrical stimulation differed between globus patients and healthy subjects. During balloon distension, seven of nine patients referred sensations to the region at or above suprasternal notch (C-spine dermatome) compared with none of controls during balloon distension (p = 0.001) (Figure 13.4A). Similarly, six of nine patients referred sensations to lower throat compared with one of controls (p = 0.007) during electrical stimulation (Figure 13.4B). There was no difference in the referred pain areas during balloon distension or electrical stimulation between globus patients and controls (all p values above 0.7)(Figure 13.5).

13.4 Conclusion

We report the first study of patients with globus that used different modes of oesophageal stimulation to investigate oesophageal perceptual responses and viscerosomatic referral. We found that globus patients studied exhibited altered perception of aversive oesophageal sensations manifested as lower thresholds in response to balloon distension rather than electrical stimulation. In globus patients the pattern and site of viscerosomatic referral of oesophageal pain was markedly different from that in healthy subjects for balloon distension and electrical stimulation.

There have been many theories for the etiology of globus. Consistent evidence is lacking to attribute globus to any specific motility. For example, UOS mechanics do not seem relevant and the pharyngeal swallow mechanism is normal (Back, Leong et al. 2000, Cook, Dent et al. 1989). Other potential factors such as an urge to swallow with increased swallow frequency might contribute to the symptom by periodically causing air entrapment in the proximal oesophagus (Schatzki 1964). Cricopharyngeal dysmotility has been a possible cause, but manometric studies did not find any supporting evidence (Caldarelli, Andrews et al. 1970). Globus is more common in conjunction with reflux symptoms, nevertheless, a strong relationship between GORD and globus has not been established (Wilson, Heading, et al. 1987). The results of temporal relationship between globus and acid infusion are also controversial (Cherry, Siegel et al. 1970, Cook 1989). Although GOR and oesophageal motility disorders can include globus in their presentations, these mechanisms are believed to play a limited role in the pathophysiology of globus (Chevalier, Brossard et al. 2003, Galmiche, Clouse, et al. 2006, Timon, O'Dwyer et al. 1991). Therefore, it has been suggested that the etiology of globus is likely to have a sensory or perceptive basis rather than a motility basis in the majority of cases (Cook, Shaker, et al. 1991).

The oesophagus receives dual sensory innervations including parasympathetic and sympathetic, whilst vagal and spinal nerves are generally most responsible for these sensory innervations (Gebhart 2000). Vagal afferents with their receptive areas are located in the oesophageal smooth muscle layer are sensitive to mechanical distension while polymodal vagal afferents whose receptive fields in the mucosa are sensitive to a variety of mechanical or chemical stimuli (Christensen 1984). Vagal afferents with their cell bodies in nodose ganglia do not play a direct role in visceral perception inside the gut, however, it has been recently suggested that they may play a role in visceral perception of oesophageal distension (Randich 1993). On the other

hand, spinal afferents, with their cell bodies in the dorsal root ganglia (DRG), are primarily mediating nociception and important to visceral perception of discomfort and pain (Mayer and Gebhart 1994). Spinal afferents with receptive fields in the muscle layer and serosa are also mechanosensitive.

The human oesophagus is sensitive to several stimuli including mechanical (balloon distention), chemical (acid reflux), and thermal stimuli under normal conditions. Oesophageal pain is usually retrosternal in location and could be similar to cardiac chest pain due to convergence of sensory afferents from the heart and oesophagus in the same spinal dorsal horn neuron at the cervical and thoracic levels of spinal cord (Garrison, Chandler, et al. 1992, Qin, Chandler et al. 2004a). In healthy subjects, non-painful distension can produce a pressing sensation in the chest, whereas a high intensity of distention produces pressing, pricking, or warm sensation. Electrical stimulation in human oesophagus can produce pricking, shooting, or warm sensation in the chest (Mehta, De Caestecker et al. 1995, Sarkar, Aziz et al. 2000). These observations clearly suggest that there is a similarity in the nature of sensations to different stimuli of the oesophagus, implying an involvement of polymodal properties of sensory afferents signaling to the central nervous system (CNS).

The evidence of visceral hypersensitivity of the oesophagus in patients with globus has been first demonstrated by Cook et al., who found most of globus patients had heightened thresholds to oesophageal balloon distension (Cook 1989). Similar to these findings, we observed lower thresholds for perception and pain during balloon inflation. Hypersensitivity defined by reduced pain and discomfort thresholds to visceral nociceptive stimuli, has been previously described in non-cardiac chest pain (Richter, Barish, et al. 1986), functional dyspepsia (Mearin, Cucala et al. 1991, Mertz, Fullerton et al. 1998), post-cholecystectomy pain syndrome (Desautels, Slivka et al. 1999) and IBS (Mertz, Naliboff, et al. 1995). Electric stimulation of the viscera which has been applied for studying the afferent pathways from the gut to the brain (Frieling, Enck et al. 1989), unlike balloon distension, allows direct stimulation of both submucosal pain receptors and afferent nerves. The observation of visceral hypersensitivity with balloon distension but not with electrical stimulation, suggesting globus sensation is more likely to be mediated by oesophageal mechanosensive afferent nerves. There are two different types of mechanoreceptors in the mucosal and muscular layers that project to the CNS (Grundy and Scratcherd 1989). It is unclear which type of receptor was more responsible for the current findings, although the

mechanoreceptors for pain perception with oesophageal balloon distension were reported to locate in oesophageal longitudinal muscle (de Caestecker, Pryde et al. 1992, Sengupta, Saha et al. 1990). Previous studies using mucosal anesthesia were able to differentiate the role and location of mechanoreceptor associated with balloon distension (Lang, Medda et al. 2001). Further work with mucosal anesthesia (e.g., lidocaine) may be helpful for obtaining more insight into actual localization of the mechanoreceptor that mediates globus sensation.

Aberrant somatic referral pattern are reported in a variety of functional bowel disorders including irritable bowel syndrome (IBS) (Mertz, Naliboff, et al. 1995), non-cardiac chest pain (Sarkar, Aziz, et al. 2000), functional dyspepsia (Mertz, Fullerton, et al. 1998), and healthy adults under duodenal stimulation (Frokjaer, Andersen et al. 2005). It is assumed that somatic (cutaneous) referral of visceral stimuli is associated with the co-localization of peripheral sensory 'afferent' nerve terminals from the gut and the skin on the same dorsal horn spinal neuron. The brain receives a signal from the dorsal horn neuron in response to visceral stimuli, which is not easily discerned from somatic sensation. The alteration of the receptive fields in dorsal horn neurons was demonstrated in animal models by experimental interruption of nerve transduction connected to the brain (Yaksh, Hua et al. 1999). Such alteration in viscerosomatic referral patterns may represent a model of neural up-modulation inside the property of the nervous system.

In this study, we also observed altered viscerosomatic referral was present in the majority of the patients with globus but not in healthy subjects. The combination of aberrant referral of oesophageal noxious stimuli to cervical dermatomes reported here and in previous studies (Qin, Chandler et al. 2004b, Sarkar, Aziz, et al. 2000, Yaksh, Hua, et al. 1999) suggests the involvement of spinal afferents from oesophagus which could project to cervical spinal cord. The spinal pathway presumably mediates visceral pain with a wide dynamic range (including pain) can account for viscero-somatic referral of pain at the spinal level, and has nerve endings in the muscularis and serosa where visceral pain is triggered. This notion is supported by the fact that blockade of mucosal (generally vagal) receptors with lignocaine (lidocaine) does not block visceral pain due to balloon distension, nor does vagotomy block abdominal pain (Mertz 2003).

The clinical relevance of visceral hypersensitivity to symptom generation and disease severity is yet unclear in globus. The evidence of shifted viscerosomatic

referral of oesophageal pain may suggest that the neuronal modulation of oesophageal visceral hypersensitivity is likely to be extrinsic to the oesophagus and possibly upper level such as spinal pathway, although gobus is defined based on positive sensation of a lump rather than pain in the throat. Treatment targeted on oesophageal visceral afferent traffic may be of potential benefit for the treatment of globus. For example, it has been shown that drugs with block 5-hydroxytryptamine-3 (5-HT-3) or 5-HT-4 receptors may directly reduce the activation of spinal afferents, or indirectly by abolishing enteric afferent neurones which may intact with spinal afferents (Mertz 2003).

The limitation of this study is that we did not determine the reproducibility of sensory thresholds for balloon distension and electrical stimulation. However, the current methodology was similar to the methodology used by Metha et al., who observed fair reproducibility by measuring coefficient of variation (Mehta, De Caestecker, et al. 1995). The other limitation is that the observed patterns of oesophageal perception may be related to response bias due to the fact that patients with globus were more likely to perceive their discomfort at lower degrees of the distension than normal subjects. This type of response bias were reported in functional bowel disease before (Bradley, Richter et al. 1993, Whitehead, Croweil et al. 1994). Altered viserosomatic referral, a parameter generally not influenced by affective sensation experience, was present in the majority of the patients with globus. This observation may make the possibility of responses bias less likely. Moreover, as has been previously reported in the IBS and oesophageal chest pain (Liss, Alpers et al. 1973, Richter, Obrecht et al. 1986), psychological disorders are also reported in patients with globus (Cook, Dent, et al. 1989, Harris, Deary, et al. 1996). This relationship is intriguing and more works need to be done to understand the complex interaction between globus, altered visceral perception, and psychiatric illness.

In conclusion, patients with globus demonstrate visceral hypersensitivity with aberrant viscerosomatic referral. The observation of differential responses to balloon stretch and electrical stimuli suggests globus sensation may be mediated by oesophageal mechanosensitive but not by electro-sensitive afferent nerves. Although the exact role of these oesophageal afferents and receptors on globus awaits further exploration, this study may provide a new insight into therapeutic approaches in patients with globus

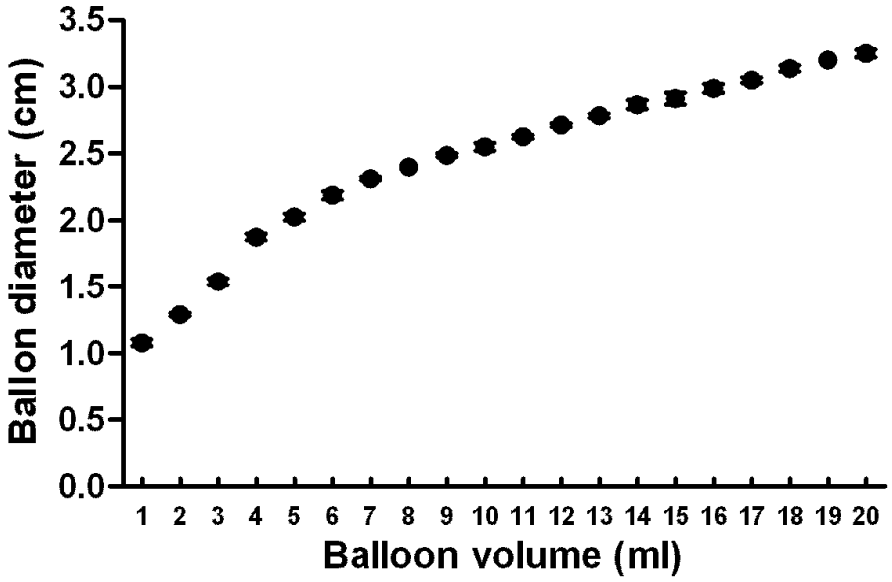
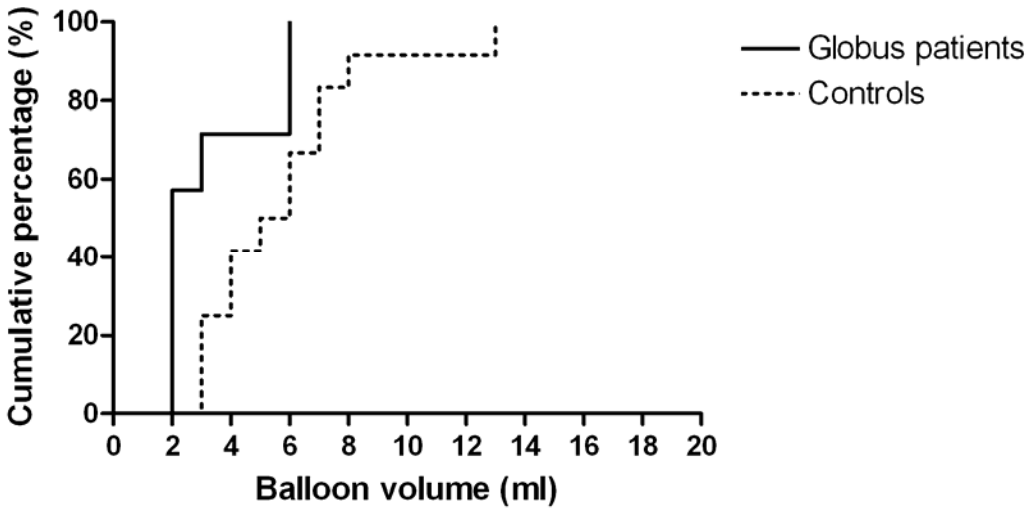


Figure 13.1: Relation of distending volume to diameter of the balloon used in this study.

A



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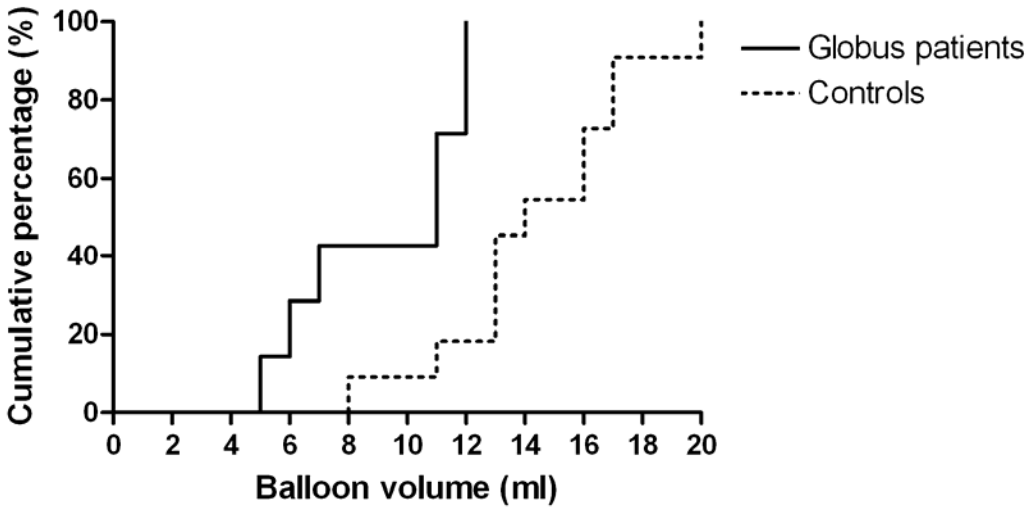
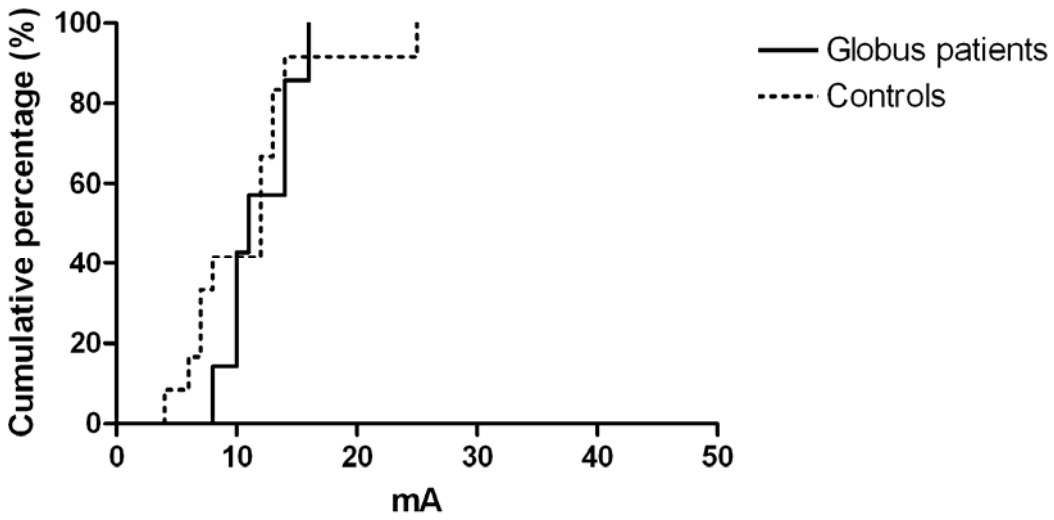


Figure 13.2: Oesophageal perception and pain with balloon distension. (A) All the patients reported their first sensation (perception) at lower volumes than controls (p = 0.03). (B) All the patients reported their pain at lower volumes than controls (p = 0.001).

A



B

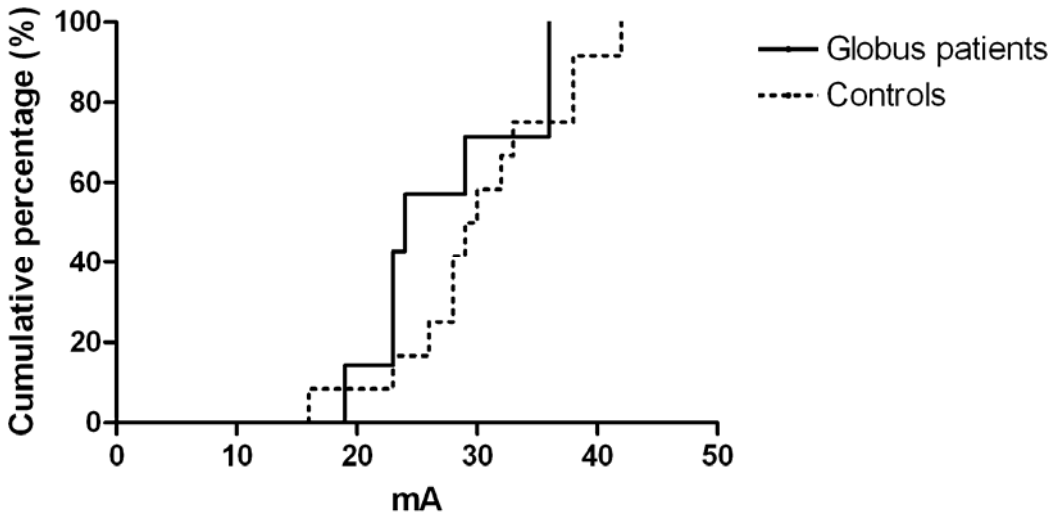
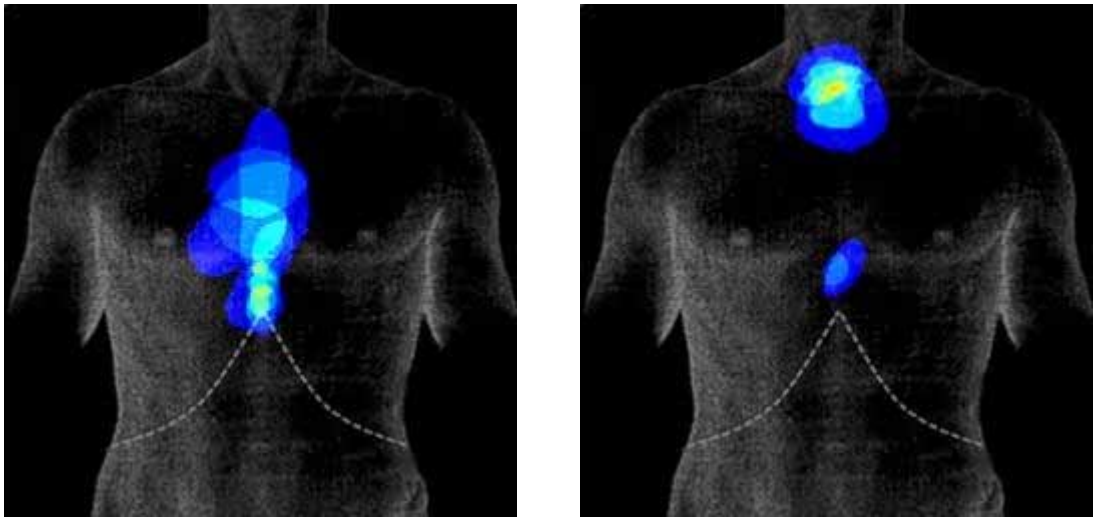


Figure 13.3: Oesophageal perception and pain with electrical stimulation. No group difference was found for either perception (p = 0.4) (A) or pain sensation (p = 0.3) (B).

A



B

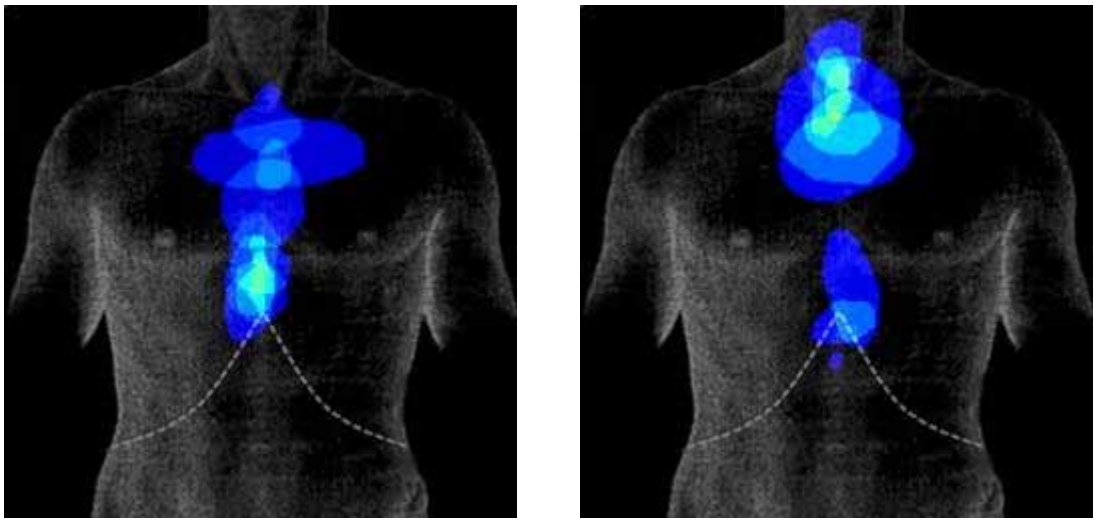


Figure 13.4: Viscerosomatic referral during balloon distension and electrical stimulation. (A) The sites of the somatic referred pain for balloon distension of the oesophagus in controls (left) and patients (right). (B) The somatic referred pain sites for electrical stimulation of the oesophagus in controls (left) and patients (right).

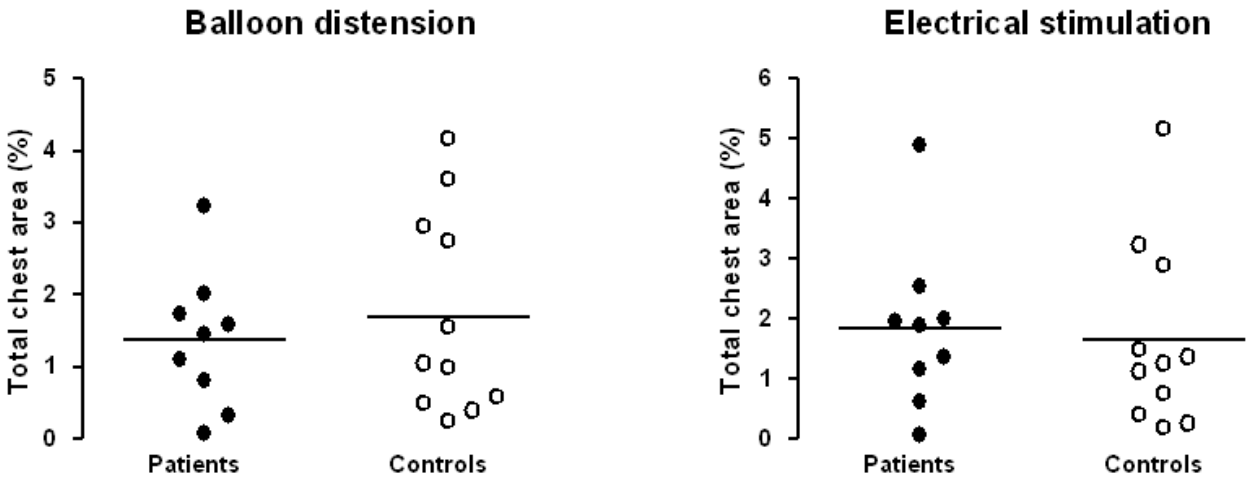


Figure 13.5: The somatic referred pain areas for balloonl distension (left) and electrical stimulation (right) of the oesophagus. Horizontal bars represent the mean referred pain area (in % of total chest area).

SECTION E

PRELIMINARY DEVELOPMENT OF TECHNIQUES TO EVALUATE OESOPHAGEAL MUCOSAL AFFERENT NERVES IN HEALTH AND GORD

Chapter 14

Genetic characterization of TRPV1 in oesophageal mucosa of patients with GORD- a preliminary report

14.1 Introduction

Patients with gastro-oesophageal reflux disease (GORD) commonly report heartburn and pain as their main symptoms. They represent a heterogeneous population, ranging from those with symptoms associated with erosive oesophagitis to those with no evidence of pathological oesophageal inflammation or acid exposure but oesophageal hypersensitivity. The perception of oesophageal sensation is mediated via vagal and spinal afferents, with the latter regarded primarily as nociceptors (Fass and Tougas 2002). Whilst acid-induced pain is likely to be mediated by intraepithelial nerve terminals of spinal afferents, the receptor mediating the symptom of heartburn remains unclear. A potential candidate receptor for mediating this symptom could be transient receptor potential vanilloid subfamily, member 1 (TRPV1). Previous studies have shown increased expression of TRPV1 fibers in human oesophageal mucosa with or without inflammation (Bhat and Bielefeldt 2006, Matthews, Aziz, et al. 2004).

Visceral hypersensitivity has been increasingly recognized to play an important role in the pathogenesis of functional disorders of the gastrointestinal tract. Changes in peripheral nerves or central processing of sensory information may both be altered and lead to discomfort or pain in response to harmless stimuli (Kellow, Azpiroz et al. 2006). Several studies in humans and animals demonstrate that inflammation of the gastrointestinal tract enhances hyperalgesia with mechanical and chemical stimulation (Fass, Naliboff et al. 1998, Lamb, Kang et al. 2003). Those responses are associated with enhanced excitability of visceral sensory neurons, which is at least in part due to changes in the properties and expression of ion channels (Bielefeldt, Ozaki et al. 2002, Dang, Bielefeldt et al. 2004). One of these channels – the capsaicin receptor (TRPV1) – has recently attracted significant attention because of its relevance in nociception (Caterina, Schumacher, et al. 1997). TRPV1 activation in primary afferent neurons evokes the sensation of burning pain and induces neurogenic inflammation by the release of substance P (SP) and calcitonin-gene related peptide (CGRP) (Caterina, Schumacher, et al. 1997). TRPV1 has been localized in the human colon, where its expression is increased significantly in patients with painful

inflammatory bowel disease (Yiangou, Facer, et al. 2001b). In addition, a recent human study is able to link rectal hypersensitivity to excessive expression of TRPV1 in rectum (Chan, Facer, et al. 2003).

The aim of this study examined the mRNA expression of TRPV1 gene and determined the hypothesis that chronic oesophageal inflammation due to acid reflux up-regulated and enhanced the expression of TRPV1 in oesophageal mucosa.

14.2 Methods

#14.2.1 Subjects

The study protocol was approved by Ethics Committee of Tzu Chi Medical Center (Taiwan). The informed written consent was obtained from each subject. GORD patients with heartburn and/or acid regurgitation of at least 6 months' duration were enrolled in the study. Controls were those patients who came for upper gastrointestinal endoscopy for clinical indication other than reflux disease and had no reflux symptoms together with normal endoscopy. Patients with diabetes and patients taking antiepileptic medications and/or oral anticoagulants were excluded. Pregnancy or nursing women were not eligible for the study. Patients were also excluded if they were unable to discontinue acid suppressive drugs, or had positive Helicobacter pylori during the examination.

14.2.2 Symptom Assessment

Before the study, each subject completed a reflux symptom scale. The patients were questioned with regard to the presence and severity of heartburn and regurgitation.. All patients were asked to grade the severity (Visual analogue scale: 0–10; 0 = absent, and 10 = maximal) of the symptoms over the last 4 weeks (Chen, Orr, et al. 2006).

14.2.3 Endoscopy

During the endoscopic examination, two biopsies of the distal oesophagus were taken. Biopsies were taken from between oesophagitis erosions at a fixed position 3 cm above the gastro-oesophageal junction in all subjects to maximize sample consistency. The extent of mucosal damage was assessed using the Los Angeles grading system (Lundell, Dent, et al. 1999). The diagnosis of non-erosive reflux disease (NERD) was based on typical reflux symptoms at least twice a week together

with no endoscopically detectable mucosal lesions such as erosions or ulcers. In additions, NERD patients had ambulatory 24-h oesophageal pH monitoring indicating pathological acid reflux [Johnson LF, J Clin Gastro 1986]. Erosive GORD was defined by the presence of endoscopically detectable mucosal breaks (erosions or ulcer).

14.2.4 RNA isolation and reverse transcription

All subjects underwent endoscopy and had paired biopsy specimens obtained from the gastrooesophageal junction. In order to stabilize and protect RNA in fresh specimens, biopsy specimens were stored in RNA*later*® Solution (Ambion, Austin, Texas, USA) at 4°C. Total RNA was extracted by using MasterPure™ RNA purification kit (Epicentre, Madison, WI, USA) from homogenizing fresh tissue and was transferred to a microcentrifuge tube. Briefly, the tissues were mixed thoroughly with 300 ul of tissue and cell lysis solution containing the 1l of 50 g/l proteinase K, and were then incubated at 65 for 15 minutes (vortex mix every 5 minutes) as well as placed on ice for another 5 min. Adding 150 l of MPC protein precipitation reagent to 300 l of lysed sample and vortexing mix vigorously for 10 sec was following. The debris was pelleted by centrifugation at 12,000 g for 10 minutes at 4 , and then the supernatant was transferred to a new microcentrifuge tube and discarded the pellet. Adding 500 l of isopropanol recovered supernatant, and then inverted the tube several (30-40) times following placing the tube at -80 for 10 min. The RNA was pelleted by centrifugation at 12,000 g for 10 minutes at 4 and the isopropanol was carefully poured off without dislodging the RNA pellet. The pellet was rinsed twice with 1 ml 75% ethanol at 10,000 g for 5 minutes at 4 , and then removed all of the residual ethanol with a pipet with drying the RNA pellet at room temperature for 5 minutes. Finally the RNA was resuspended with 25 l DEPC-H_2O on ice for 30 minutes and quantified by spectrophotometry. All RNA samples were reverse transcribed into cDNA at the same time. The first-strand cDNA synthesis was carried out with using the ImProm-II™ Reverse Transcription System (Promega, Madison, WI, USA). Denatured total RNA (1 μg) was used as a template in a 20-μl cDNA synthesis reaction. The RNA samples were incubated with oligo$(dT)_{20}$ primer (0.1 μg/μl) and random hexamers (0.05 μg/μl) at 70 for 5 minutes and then immediately chilled on ice. Master mix contained (per sample): ImProm-II 1×reaction buffer, 3mM $MgCl_2$, 0.5mM each dNTP and ImProm-II Reverse Transcriptase. Extension was carried out

for 60 minutes at 50 . Finally, reverse transcriptase was thermally inactivated by incubation at 70 for 15 minutes and stored at -20 .

14.2.5 Real-time quantification polymerase chain reaction (qPCR)

The primers used in real-time qPCR were designed using Primer Express Software V2.0 (Applied Biosystems, Foster City, CA, USA) based on sequence information from the National Center for Biotechnology Information database. Homo TRPV1 forward primer, **5'**-GAGTTTCAGGCAGACACTGGAA-3', reverse primer, **5'** -CTATCTCGAGCACTTGCCTCTCT-3'; Homo β-actin forward primer, **5'**-CTCCTCCTGAGCGCAAGTACTC-3', reverse primer, **5'** -CTGCTTGCTGATCCACATCTG-3'. Quantification of cDNA was performed with the iTaq™ SYBR® Green Supermix with ROX (Bio-Rad, Hercules, CA, USA) within ABI PRISM® 7300 Real-time PCR System (Applied Biosystems). Real-time qPCR conditions were: 95 , 10 minutes followed by 40 cycles of 95 , 15 seconds and 60 , 1 minute. Specificity and identity of the PCR product was validated by adding melting curve analysis step into program. The qPCR mixture (total volume 15 μl), which was prepared with RNase/DNase free water, contained 7.5 μl iTaq SYBR Green Supermix with ROX (Bio-Rad), 200 nM of each primer, together with 5 μl cDNA or negative control. The qPCR reaction for each sample was carried out in triplicate for all cDNA and β-actin control. The relative change in gene expression was determined by the fold-change analysis, in which fold change equals $2^{-(\ Ct)}$, where Ct= $(Ct_{TRPV1}-Ct_{\beta\text{-actin}})_{patient}$ - $(Ct_{TRPV1}-Ct_{\beta\text{-actin}})_{control}$. Note that the Ct value is the cycle number at which the fluorescence signal crossed the threshold.

14.2.6 Statistical analysis

We analyzed the data with a commercially available statistical software package (Prism 3.0, GraphPad Software, Inc, San Diego, CA, USA). Analysis of variance with a post-hoc correction was used to compare differences in TRPV1 expression among different groups of the patients and controls. A *p* value of less than 0.05 is defined as significant.

14.3 Results

Fourteen controls (7 women, age 30 ± 2 years) and 30 patients with reflux disease were studied. The reflux patients included 12 with NERD (6 women, age 36.0

± 2.2 years), 11 patients with LA grade A oesophagitis (6 women, 38 ± 2.5 years), and 7 with LA grade B& C oesophagitis (4 women, mean age 40.7± 1.4 years). The severity of reflux symptoms did not differ among the patient groups.

The relative mRNA expression of TRPV1 was significantly greater in patients with LA grade B-C oesophagitis than controls and other patient groups including NERD and LA grade A oesophagitis (Figure 14.1). The relative mRNA expression of TRPV1 was significantly different among controls, NERD patients, and patients with LA grade A oesophagitis (Figure 14.1).

14.4 Discussion

We have shown in this study that reflux patients with severe oesophagitis, i.e., LA grade B &C, have greater gene expression of TRPV1 in oesophageal mucosa compared to controls, mild reflux patients, and NERD patients. Although the present data is still preliminary due to small sample size, our findings are consistent with the hypothesis that chronic oesophageal inflammation due to acid reflux could up-regulate the expression of TRPV1 in oesophageal mucosa. The current results are in the same line with an earlier investigation which demonstrated TRPV1-expressing nerve fibres are present in human oesophagus, and suggested greater TRPV1 expression in oesophagitis patients than healthy controls (Matthews, Aziz, et al. 2004).

In this study, we did not detect any difference in TRPV1 expression between controls and NERD or LA grade A oesophagitis. Despite a positive correlation between oesophageal acid exposure and mucosal TRPV1 expression in NERD patients (Bhat and Bielefeldt 2006), an earlier study did not observe any subtle difference in the expression of TRPV1 between healthy controls and NERD. The reason for this is still unclear, but might be potentially explained by the fact that the clinical spectrum of NERD is heterogeneous and can be further sub-classified into that with and without normal acid exposure (Martinez, Malagon et al. 2003). Further studies in the subclasses of GORD patients may help address this concern.

In summary, our study suggests that TRPV1 up-regulation occurs within the oesophageal mucosa in patients with severe oesophagitis. The observed in increased TRPV1 expression with oesophageal mucosa and its potential for therapeutic target mediating oesophageal inflammation and hyperalgesia may be of substantial value in clinical management of GORD.

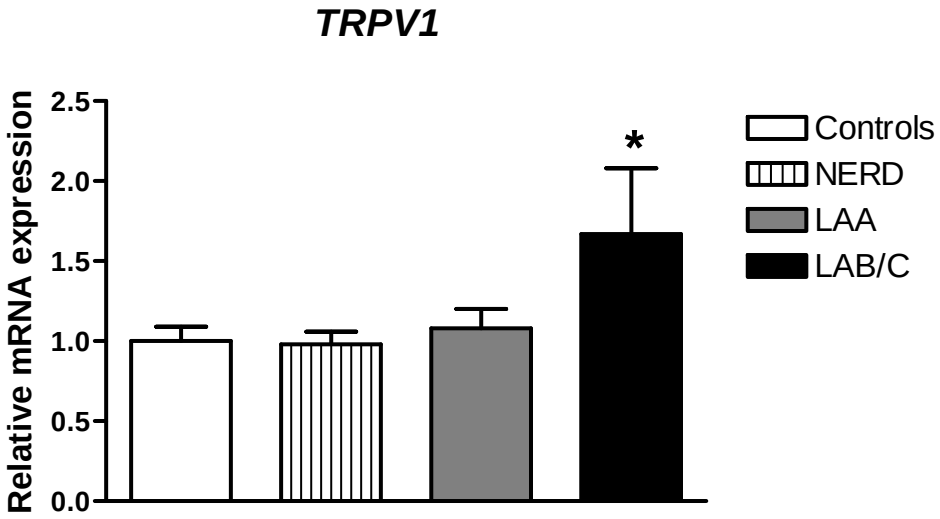


Figure 14.1: Patients with LA B/C oesophagitis had greater mRNA expression of than any other group of the subjects (*$p < 0.01$). The relative mRNA expression of TRPV1 was similar among controls, NERD, and LA grade A oesophagitis.

SECTION F

SUMMARY AND CONCLUSION

Chapter 15

Summary and conclusion

To review the extent to which this thesis has advanced our knowledge of the relationships among oesophageal neural afferent processing, peristaltic function and bolus transport, I will discuss my findings and conclusions in four broad areas:

1. Advances in the application of impedance and its utility in distinguishing clearance characteristics between primary and secondary peristalsis.
2. Advances in our understanding of the relationships among peristalsis, oesophageal bolus clearance and symptom perception in dysphagia syndromes.
3. Peristaltic dysfunction, impaired bolus clearance and symptom perception in patients with gastro-oesophageal reflux disease (GORD) and globus.
4. Clinical implication for transient receptor potential vanilloid subfamily, member 1 (TRPV1) expression in patients with GORD.

15.1 Advances in the application of impedance and its utility in distinguishing clearance characteristics between primary and secondary peristalsis

Combined measurement of oesophageal impedance and pressure allows one to obtain information on both oesophageal peristalsis and bolus transit during the same swallow (***Chapter 3)***. Combined multichannel intraluminal impedance and oesophageal (MII-EM), from the patient's perspective, is no more invasive than conventional manometry. Although the combined technique has been validated previously in the evaluation of primary peristalsis, normative data characterizing bolus clearance by secondary peristalsis is scarce. The first challenge in this work was to apply this novel technique to evaluate the relationship between the motor characteristics of secondary peristalsis and bolus clearance and to compare this relationship between secondary and primary peristalsis.

#15.1.1 Establishment of normative data using combined manometry and impedance to assess oesophageal bolus transit and clearance in a Chinese population

The present study is the first prospective study to provide normal values for a Chinese population using combined MII-EM. Comparing with the data reported in

Caucasians some differences in MII-EM arising in our study are: (i) lower esophageal contraction amplitude and duration occur in our subjects; (ii) our subjects have lower values for total bolus transit time (TBTT), i.e. rapid esophageal transit; and (iii) Chinese subjects with ineffective peristalsis during viscous bolus swallows are more likely to have complete bolus transit (***Chapter 6***). Normal values for impedance parameters in combination with manometry have been recorded in a Chinese population, which will help with standardization of this diagnostic technique for routine clinical use as well as for future research.

However, there are several issues to be resolved before the technique can be rendered more reliable and suitable for routine clinical use. The equipment, particularly the spatial arrangement of impedance segments and pressure transducers should be standardized, since this is the most important factor for the analysis of the relationship between manometric and impedance events during simultaneous monitoring. The impedance values obtained are the results of an integrative change of intraluminal conductivity and cross-sectional area in the whole recording segment, which may differ from actual corresponding pressure transducer. In our study, since the pressure transducer is located between the impedance rings, the timing of the initiation of the contractile wave may not be identical to that of the departure of the bolus tail defined by impedance. Further works would be necessary to clarify whether the location and spatial arrangement of the pressure transducer, i.e., at the end *vs*. between the impedance segments, would affect the data accuracy, or perhaps, based on velocity data, a systematic application of a correction factor might achieve this.

Impedance analysis is time consuming and therefore not appropriate for routine clinical studies. Analysis has since been simplified in light of recent studies which have suggested bolus entry is considered to occur at the 50% point between impedance baseline and nadir during bolus passage, and bolus exit is determined as 50% point on the impedance recovery curve (Tutuian, Vela, et al. 2003). Because of the same level of impedance used for the bolus head (entry) and the bolus tail (exit) regardless of the bolus consistency (liquid and solid boluses), this definition implied that bolus geometry remains constant independent from bolus characteristics. However, fluoroscopic studies do not support this assumption (Kahrilas, Dodds, et al. 1988b). Considering the impedance tracing during a bolus passage, bolus entry is associated with a very rapid drop of impedance; thus, some variation in impedance will not yield significant differences regarding the determination of the bolus head.

Nevertheless during bolus exit, impedance increases slowly, and 5-10% variation will lead to significant differences regarding the determination of the bolus tail. Therefore, constant impedance levels may not be ideal to determine bolus transport under various conditions, particularly for the definition of bolus exit as a parameter for completion of bolus transit (Tutuian, Vela, et al. 2003). This aspect might be explored in the future. Due to the fact that body position might affect the results of bolus transport consequent from the degree of inclination associated with the gravity (Tutuian, Elton, et al. 2003), further studies may be performed with subjects at supine or recumbent position to avoid this gravity affect.

#15.1.2 Secondary peristalsis is less effective than primary peristalsis in oesophageal liquid bolus transport and clearance

This study utilized concurrent impedance and manometry to quantify the relationships among secondary peristalsis and bolus transport as well as effectiveness of oesophageal clearance by this motor pattern. Oesophageal bolus transit time response to secondary peristalsis was comparable to that of primary peristalsis, although complete bolus transit by secondary peristalsis was seen less frequently than that seen during primary peristalsis. In addition, bolus dwell times in regional oesophageal segments were longer during secondary peristalsis than during primary peristalsis.

We are not aware of any published data reporting oesophageal bolus transport by secondary peristalsis. It has been shown that mechanisms regulating the dynamics of bolus propulsion are complex in the oesophagus, and different parts of a bolus have different propulsion behavior (Nguyen, Silny, et al. 1997). For determining bolus clearance of secondary peristalsis (***Chapter 7***), we measured the transit of a bolus tail which is directly induced by a sequence of peristaltic contractions (Kahrilas, Dodds, et al. 1988b, Ren, Massey, et al. 1993). We observed oesophageal bolus transit of secondary peristalsis was similar to that of primary peristalsis. Nevertheless, we found in every regional oesophageal segment that bolus dwell time was longer in secondary peristalsis than that of primary peristalsis. The reasons for these findings were unclear but may relate to several factors such as the integrative aspects of bolus transport resulting from the global traction force of the oesophageal wall, different peristaltic responses between primary and secondary peristalsis, the location of the stimulation, and the existence of pharyngeal pump during swallowing, etc. Furthermore, as bolus

dwell time was determined from the time of bolus entry, the relationship between bolus entry and the start of peristalsis is likely to differ between primary and secondary peristalsis, which may potentially explain for this difference.

The mechanisms underlying impaired bolus transit in secondary peristalsis in response to saline injection remain to be defined, but inferences are possible from the pattern of the manometric responses. Our data suggest that the effectiveness of secondary peristaltic responses is an important determinant of oesophageal bolus clearance by secondary peristalsis. On the other hand, most non-peristaltic responses are associated with incomplete bolus transit. The association of prolonged clearance and defective motor response to intraluminal distension has been noted in previous work done by Kendall et al., who have demonstrated that abnormality of secondary clearance mechanism occurs in oesophageal clearance disorders (Kendall, Thompson, et al. 1987). Our results are substantiated by a recent study which has shown prolonged TBTT occur more with ineffective oesophageal motility (IEM) than with manometrically normal motility (Tutuian, Vela, et al. 2003).

Regarding methodological points, it may be worthy to further investigate whether the attachment of the polyvinyl catheter (for injection of saline or air) would influence the quality of data from the manometry and impedance signals. Since secondary peristalsis was stimulated by rapid mid-oesophageal injections of air (0.5 second) and saline (3 seconds), it would be interesting to document the role of different methodological approaches using different volume (i.e., 20 ml saline swallow *vs.* injection) or location (proximal, mid, or distal oesophagus) for generation of secondary peristalsis due to the previous notion that different regions in the oesophagus have different compliance and distension which might yield different type of response (Patel and Rao 1998). In addition, slow infusion of 20-ml saline within 3 second is likely to produce dispersion of the bolus and less significant distension in the mid-oesophagus, especially since mechanical distension likely triggers secondary peristalsis, whereas a rapid injection could potentially result in localized mechanical stimulation to a higher extent. Thus, the caliber of the injection port would influence and limit the amount of fluid injected, although our studies demonstrated that 13 ml of saline can trigger secondary peristaltic response in all healthy subjects. Further work utilizing a smaller but more rapid infusion could potentially show further differences between normal controls as well as symptomatic subjects with non-obstructive dysphagia. Furthermore, the unique

finding of retrograde bolus movement proximal to the injection site could be of particular interest. I speculated that this retrograde movement may relate to a global motor inhibition of the oesophagus induced by the sudden distension of mid-oesophagus as it reaches the upper part of the oesophagus immediately before the peristaltic contraction starts. Further work will be needed to elucidate this speculation.

15.2 Advances in our understanding of the relationships among peristalsis, bolus clearance and symptom perception in dysphagia syndromes

#15.2.1 Peristaltic dysfunction and impaired bolus clearance may play a limited role in the generation of the symptom of dysphagia

Although motility disorders can be demonstrated in non-obstructive dysphagia (NOD) patients, a significant minority of patients with dysphagia will not show any anatomic or motility abnormality.(Katz, Dalton, et al. 1987). Thus, in spite of some motility abnormalities associated with NOD, it is difficult to understand how actually the observed oesophageal dysmotility will provoke symptom such as dysphagia. To do so, it will be more appropriate to assess the oesophageal function and identify motor abnormalities associated with complaint rather than categorization of motor disorders based on morphology of oesophageal contractions. The novelty of this body of work is the analysis of the relationship between dysphagia perception and motility of individual swallow, as assessed by both manometry and impedance, with both liquid and viscous swallows. Our work has shown that, although oesophageal dysmotility occurs in NOD patients, there is a poor correlation between perception of dysphagia and oesophageal dysmotility in terms of poor contractility and impaired bolus transport (see ***Chapter8***).

The work poses the question whether impaired oesophageal sensitivity might play a role in the genesis of the complaint of dysphagia. Impairment in oesophageal sensitivity has been described in patients presenting with non-cardiac chest pain (Barish, Castell, et al. 1986) or association with dysphagia (Katz, Dalton, et al. 1987). A recent study also showed that oesophageal mechanical hypersensitivity was observed in patients complaining of isolated dysphagia without chest pain by application of oesophageal balloon distension (Bohn, Bonaz, et al. 2002). In addition, the study (Bohn, Bonaz, et al. 2002) did not find any correlation between the sensitivity to distension threshold and the manometric parameters during swallowing.

It was speculated that visceral hypersensitivity may be caused by the alteration in either the biomechanical characteristics or receptors or by impaired afferent neuronal transmission. Furthermore, it is conceivable that such perceptual difference may be caused by impaired cortical perception, inasmuch as decreased sensory perception has been observed at oesophagus in non-cardiac chest pain (Sarkar, Aziz, et al. 2000). These aspects merit further study.

The current investigation seems to be helpful for further understanding the pathogenesis underlying dysphagia in NOD patients. Future works will be needed to determine the exact role of oesophageal sensitivity predisposing to NOD.

#15.2.2 Triggering of secondary peristalsis is impaired, and impedance measures demonstrate impaired bolus clearance by both morphologically normal and aberrant secondary peristaltic sequences in patients with NOD

Evidence exists suggesting that secondary peristalsis may be impaired in patients with NOD (Schoeman and Holloway 1994b). The hypothesis regarding the relationship between such changes and alterations in bolus transport, if any, has not been studied. We investigated whether the triggering of secondary peristalsis and its effectiveness in oesophageal bolus clearance is impaired in patients with NOD. In patients with NOD, it was demonstrated that triggering of secondary peristalsis is less efficient, and impedance measures also reveal impaired bolus clearance by both morphologically normal and aberrant secondary peristaltic sequences (ineffective and synchronous). From the evidence already shown herein (See ***Chapter 9***), current data would suggest that abnormal secondary peristalsis with defective bolus clearance may account for, in part, clinical presentation of dysphagia.

The success or failure of the contractile front regarding bolus clearance is further dependent on the downstream resistance that must be overcome. More specifically, owing to the timed nature of peristalsis, clearance is dependent on the instantaneous intraluminal relationship among clearance force (intra-bolus pressure), closure force (peristaltic amplitude), and outflow resistance. An earlier study (Kahrilas, Dodds, et al. 1988b) combining manometry with fluoroscopy demonstrated that most instances of impaired bolus clearance occurred in the distal oesophagus, suggesting that the downstream resistance from the proximal oesophagus was minimal compared with that at the oesophagogastric junction. Therefore, it is of important to investigate and further quantify secondary peristalsis related bolus flow across this junction. Such

information will be better obtained by the novel technique such as high-resolution manometry (Ghosh, Pandolfino et al. 2006). In addition, it has been recently demonstrated, by high-resolution manometry, that a transition zone existing between the proximal and mid-oesophagus, which has been suggested to play a role in bolus escape leading to abnormal bolus transit and the sensation of dysphagis (Fox, Hebbard et al. 2004). Data acquisition with combined high-resolution manometry and impedance could enhance detailed characterization regarding the function of primary and secondary peristalsis in both healthy subjects and symptomatic patients such as patients with NOD and reflux disease, particularly in respect of bolus escape at the recognized transition zone mid-oesophagus.

It must be emphasized that our patients were highly selected, having been extensively investigated previously. The motor abnormalities demonstrated in this study were clinically significant, but the current findings may not always fit the conventional classification of motility disorders. Nevertheless, our data could provide a pathological basis for functional disturbance and symptoms in patients with NOD. Secondary peristalsis is generally believed to play a role in facilitating the volume clearance of the oesophagus from the ingested material left behind after a swallow or from the refluxate after reflux episodes. Defective bolus clearance by secondary peristalsis found in NOD patients may conceptually lead to a failure to expel the retained bolus downward the oesophagus, and might therefore contribute to the feeling of dysphagia. Even when current findings do not lead to specific treatment, current pathophysiological explanation for the presence of dysphagia may help develop further clinical investigation and management of NOD.

#15.2.3 Clinical implication of current findings in dysphagia syndromes

Based on previous studies, it has been hypothesized that delayed bolus transit, consequent to defective secondary peristalsis, along the oesophagus may contribute to the development of dysphagia (Schoeman and Holloway 1994b). This hypothesis had not been tested since then, and my finding that NOD patients are characterized by abnormal secondary peristalsis and consequently delayed bolus clearance, confirms this hypothesis in patients with NOD. In patients with NOD who were demonstrated to have normal primary peristalsis and abnormal secondary peristalsis, the efferent pathways seem to be intact and this may implicate that there might be a defect in the afferent limb of the reflex pathway with impaired oesophageal sensitivity to

distension.

The other important finding is that, in NOD patients, a poor correlation exits between dysphagia perception and oesophageal dysmotility in term of oesophageal hypocontactility and delayed bolus clearance during primary peristalsis (See ***Chapter 8***). The pathogenesis of NOD might be multifactorial (see ***Chapter 4***), and this work implicate that oesophageal hypersensitivity could be a potential role in the genesis of dysphagia complaint. Therefore, restoring normal sensitivity could be an attractive target for pharmacological interventions in these selected patients. Recent studies suggest that functional brain imaging may provide valuable insights in how certain drugs may modulate the viscerosensory processing at the level of the brain (Mayer, Berman et al. 2002). Such technique may help to select patients for future studies addressing the potentially differential clinical efficacy of visceral modulating agents in hypersensitive vs. normosensitive NOD patients.

15.3 Peristaltic dysfunction, impaired bolus clearance and symptom perception in GORD and globus

#15.3.1 Impaired bolus clearance occurs in reflux patients with or without peristaltic dysfunction

As discussed in **Chapter 11**, impedance can provide physiologically and clinically relevant information in reflux patients with potentially oesophageal dysmotility in whom traditional manometry could provide less definite results. Our findings were similar to a previous study which showed, in GORD patients, these motor abnormalities lead to substantial impairments in oesophageal clearance (Quiroga, Cuenca-Abente et al. 2006). We found that whereas manometry identified motility abnormalities in approximately one-fourth of GORD patients, impedance found that the majority of these, as well as some additional patients in whom manometry results appeared normal, had defective bolus clearance. The fact that none of our asymptomatic subjects had abnormal bolus clearance strongly suggests that the abnormalities we found appear to be highly specific to GORD patients. The ultimate significance of this relatively high prevalence of defective clearance in the pathogenesis of dysphagia or GORD remains to be determined. However, this notion might be partially relevant to the fact that disruption of oesophageal peristalsis affects both volume clearance (Kahrilas, Dodds, et al. 1988b) and delivery of swallowed saliva to the distal oesophageal body. The other abnormality found in patients with

mild oesophagitis was an increased basal impedance gradient (Domingues, Winograd, et al. 2005). This finding suggests that persistence of bolus residues in the distal oesophagus might be a consequence of impaired distal oesophageal motility and underlying prolonged acid clearance. In addition, a recent detailed analysis of the relationship between oesophageal contractions and bolus transit in 70 patients with IEM suggested that oesophageal transit depends on the number of sites with decreased contraction amplitude (Tutuian and Castell 2004a).

#15.3.2 Impaired bolus clearance occurs more in patients with erosive GORD than NERD, whereas peristaltic dysfunction occurs equally between the two groups of the patients

This work has shown that patients with erosive GORD were characterized by delayed oesophageal bolus clearance and increased oesophageal acid exposure, whereas their manometry was comparable to patients without erosive GORD (see ***Chapter 12***). Our data do not definitely clarify the causal interrelationship among delayed bolus clearance, excessive acid reflux, and severity of endoscopy-defined oesophagitis. However, because both groups of patients exhibited greater motor dysfunction than normal controls, the findings would suggest that the noted differences in oesophageal bolus clearance may reflect a continuum of dysfunction consequent to increasing oesophageal mucosal damage which was paralleled by an increase in oesophageal acid exposure.

The manomteric findings in our study are in agreement with a previous work which did not reveal any significant difference in oesophageal motility as investigated by traditional manometry (Lemme, Abrahao-Junior, et al. 2005). We did not notice any difference between both groups in ineffective motility (%), and normal peristalsis (%) during each manometry. These results suggest that IEM alone is unlikely to be the major determinant of pathological acid reflux, i.e., abnormal oesophageal acid exposure, and could not be a prerequisite for the development of oesophagitis. However, data presented from the literature still support a potential association between GORD and IEM (Kahrilas and Pandolfino 2003). Therefore, IEM could be an integral part of GORD, but may not be always associated with oesophageal erosions such as in erosive reflux disease. In the future, it would be of interest to establish casual interrelationships among abnormal bolus clearance, mucosal damage, and excessive acid exposure.

In this thesis we found the prevalence of normal peristalsis was similar between erosive and non-erosive GORD, would it be possible to identify any difference in oesophageal bolus clearance regarding endoscopic reflux severity? We hypothesized that oesophageal motility as determined by combined MII-EM would differ between patients with erosive and non-erosive GORD. We did observe that patients with erosive esophagitis exhibit an increase in the frequency of incomplete bolus transit, which is also accompanied by more prolonged oesophageal bolus transit. As discussed earlier, such difference in oesophageal bolus transit can be explained by differences in oesophageal inflammation and tone. Another important factor associated with impaired oesophageal bolus clearance is the presence of hiatal hernia in GORD. Although we observed a slight increase (without statistical significance) in the presence of hiatus hernia in erosive GORD, it was previously suggested that the efficacy of oesophageal emptying is influenced by peristaltic dysfunction as well as hiatus hernia (Lin, Ke, et al. 1994). The phrenic ampula was the main site of impaired emptying, possibly representing a hiatal hernia and not tubular oesophagus in many cases. This indicates that the presence of hiatal hernia may be a dominant influence on oesophageal bolus clearance (Dent 1999). Further work would be necessary to directly investigate this effect on oesophageal bolus clearance as determined by the impedance technique.

#15.3.3 Evidence for oesophageal hypersensitivity with aberrant visceral referral in patients with globus

As demonstrated in **Chapter 13**, the work confirmed the hypothesis that visceral hypersensitivity with associated aberrant viscerosomatic referral of the oesophageal stimuli is an important pathological mechanism for symptoms in globus. The differential responses to balloon stretch and electrical stimuli may indicate that globus sensation is more likely to be mediated by oesophageal mechanosensitive but not electro-sensitive afferent nerves. The exact clinical implication of our findings is still unclear and to be further investigated in a large number of patients with globus.

The evidence of shifted viscerosomatic referral of oesophageal pain implies that the neuronal modulation of oesophageal hypersensitivity is likely to be extrinsic to the oesophagus and possibly at upper level such as spinal pathway, although globus symptom is defined based on positive sensation of a lump rather than pain in the throat. Therefore, therapy targeted on oesophageal visceral afferent traffic may be of

potential benefit for the treatment of globus. For example, it has been shown that drugs with block 5-hydroxytryptamine-3 (5-HT-3) or 5-HT-4 receptors may directly reduce the activation of spinal afferents, or indirectly by abolishing enteric afferent neurones which may intact with spinal afferents (Mertz 2003). On the other hand, the 5-HT_4 partial receptor agonists tegaserod reduced visceral afferent firing during colorectal distension in cats (Schikowski, Thewissen, et al. 2002). Therefore, application of both 5-HT agonists and antagonists, acting on different 5-HT receptors, are potentially valuable for the treatment of visceral hypersensitivity (see ***Chapter 1***). However, the clinical effectiveness of this type of therapy needs to be further investigated in patients with globus.

15.4 **Clinical implication for TRPV1 expression in patients with GORD**

TRPV1 has been previously recognized as the receptor for capsaicin, the pungent ingredient in red pepper fruits of the genus *Capsicum*. TRPV1 behaves as a multimodal nocisensor of afferent neurones and is hypothesized to be a key player in the hyperalgesia associated with inflammation. Increased expression of TRPV1 has been observed in patients with or without erosive GORD (Bhat and Bielefeldt 2006, Matthews, Aziz, et al. 2004). In agreement with these finding, we also found that reflux patients with severe oesophagitis have greater gene expression of TRPV1 in oesophageal mucosa compared to controls, mild reflux disease, or non-erosive reflux disease (NERD). Although our data are still preliminary due to small sample size, our findings support the hypothesis that chronic inflammation may lead to the release of mediators which may modulate function of primary sensory neurons.

The validity of the concept of TRPV1 as a biological marker in GORD is still unproved. Although longitudinal studies are needed to definitively address this concern, the previous study found a significant relationship between oesophageal acid exposure and innervation density from patients without macroscopic evidence of oesophagitis (Bhat and Bielefeldt 2006). Considering the potential importance of inflammatory mediators in the modulation of structure and function of nerve terminals, it would be interesting to relate microscopic signs of inflammation to the observed changes in mucosal innervations. Experimental inflammation can trigger structural changes of nerve endings which have also been seen in humans, with an increase in TRPV1 immunoreactive fibers in patients with other clinical disorders (Chan, Facer, et al. 2003, Yiangou, Facer, et al. 2001b). Taken together, these findings suggest that

chronic inflammation, even in the absence of macroscopic injury, may lead to the release of mediators which may modulate the structure and/or function of primary sensory neurons.

The role of acid in mediating oesophageal visceral hypersensitivity has been investigated by a previous work, which demonstrated that acid infused into the distal oesophagus can lower pain threshold to electrical stimulation in both the distal and the proximal oesophagus (Sarkar, Aziz, et al. 2000). Such primary and secondary allodynia has been demonstrated in somatic models of pain mediated by an increase in sensitivity of both primary afferents and spinal dorsal horn neurons; it is termed peripheral and central sensitization, respectively. Therefore, in their visceral model of acid-induced hypersensitivity, both peripheral and central sensitization of spinal neurons might be responsible for the primary and secondary allodynia observed. Given that in this model, acid acted as the noxious mucosal stimulus for peripheral sensitization of afferent neurons, which drove the central sensitization, it is possible that TRPV1 might act as a biological marker of peripheral sensitization in a subset of functional oesophageal disorders characterized by oesophageal hypersensitivity. It would be of interest to do this investigation with a combined biological and physiological approach in order to identify whether there is a significant association between visceral hypersensitivity and increased TRPV1 expression. If this hypothesis can be proved, TRPV1 channel blocker may be of substantial value in reducing GI discomfort that arise from upregulation and sensitization of TRPV1 in gut. Furthermore, this approach may also help elucidate its role in sensitization of visceral afferents in susceptible individuals such as functional heartburn and non-cardiac chest pain.

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APPENDIX

Comments of Reviewer 1; responses and modifications to thesis

1. Though there appear to be no significant errors in Section A (chapters 1-5), I find it too detailed on some matters, e.g. pages 5-9 on extrinsic and intrinsic innervation. The same accounts for the section on neuropharmacology where a table…The literature review is quite extensive but this thesis is focused on the oesophagus and it is difficult to know data obtained in other parts of the GI tract apply the to the oesophagus This especially relevant to finding on the different types of mechanoreceptors (IGLEs, IPANS, etc).

Response: Appropriate corrections are made according to the suggestions of the reviewer. One of the problems in understanding oesophageal innervation is the relative lack of studies specifically in this organ, at least in comparison with other regions of the gut. Hence, a reasonably comprehensive description of the immunohistochemistry and neuropharmacology of other regions has been given to indicate, by analogy, the possible or likely structure and function of the oesophageal afferents where direct evidence is lacking. Nonetheless, I have abbreviated a lot of the material on neuropharmacology and mechanoreceptors, and I have made these data more specific and simplified. Note: 1. Page 5-8, extrinsic and intrinsic innervations. 2. Page 10-13 (top 2 paragraphs)[neuropharmacology].

2. Page 2, Line 9. Findings dating back 14 years can hardly be said to be recent.

Response: I have corrected this statement (Page 2, Line 12) to read:
"Attention has been paid to the role of visceral sensitivity in the pathophysiology of functional gastrointestinal disorders (Mayer and Gebhart 1994)."

3. The evidence in Zagorodnyuks papers on IGLEs reacting to active and passive tension is not fully convincing.

Response: I have replaced the reference with *Zagorodnyuks and Chen et al, J Physiol 2003.* Note: Page 5, 6-7 from the bottom.

4. Figures. In each chapter the figure numbering starts again from figure 1. I suggest to make them consecutive or to incorporate the chapter numbers..

Response: Figure numbering revised as suggested

5. The candidate may want to check the thesis for misspellings. Examples are page iv (acknowdgements), page vi …

Response: Thesis has been proof read again and corrections extensively made.

6. The thesis can be more consistent with respect to the use of abbreviations (abbreviate the first time in the main txt…)

Response: Abbreviations minimized and format revised as suggested throughout.

7. The extensive description of the many oesophagus-related reflexes on page 36-44 seems too long……. I suggest shortening the text.

Response: This description has been shortened. Note: Page 37-40 (first paragraph).

8. Page 60. Sinyl et al was not the first to describe intraluminal impedance in 1991….Other have used impedance measurements to evaluate flow before Sinyl..

Response: I have replacing it with *Fisher MA and Hendrix TR et al, Gastroenterology 1978. (*Note: Page 56, Line 6-7 from top).

9. I do not think that the very brief text in chapter 5 justifies a separate chapter. Consider to implement it into chapter 4.

Response: Ch 5 now incorporated into Ch 4. (Note: Page vi, Line 6 from the top).

Comments of Reviewer 2; responses and modifications to thesis

1. Language, grammar and spelling: Overall, the thesis is well organized and well written. However threw are numerous grammatical and typographical errors.

Response: Thesis has been proof read again and the grammatical and typographical errors have been extensively corrected.

2. Figures and figure legends: please label the figures clearly and describes the legends. Please reference the figures and tables appropriately as you are describing the text.

Response: I agree that some of figure legends have been revised extensively to truly and clearly reflect the message from the respective figures and to ensure that this message is relevant to the main text.

3. Please consider writing review articles pertinent to this thesis topic.

Response: I plan to continue my research in this area in Taiwan and hope to find the time to write a comprehensive review of this area.

4. Within the chapters, consider bold headings and sub-headings.

Response: As suggested, each chapter has been revised in such manner.

5. Please highlight the importance of chapter 6 in the introduction. This chapter provides a comprehensive overview of the entire work.

Response: With the amalgamation of chapters 4 & 5, chapter 6 becomes chapter 5. In fact, the importance of Chapter 5 (Aims and Hypotheses) has been addressed and incorporated into " Summary". Note: Page xii – xv.

6. Abbreviations are too many and are unavoidable. For clarity, please expand the applied abbreviation at least once in each section.

Response: This suggestion has now been applied throughout the thesis.

7. Reproducibility of analysis may be considered and coefficient of variation reported. This will improve the confidence of the methods and tests.

Response: True, I agree with the concern from the reviewer. However, since most of the thesis work has been published. We would perform such analysis in the future work.

8. Describe the clinical characteristics of controls and patients with reference to the scientific appropriateness for their participation. In some respects, data are similar between the groups. I wonder if there are any underlying clinical similarities.

Response: My healthy volunteers were recruited, by advertisement, from the community and university campus. All volunteers were interviewed carefully in order to be sure that they had no prior or current gastrointestinal symptoms or disease. All the patients were appointed after thoroughly reviewing their suitability for the study. Although some measures did not differ between patients and controls, I believe the eligibility and exclusion criteria for both groups was sufficiently robust not to attribute any lack of differences to occult disease confounding the data from controls.

Comments of Reviewer 3; responses and modifications to thesis

1. A limitation of this thesis is the fact that high resolution manometry was not

utilized to evaluate esophageal peristalsis. With high resolution manometry, esophageal contraction segments have been clearly identified. A trough of low pressure has been identified between the proximal skeletal muscle contraction and the distal smooth muscle contraction segments. Recent reports have focused on an extended or lengthy trough between the skeletal and smooth muscle contraction segments as potentially playing a role in bolus escape contributing to abnormal bolus transit and the sensation of dysphagia.

Response: I agree that high resolution manometry would potentially provide additional insights into mechanisms and prevalence of bolus escape at this transition zone described by the reviewer. Although during my work co-workers in the laboratory did develop combined impedance/manometry for preliminary validation studies in the pharynx, my work was well advanced using standard techniques by the time the newer technique might have been feasible to adapt to my work. However, as suggested, the discussion has been re-stated according to this reviewer's suggestion. *"In addition, it has been recently demonstrated, by high-resolution manometry, that a transition zone existing between the proximal and mid-oesophagus, which has been suggested to play a role in bolus escape leading to abnormal bolus transit and the sensation of dysphagis (Fox M, Neurogastro Motil 2004). Data acquisition with combined high-resolution manometry and impedance could enhance detailed characterization regarding the function of primary and secondary peristalsis in both healthy subjects and symptomatic patients such as patients with NOD and reflux disease, particularly in respect of bolus escape at the recognized transition zone mid-oesophagus."* Note: Page 208, first paragraph.

2. Another area of minor concern is the fact that a slow (3 second) 20 mL infusion into the mid esophagus was used for triggering of secondary peristalsis. The slow speed of infusion could have contributed to dispersion of the bolus and less significant distension, especially since mechanical distension likely triggers secondary peristalsis. …… Utilizing a smaller but more rapid infusion could potentially have demonstrated further differences between normal controls and subjects with non-obstructive dysphagia.

Response: The advice is acknowledged in the discussion. I add this statement in the discussion, *"In addition, slow infusion of 20-ml saline within 3 second is likely to produce dispersion of the bolus and less significant distension in the*

mid-oesophagus, especially since mechanical distension likely triggers secondary peristalsis, whereas a rapid injection could potentially result in localized mechanical stimulation to a higher extent. Thus, the caliber of the injection port would influence and limit the amount of fluid injected, although our studies demonstrated that 13 ml of saline can trigger secondary peristaltic response in all healthy subjects. Further work utilizing a smaller but more rapid infusion could potentially show further differences between normal controls as well as symptomatic subjects with non-obstructive dysphagia." **Note: Page 205, Line 1-10 from the bottom.**

3. Abbreviations: Since multiple abbreviations were used throughout the thesis, only having a key at the beginningof the thesis was insufficient. Full forms of the less common abbreviations in each chapter (when the abbreviation occurred for the first time in the text) would have been ideal.

Response: Abbreviations have been thoroughly revised – see above.

4. Typographical errors:

Response: I thank the reviewer for taking the time to point out these errors (not listed again here for brevity); all of which have now been rectified. The entire thesis has been proof read again and further corrections made that will enhance its readability.