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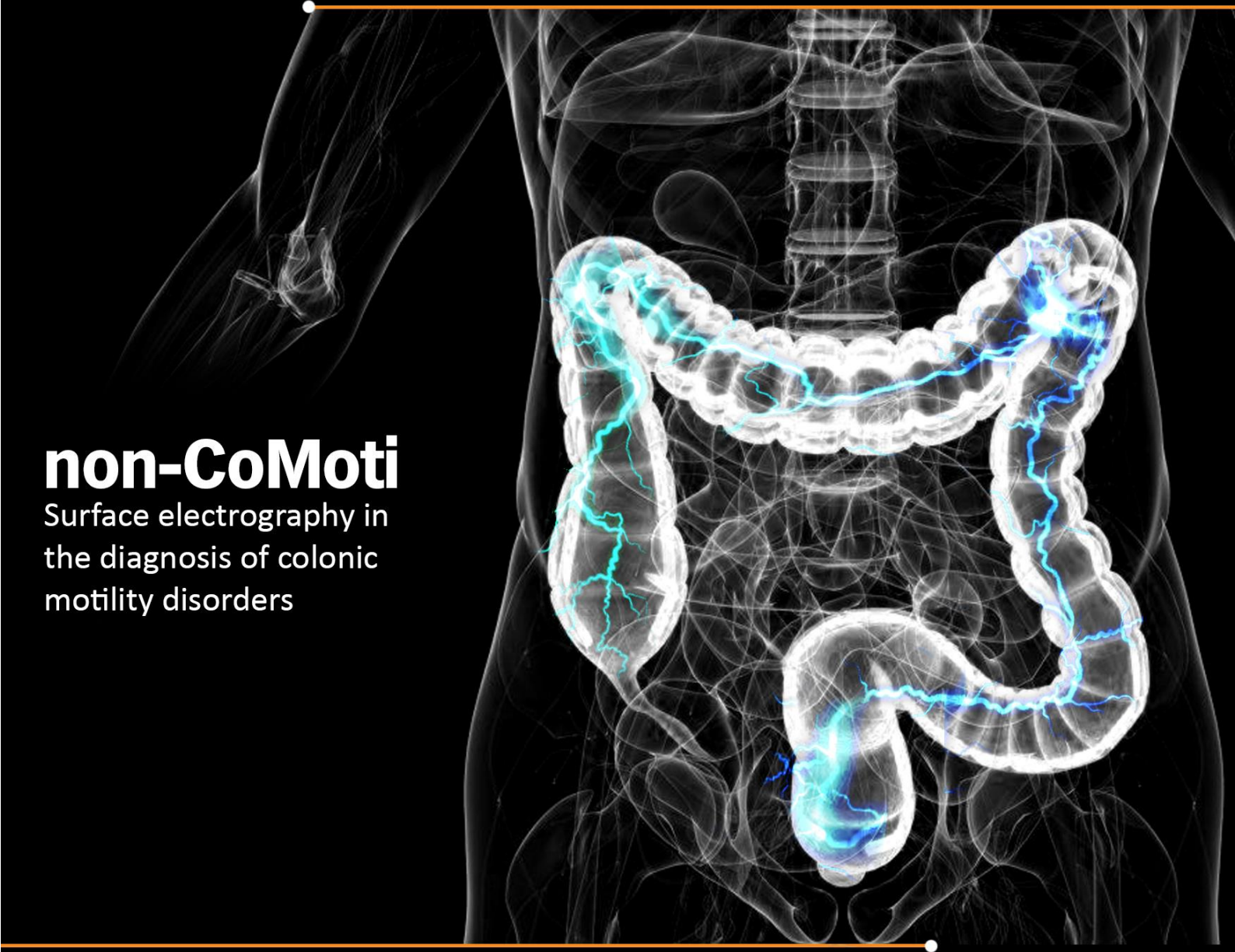

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Surface electrography in the diagnosis of colonic motility disorders

Master Thesis - Technical Medicine
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Abstract

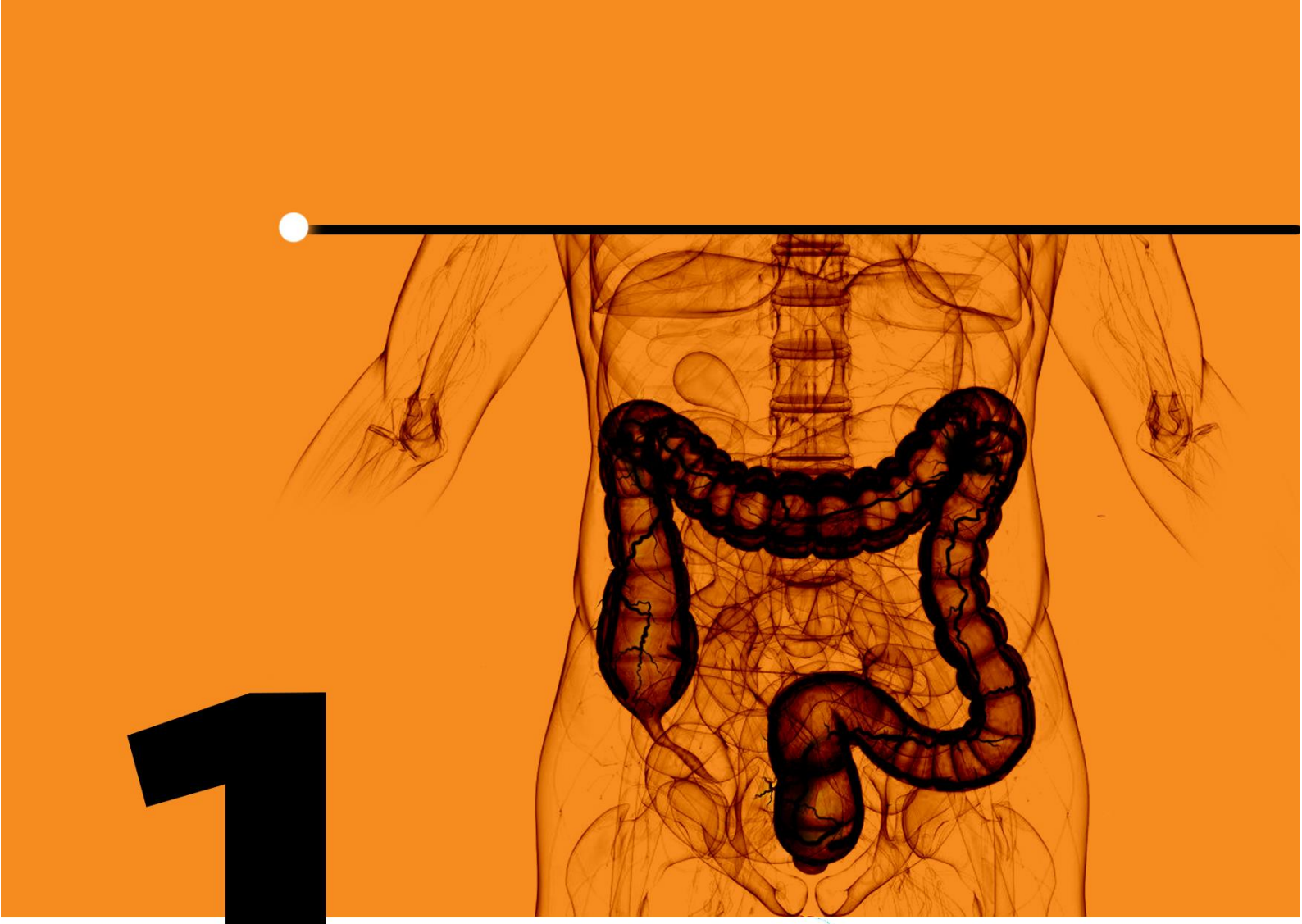
Introduction Colonic motility disorders affect many adults as well as children, but diagnostic procedures are often invasive, require complex skills and are not always conclusive.

Aim The aim of this study is fourfold. First, it was investigated whether surface electroenterography (sEEnG) could measure the gastrocolic reflex in healthy adults. Secondly, interference from gastric activity on sEEnG measurements at the sigmoid was investigated. Next, differences between sEEnG recordings of healthy and constipated subjects were studied and finally, the experience of the sEEnG procedure was compared with the experience of the gold standard procedure which was used to diagnose the patients initially.

Methods For the first two aims, healthy adults aged 18 years and older were included, whereas for the third and fourth aim, healthy subjects and subjects suffering from a colonic motility disorder aged 18 years or older or 11 years or younger were included. In all subjects, sEEnG recordings in both pre- and postprandial states were performed. The primary outcome for the first and third aim was the postprandial power increase. For the second aim, differences and coherence between measured gastric and sigmoidal activity are determined. For the final aim, patients and/or their parents completed custom-made questionnaires regarding the sEEnG procedure as well as the procedure with which the patients were initially diagnosed. All values are presented as median with interquartile range.

Results In ten healthy adults, postprandial power was significantly larger than preprandial power, 307 μV^2/cpm (186 – 450) vs. 704 (437 – 1,458), $p = 0.013$. In six other healthy adults, no differences in dominant frequency, amplitude and power between gastric and sigmoidal activity were found. Nevertheless, relevant interference from gastric activity on the sEEnG signal at the sigmoid was also not observed. Comparison of sEEnG recordings between four healthy and seven constipated children showed no significant differences, although preprandial power tended to be higher in constipated children (2,270 μV^2/cycles per minute (cpm) (318 – 8,356) vs. 494 μV^2/cpm (265 – 2,670), $p = 0.07$). In contrast, relative postprandial power increase tended to be lower in constipated children (26% (-37 – 324) vs. 283% (104 – 699%, $p = 0.13$). In general, the sEEnG procedure was experienced more positive than the gold standard procedure (9 (8.5 – 10) vs. 6.5 (5 – 7.5), $p = 0.046$).

Conclusion The gastrocolic reflex can be measured well using sEEnG recordings in healthy subjects and interference from gastric activity is not an issue. There seem to be differences between healthy and constipated children, although further study is necessary to correctly interpret these differences. Nevertheless, sEEnG shows potential to play a future role as a non-invasive, easy to use and readily available diagnostic tool for colonic motility disorders.



1

INTRODUCTION

1. Introduction

When Sven was just 5 days old, he had to undergo intestinal emergency surgery because he vomited bile and his abdomen was distended. However, no cause for his problems were found. After surgery, things seemed to improve, but after starting with solid food, his problems rapidly increased. Sometimes, Sven did not defecate for 10 days straight and then defecated continuously for the next 2 days. After being referred to a gastroenterologist by the pediatrician, a lot of diagnostic procedures were performed to find the cause of Sven's problems. However, these procedures could not reveal the underlying cause of the problems. A rectal biopsy was postponed each time due to its invasive nature and because it could only be performed at an academic center. Finally, after three months of worrying, multiple diagnostic procedures and waiting for results, rectal biopsy was performed and showed that Sven suffered from Hirschsprung's disease. After surgery, in which the afflicted part of the colon was removed, Sven finally became quite the happy baby who could eat and defecate just like other children.

The story of Sven, unfortunately, is not just a single story, but a reality for a lot of patients who are suffering from gastrointestinal motility disorders. A recent multi-national, survey-based study found that among 73,076 adult respondents, up to 40.3% met the criteria for having at least one functional gastrointestinal disorder [1]. In children, functional constipation comprise 1-5% of pediatric consultations and 25-30% of referrals to pediatric gastroenterologists [2]. Rouster et al. even reported that more than two thirds of the newly presented pediatric gastrointestinal clinic patients met the Rome III criteria for having a functional gastrointestinal disorder [3]. Such disorders are often but not exclusively accompanied by difficulty with defecation, abdominal pain, malnutrition and increased risk of infection and may even result in early mortality [4, 5]. For adequate treatment of gastrointestinal motility disorders, a correct diagnosis is paramount. However, gastrointestinal motility disorders are often misunderstood and current diagnostic tools do not always suffice in providing a clear-cut diagnosis [6].

An important factor impeding an adequate diagnosis is the fact that just 5% of all gastrointestinal motility disorder have an organic etiology [2]. This means 95% of all gastrointestinal motility disorders are all functional in nature and no anatomical or biochemical abnormalities can be found. Functional gastrointestinal motility disorders comprise, among others, gastroparesis, chronic intestinal pseudo-obstruction, slow-transit constipation, functional constipation and rectal outlet obstruction. Assessment of these disorders often involve multiple diagnostic tools, including clinical history, physical examination, scintigraphy

studies, transit studies using markers and x-rays, gastro- or colonoscopies and esophageal or anorectal manometry [5-7]. However, many of these diagnostics are invasive, require complex skill or are painful and traumatizing and cannot always provide an adequate diagnosis.

For gastrointestinal motility disorders with an organic etiology, nevertheless, providing a correct diagnosis is also challenging. The most prevalent of these disorders is Hirschsprung's disease (HSCR), a congenital disorder characterized by partial absence of colonic nerve cells in the distal colon leading to impaired colonic motility [8]. The gold standard in diagnosing HSCR is a rectal biopsy [9]. This is a transanal procedure and can be performed at the outpatient clinic, but for children older than six months, it is often performed under general anesthesia [10]. Even though a rectal biopsy is the gold standard for the diagnosis of HSCR, several disadvantages are present. Firstly, rectal biopsies often need to be performed under general anesthesia, which especially in young children should be avoided whenever possible [11]. Furthermore, a recent review stated that in 8-26% of cases, specimens obtained with rectal biopsy were of insufficient quality and had to be reperformed [10]. This does not only result in a diagnostic delay and increased parental anxiety, but also another procedure under general anesthesia is often necessary [11, 12]. Thirdly, rectal biopsies can only confirm if aganglionosis is present, but cannot determine the extent of affected colon, which is important for expectation management as well as for planning the surgery [8]. Finally, rectal biopsies are only performed in certain centers in the Netherlands and may thus also contribute to a delayed diagnosis due to increased waiting time.

Although HSCR is a rare condition, with an incidence of 1 in 5.000 live births, rectal biopsies are performed quite often [4]. A recent study has shown that only 8.3% of the patients who underwent a rectal biopsy, as indicated by the U.K. National Institute for Health and Clinical Excellence, did actually suffer from HSCR [13]. This means that more than 90% of the patients underwent this procedure without getting a diagnosis. Nevertheless, these patients still exhibit signs of a motility disorder and have an indication for further diagnostic testing. Therefore, the invasiveness, limited efficacy and time-consumption of current gastrointestinal diagnostic procedures advocate for a less invasive and readily available method for the diagnostics of HSCR and other motility disorders of the colon.

Surface electroenterography (sEEnG), a non-invasive and easily accessible tool, may be such a gastrointestinal diagnostic method. During an sEEnG procedure, several surface electrodes

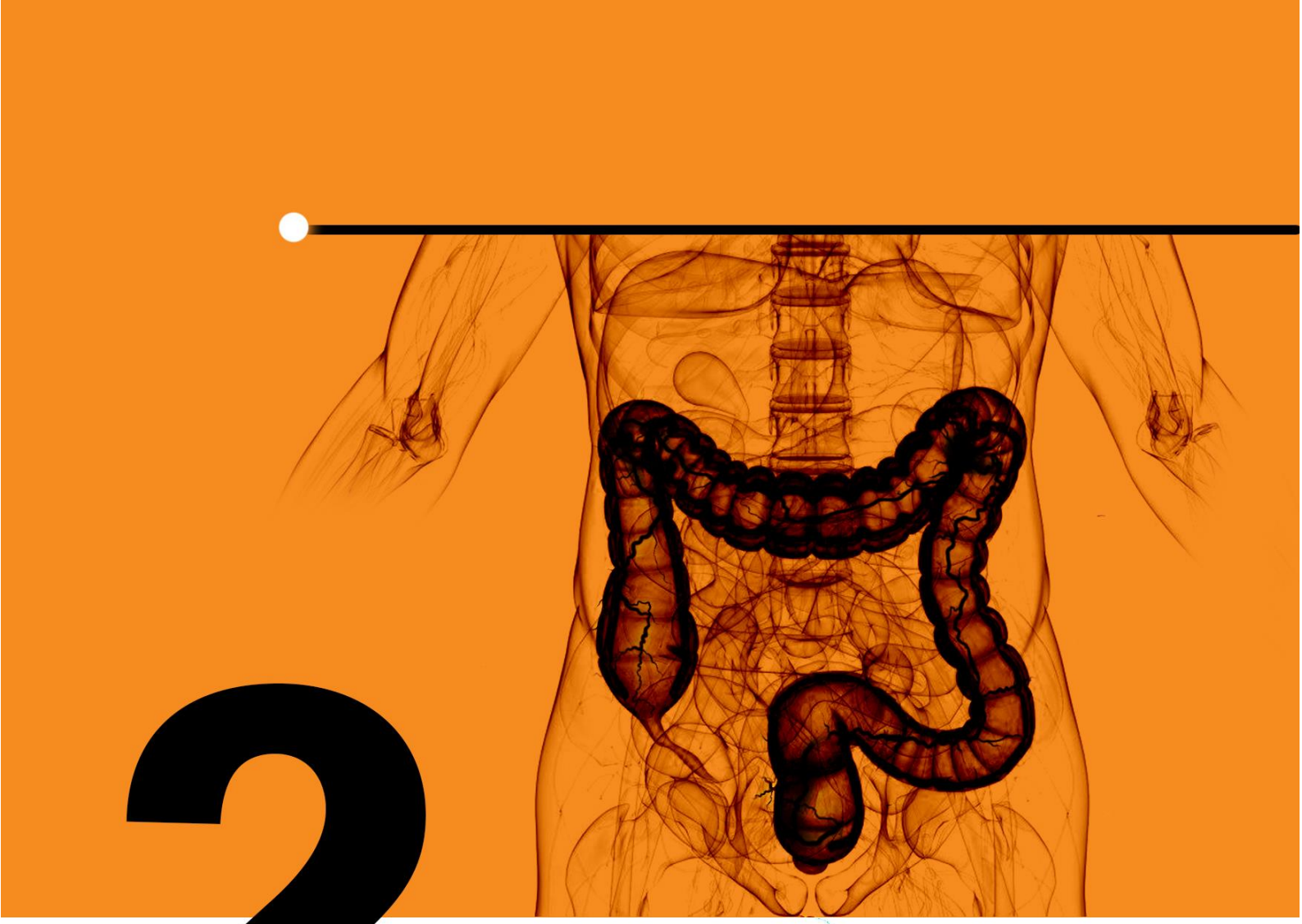
are placed on the abdominal skin close to the colon to measure colonic activity [14]. This method, however, has not been investigated extensively yet. Evidence of sEEnG being able to differentiate between healthy subjects and patients suffering from colonic motility disorders has therefore not been well established. In addition, not much is currently known about the possible interference of gastric activity, having the same characteristics as colonic activity, on the sEEnG signal. To investigate the potential of sEEnG as a diagnostic method for colonic motility disorders, this thesis aims:

1) *To study whether surface electroenterography recordings are able to measure colonic activity in healthy adults.*

2) *To study whether gastric electrical activity interferes with surface electroenterography measurements at the sigmoid colon in healthy adults.*

3) *To study whether surface electroenterography can detect differences in colonic activity between healthy subjects and patients suffering from a colonic motility disorder in both adults and children.*

4) *To compare the experience of patients and/or their parents between the surface electroenterography procedure and the gold standard diagnostic method.*



2

BACKGROUND

2. Background: Colonic motility and electrography in relation to Hirschsprung's disease

2.1 Anatomy

The colon is one of the last parts of the digestive tract. Its functions are to absorb water and fatty acids from indigestible residues of the liquid chyme and to serve as temporary storage of feces before leaving the body via the rectum and anus. The colon is situated in the abdominal cavity and can be divided into four parts: the ascending, transverse, descending and sigmoid colon. The ascending colon lies retroperitoneal and passes superiorly from the right lower quadrant of the abdomen to the right lobe of the liver. Here, it changes direction to the left at the hepatic flexure and is now called the transverse colon, located intraperitoneal. The transverse colon traverses to the left upper quadrant of the abdomen and changes direction to caudal at the splenic flexure, retroperitoneally continuing as the descending colon. In the left lower quadrant of the abdomen, the descending colon makes an intraperitoneal S-shaped turn, known as the sigmoid colon, and connects to the rectum [15].

Wall lining

The wall of the colon consists of several layers, from inside to outside: the mucosa, submucosa, muscularis externa and serosa, see Figure 1. The mucosa is built up of epithelial cells, connective tissue and the muscularis mucosae. Its main functions are to resorb water and ions and to produce mucus to ensure smooth passage of colonic contents. The muscularis mucosae also enables the mucosa to move independently to a certain extent, optimizing contact between the colonic wall and colonic contents. Furthermore, the muscularis mucosae also prevents perforation of the mucosa by contracting upon sharp objects touching the colonic wall. This reflex is controlled by Meissner's plexus, which is located in the submucosa. The submucosa also holds connective tissue, which is rich in blood- and lymph vessels. The muscularis

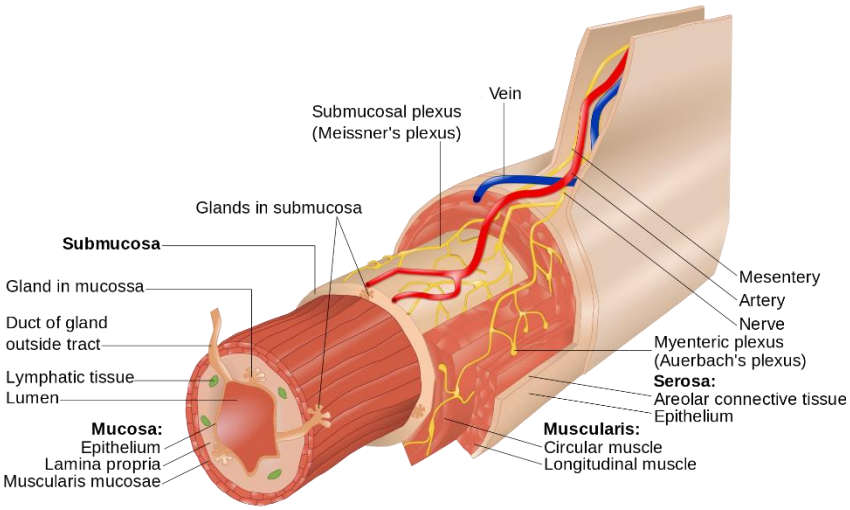


Figure 1. Anatomy of the colonic wall [16].

externa, surrounding the submucosa, is comprised of two layers: a thicker, circular and a smaller, longitudinal smooth muscle layer. In between these layers, Auerbach's plexus is located, which is responsible for the coordination of the contraction of the muscle layers. The pacemaker cells of colonic muscular activity, however, are the interstitial cells of Cajal (ICC), located within the muscle layers. The outer layer, the serosa, is comprised of connective tissue containing blood- and lymph vessels and is lined with a layer of mesothelial cells [17].

Blood supply

Arterial blood supply of the colon is provided by the superior and inferior mesenteric arteries, both branches of the aorta. The superior mesenteric artery runs in the root of the mesentery and ramifies into the right and ileocolic artery supplying the ascending colon and into the middle colic artery supplying the transverse colon. The descending and sigmoid colon, on the other hand, are supplied by the inferior mesenteric artery, which branches into the left colic artery and sigmoid arteries. In addition, the inferior mesenteric artery also supplies the proximal part of the rectum, whereas the midrectum and distal rectum are supplied by branches of the internal iliac and internal pudendal artery respectively. Venous drainage of the colon corresponds to the pattern of arterial supply to the colon and the superior and inferior mesenteric veins drain on the hepatic portal vein, transporting the venous blood to the liver [15].

Nerve supply

Colonic activity is primarily controlled by a neural entity called the enteric nervous system (ENS). Neurons of the ENS are mainly situated in either Meissner's or Auerbach's plexus in the intestinal wall and are only found in the gastrointestinal tract. As the ENS has both sensory and motor properties, it is a unique system and can mediate behavior independently of the central nervous system (CNS). However, it can still be modified by input from the brain through the vagal and pelvic nerves [18].

Similar to arterial supply, nerve supply from the CNS to the colon varies for different parts of the colon. The ascending and proximal two-thirds of the transverse colon receive their sympathetic nerve supply from nerves branching off the superior mesenteric plexus. Parasympathetic nerve supply to this region is provided by the vagus nerve. The distal part of the colon, consisting of the distal third of the transverse, the descending and sigmoid colon is

sympathetically innervated by the inferior mesenteric plexus, while parasympathetic innervation is supplied by the pelvic splanchnic nerves. Sympathetic nerve fibers act as inhibitors on colonic activity, whereas parasympathetic fibers are stimulatory [19].

2.2 Physiology

Motor activity of the colon can be divided into three types: churning, propulsion and acting as a reservoir. In the proximal colon, segmental contractions result in increased mixing (churning) of colonic contents and one to three times a day shows mass peristalsis (propulsion). This mass peristalsis is a result of progressive slow waves of relaxation followed by contractions and may be initiated by eating through the gastrocolic reflex. In the distal colon, motor activity consists primarily of churning by segmental contractions. For the distal colon to act as a reservoir, the anal sphincters contract until colonic content is propagated to the rectum by mass peristalsis, after which the sphincters relax, leading to defecation [18].

Churning and propagating colonic content is facilitated by the ICC, the ENS and the smooth muscle cells. Of these components, the ICC initially generate slow wave activity with a frequency of 2-4 cycles per minute (cpm) [20]. This slow wave activity is a function of the membrane voltage (V_m) of the smooth muscle cells. The origin of these slow waves is similar to that of action potentials in skeletal and cardiac muscle, but polarization of the cell is mediated by voltage-gated Ca^{2+} channels instead of Na^+ channels. As Ca^{2+} channels open more slowly than Na^+ channels, de- and repolarization of the muscle cells is also slower, resulting in slow waves lasting several seconds, see Figure 2. These slow waves may or may not reach action potential threshold, resulting in slow wave generation either with or without high frequency action potentials superimposed on them [18]. However, ICC are not able to effectuate coordinated contraction leading to churning or propulsion on their own. For these contractions, the ENS

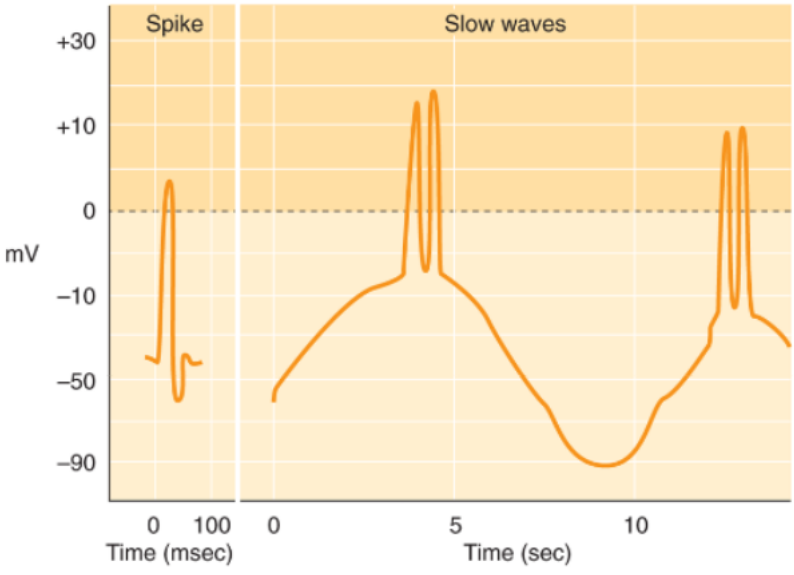


Figure 2. Left: fast action potential (spike) of skeletal muscle. Right: slow waves, as seen in colonic smooth muscle cells [18].

is essential as regulator of widespread coordination and modulation of amplitude and frequency of the contractions of the smooth muscle cells. Furthermore, the gap-junctions between colonic smooth muscle cells also assist in spreading activity to neighboring cells [21].

Gastrocolic reflex

The gastrocolic reflex is a physiological reflex that controls colonic motility following a meal. Upon food entering the stomach, the gastrocolic reflex initiates and controls peristalsis by moving colonic contents towards the rectum. This allows room for the consumption of more food [19]. The mechanisms responsible for this reflex are complex and not yet completely understood, although cephalic, neurally mediated and hormonal pathways seem to play a role [22, 23]. The gastrocolic reflex is often strongest in the rectosigmoid, but affects the entire colon [24, 25] The magnitude of the gastrocolic reflex is also dependent on the content and size of a meal, whereas factors such as age, sex, physical activity, the menstrual cycle and pregnancy may affect colonic activity in general [22, 26, 27].

Pathophysiology of Hirschsprung's disease

Hirschsprung's disease is a congenital intestinal disorder characterized by the absence of Meissner's and Auerbach's plexus in the distal colon, which may extend to the entire colon. This leads to impaired colonic motility in the affected part of the colon, as the smooth muscles remain in a tonic state [18, 21]. Patients suffering from HSCR generally present in their early stage of life with symptoms such as no or delayed (> 48 hours) meconium passage, vomiting, diarrhea, abdominal pain and malnutrition [4]. The gold standard in diagnosing HSCR is a rectal biopsy, in which the absence of Meissner's and Auerbach's plexuses and the presence of acetylcholinesterase positive hypertrophic nerve fibers can confirm HSCR [4, 9]. Aside from the absence of these plexuses, no evidence of deficits in other neural components relevant for coordination of colonic motility, such as the CNS, are described. This is in concordance with embryonic development of the gastrointestinal tract: the nerve plexuses absent in HSCR originate from the neural crest cells, whereas the motor function of the vagus nerve derives from the basal plate of the medulla oblongata and the ICC arise from mesenchymal precursor cells [19]. Nevertheless, consensus about the role of ICC in HSCR has not yet been reached as different authors demonstrated different results regarding this subject [28]. Treatment of HSCR consist of surgically removing the affected part of the colon and creating a coloanal anastomosis [4].

2.3 Surface electrography

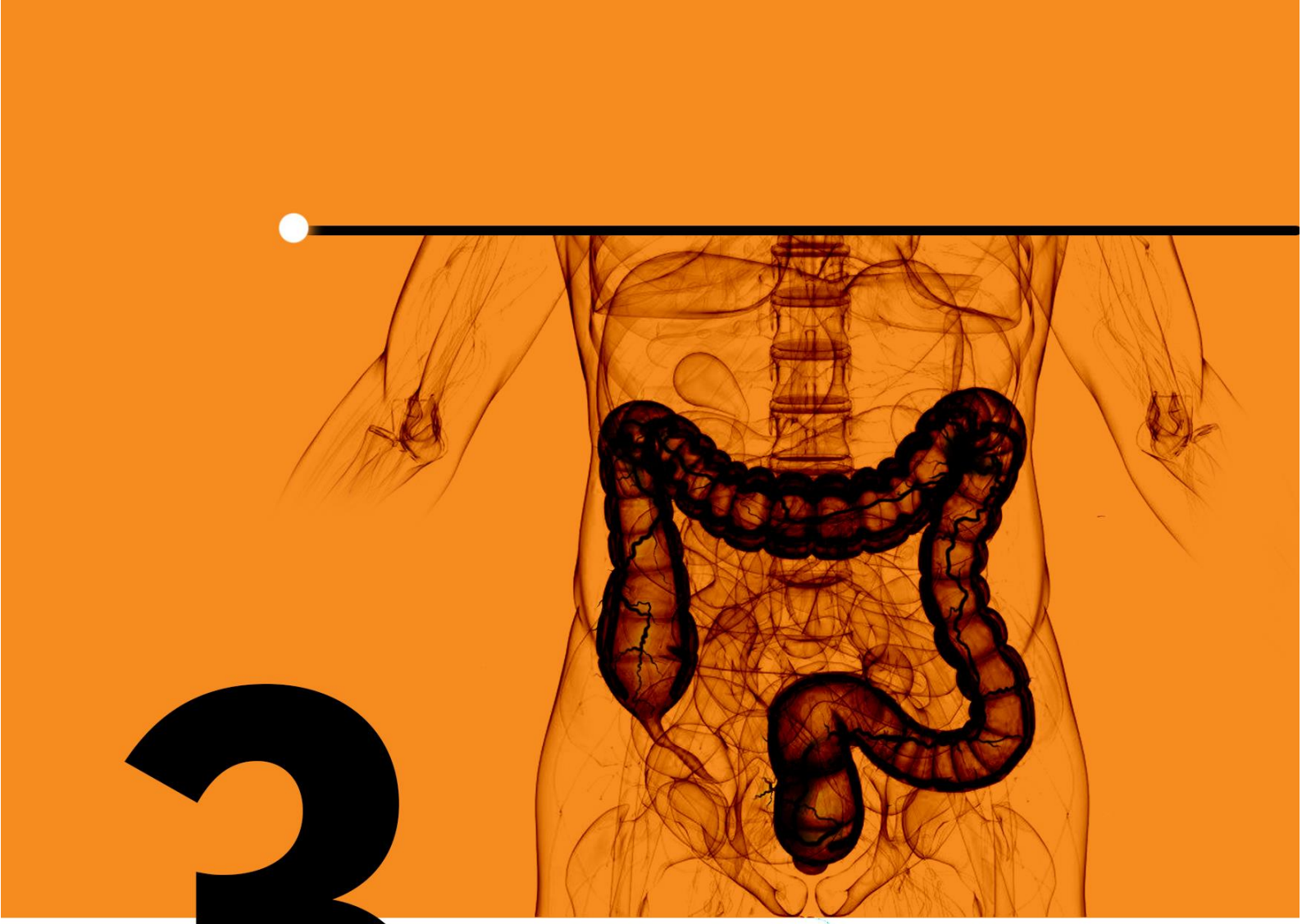
Surface electrography is the transcutaneous measurement of biopotentials originating from excitable cells underneath the body surface. These potentials are a result of de- and repolarization of muscle cells or neurons, leading to changes in membrane voltage and thus ionic currents. Before these currents can be measured by surface electrodes, they need to traverse through surrounding tissues until it reaches the skin. Different factors such as travelled distance and type of tissue influence the magnitude and thus the registration of voltage by the electrode. The type of electrode also plays an important role in correct signal registration. Polarizable electrodes behave like a capacitor, meaning charge cannot transfer across the electrode-skin interface and the electrode acts as a high-pass filter. Non-polarizable electrodes, in contrast, allow charge to transfer across the electrode-skin interface and can hence measure up to 0 Hz voltages. For non-polarizable electrodes, adequate electrode-skin contact is paramount. Insufficient or unstable contact may lead to disturbances in the charge transfer and thus incorrect signal registration. Because colonic slow waves are very low in frequency (3 cpm = 0.05 Hz), non-polarizable electrodes are necessary to record these waves properly [29, 30].

For the amplification of biopotentials, one of two measurement principles can be applied: bipolar or monopolar. In bipolar measurement, each measured signal is the potential difference between a pair of electrodes. In monopolar measurement, all measured signals are amplified against a single reference signal, which can either be a reference electrode or the average signal of all electrodes combined. When using multiple monopolar electrodes, it is preferable to amplify the signals with respect to the average of all electrodes combined instead of one single reference electrode. This way, the reference signal is not solely dependent on one electrode, meaning the recording cannot be invalidated by just one electrode conducting improperly or falling off. Another important aspect of electrography is the ground electrode. The function of this ground electrode is to reject the common mode, which is a general interference of the signals such as power line noise [30, 31].

Electroenterography

Over the past decades, the electrographic properties of the gastrointestinal tract have been studied by several researchers, either using intraluminal or transcutaneous electrodes [32-35]. Nevertheless, the exact electrographic nature of colonic activity is not yet completely known, although certain properties are demonstrated by the majority of the authors. The most

dominant of these properties is the presence of a slow wave activity of 3 cpm, most strongly present in the rectosigmoid region [14, 25, 35]. This type of activity is probably correlated to retrograde motor events limiting rectal filling. Several authors also described measuring the gastrocolic reflex, represented as a postprandial power increase when compared to preprandial measurements [14, 34-36]. Aside from a dominant frequency of 3 cpm, some authors also showed slow wave activity of 6 cpm, although the results from pre- versus postprandial measurements in this frequency band, and thus its nature, remains controversial [34, 36-39]. Furthermore, activity bursts with a frequency up to 18 cpm are reported to originate in the colon, but such activity bursts are never described on transcutaneous recordings [40].



3

METHODS

3. Methods

3.1 Design and setting

In this prospective, observational study, sEEnG measurements were performed in both pre- and postprandial states in healthy adults and children as well as in adults and children suffering from a colonic motility disorder. First, it was investigated whether sEEnG is able to measure the gastrocolic reflex in healthy adults. Secondly, possible interference of gastric electrical activity on sEEnG recordings of the sigmoid colon was studied. Furthermore, differences between the sEEnG recordings of healthy subjects and subjects suffering from a colonic motility disorder were investigated in both adults and children. Finally, the experiences of the sEEnG procedure and the gold standard diagnostic procedure were evaluated using custom-made questionnaires. This study started in December 2020 in the Radboud University Medical Center, Nijmegen, the Netherlands, and recruitment is still ongoing.

3.2 Participants

In this study, healthy adults over 18 years old were included. Exclusion criteria were a body mass index (BMI) higher than 27 kg/m^2, pregnancy, diabetes, any food intolerances, presence of an intestinal stoma, use of continuous tube feeding, the presence of known gastrointestinal diseases or complaints and the use of laxatives in the past two years. For the third and fourth aim of the study, patients over 18 years old were also included. The additional inclusion criterion for patients was suffering from a functional colonic motility disorder or surgically untreated HSCR as previously diagnosed using the gold standard method. Exclusion criteria for patients were the same as for healthy subjects, except that the presence of known gastrointestinal diseases or complaints and the use of laxatives in the past two years were omitted. Additional exclusion criterions were suffering from an inflammatory bowel disease and previous intestinal surgery.

In- and exclusion criterions of healthy children as well as pediatric patients for the third and fourth part of the study were similar to the criterions for adults. However, children were included if they were aged 11 years or younger and the exclusion criterion of a BMI > 27 kg/m^2 was replaced by a weight-for-length Z-score > 2.5 standard deviations as defined by the World Health Organization Child Growth Standard. All subjects or their parents signed informed consent prior to participation. During participation, subject characteristics such as weight,

length, time since last defecation, defecation frequency, Bristol stool scale, time since last menstruation (for women) and medication use were registered.

3.3 Measurement protocol

The measurement protocol was nearly identical for all subjects and started with fasting for at least 4 hours (3 hours for children < 1 years). After fasting, an experienced radiologist positioned eight surface electrodes on the abdomen using ultrasound guidance, as depicted in Figure 3. For the second aim, focusing on the interference from gastric electrical activity on sEEnG measurements at the sigmoid, only two electrodes were used and these were located using anatomical landmarks. The electrogastrogram (EGG) electrode was placed just caudal to the ribs, on the line originating in the middle of the xiphoid process and the umbilicus and moving craniolateral towards the stomach with a 45° angle. The sEEnG electrode, targeted at the sigmoid colon, was positioned just medial from the left anterior superior iliac spine.

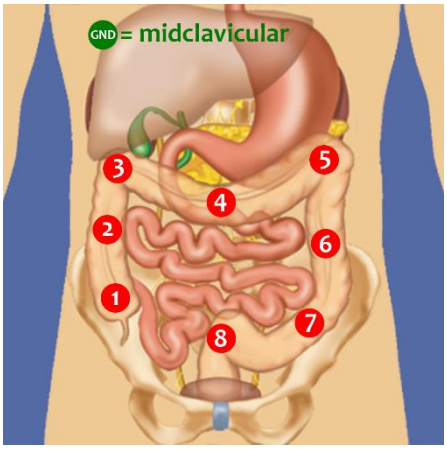


Figure 3. SEEnG electrode positions. Eight electrodes are positioned enabling the registration of all parts of the colon (cecum, ascending, transverse, descending and rectosigmoid). Image adapted from [41].

After electrode placement, a 20-minute sEEnG recording was obtained with the subjects in supine position. Next, subjects were asked to consume a meal consisting of at least 1/6th of their daily recommended calorie intake, based on the advice of the Voedingscentrum [42]. Babies were given their usual amount of either breastmilk or formula. Directly after the meal, another 20-minute sEEnG recording in supine position was performed. All measurements were acquired using Ag/AgCl electrodes (30 x 24 mm, Covidien, Massachusetts, USA), an amplifier (Porti7, TMSi, Oldenzaal, the Netherlands) and the Polybench software (version 1.34.0, TMSi, Oldenzaal, the Netherlands). The signals were sampled at 2048 Hz.

After the sEEnG procedures related to the third aim of the study, all subjects and/or their parents were asked to complete a questionnaire regarding their experienced burden of the sEEnG procedure. Patients and/or their parents were also asked to complete an identical questionnaire regarding the (gold standard) procedure used to diagnose their motility disorder. The questionnaire consisted of six or seven multiple choice questions, which could be scored

between 0 and 10, with 10 being the most positive and 0 the most negative answer, see Appendix A. For patients under four years, only their parents filled out the questionnaires, whereas for patients aged 4-11 years old, both the patient and one of their parents completed the questionnaires. Pediatric patients were aided by a smiley scale to score the questions. The questionnaires were custom-made with the help of a child psychologist, representatives of the Dutch patient organization for Hirschsprung's disease and a pediatric surgeon.

3.4 Data preprocessing

Data preprocessing was similar for all recordings in adults and were performed separately but identical for the pre- and postprandial data. First, offset and irrelevant signal contents were removed using a 4^{th} order Butterworth bandpass filter with cut-off frequencies of 1.5 and 20 cpm. Next, visual inspection of the signals was performed and clear artefacts, appearing as high (>> 200 μV) amplitude action potentials, were removed. This was done by manually selecting and subtracting the artefact, multiplied by a Hann-window to ensure signal continuity, from the signal.

Visual inspection of the sEEnG recordings in children showed that, even after 1.5 to 20 cpm bandpass filtering, movement artefacts were still clearly present. Because removing them manually could lead to arbitrary choices, artefacts were automatically removed using the artifact rejection methodology previously described by Gharibans et al. [43]. They originally developed this method to remove movement artefacts from electrogastrography recordings. However, since the electrogastrogram is comparable to the electroenterogram in terms of dominant frequency and amplitude, we found it justified to employ this method in our study. The general idea behind the method is that the local variation during a movement artefact is larger than the average variation of the entire signal. Therefore, signal epochs with a high variation should be attenuated. However, this filter did not completely remove all movement artefacts and in some signals, it attenuated signal epochs which we assumed to represent colonic activity. Hence, we added an algorithm which applied the filter as many times as necessary to remove all movement artefacts while leaving signals without movement artefacts intact. The algorithm operated by comparing the variation of the estimated artefacts with the variation of the complete signal. If the variation of the artefact signal was greater than 20% of the variation of the original signal, the artefact was removed from the signal. This procedure was repeated until the artefact variation was lower than 20% of the variation of the preceding signal. Before applying the algorithm, sample frequency was decreased 100-fold to improve

computational speed. Two other methods of data preprocessing we tried, are described in Appendix B.

3.5 Data analysis

Several time and frequency domain features were calculated for all sEEnG analyses, being mean amplitude, dominant frequency, mean power density (MPD) and percent power difference (PPD). Mean amplitudes were calculated by averaging the absolute values of the signals and multiplying them by two. For the other features, the signals were transformed to the frequency domain using Welch's method with Hann-windowing and a frequency resolution of 0.75 cpm. Now, dominant frequency was defined as the frequency with the largest power spectral density and MPD was defined as the mean power spectral density in a 2-cpm bandwidth around the dominant frequency. PPD, resembling the relative power increase between pre- and postprandial recordings, is expressed as

$$PPD = \frac{postMPD - preMPD}{preMPD} * 100\%, \quad (1)$$

with preMPD and postMPD the MPD's in fasting and postprandial states, respectively.

Gastrocolic reflex in healthy adults

Because the gastrocolic reflex is dynamic in nature, time to peak (TTP) was also determined for the first aim of the study. TTP represents the time from the start of the meal to maximum power during the postprandial measurement. The first step in determining the TTP was obtaining spectrograms based on the short-time Fourier transform with Hann-windowing and a frequency resolution of 0.1 cpm. Next, power over time was determined by finding the maximum average power density in a 2-cpm bandwidth at each point in time. TTP was subsequently defined as the point in time with the highest mean power density. The spectrograms were also visually inspected for a descriptive analysis.

Interference from gastric activity on sEEnG at the sigmoid in healthy adults

Additional analyses of the second part of the study incorporated investigating the dynamic relationship between the EGG and sEEnG signal. To this end, continuous wavelet transforms and wavelet coherence, as described by Maraun et al., were determined [44]. This method of determining wavelet coherence provides a means to identify patches of statistically significant

coherence in the time-frequency domain. Such patches were determined for EGG and sEEnG signals in a 3-cpm frequency band around their mean dominant frequency and reduced to the time domain. For a more detailed explanation of this process, see appendix B. Even though wavelet coherence provides insight in the dynamic relationship of EGG and sEEnG measurements, it does not explicitly include information about a possible time delay between both signals. Therefore, normalized temporal cross-correlation between both signals at lags ranging from -10 to +10 minutes were determined and the maximal cross-correlation and corresponding lag were investigated.

Differences in sEEnG recordings between healthy and constipated children

Analysis of differences between patients and healthy controls was limited to the dominant frequency, mean amplitude, MPD and PPD. Furthermore, sub-analysis of these variables in patients suffering from HSCR will be performed, only taking electrode 8, targeted at the rectosigmoid colon, into account. All data preprocessing and analyses were performed using MATLAB (version R2018a, The Mathworks Inc., Natick, Massachussets).

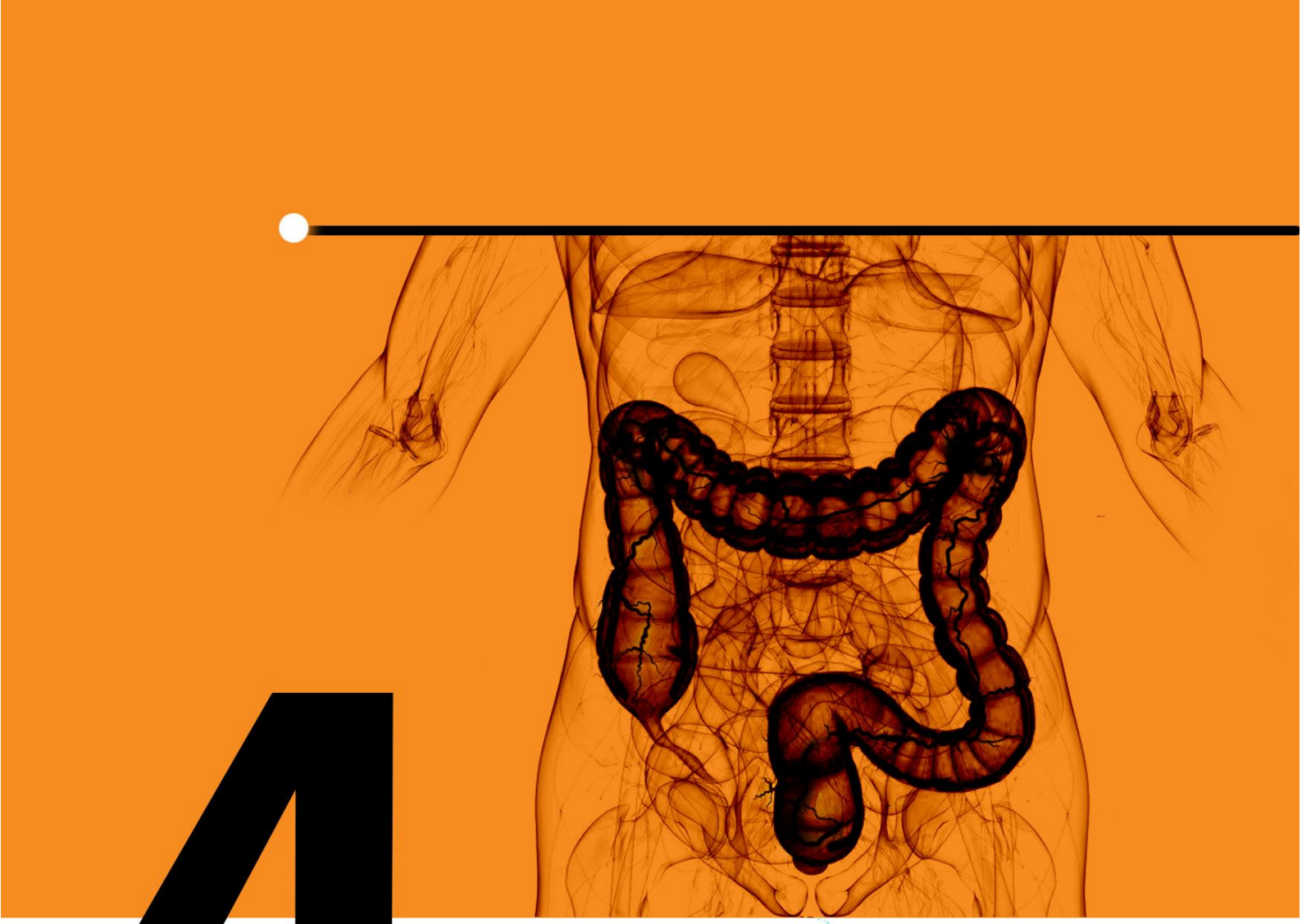
3.6 Statistical analysis

For the first part of the study, all values are presented as mean ± standard deviation or median with interquartile range (IQR), when appropriate. Differences between men and women were tested using t-tests or Mann-Whitney U tests, when appropriate, whereas differences between pre- and postprandial recordings as well as interelectrode differences were tested using Wilcoxon signed-rank tests.

For the second part of the study, all values are presented as median with IQR. Differences between EGG and sEEnG features are tested using Wilcoxon signed-rank tests. Statistical significance of wavelet coherence is determined using the algorithm provided by Maraun et al. [44]. For temporal cross-correlation, values > 0.8 are considered to indicate a relevant cross-correlation.

For the third part of the study, all values are presented as median with IQR. Differences between healthy controls and patients were tested using Mann-Whitney U tests. To investigate the gastrocolic reflex in healthy children, differences between pre- and postprandial values were tested using Wilcoxon signed-rank tests.

Outcomes of the questionnaires will be presented as median with IQR. Differences between questionnaires will be tested by Mann-Whitney U tests. For all tests, p-values < 0.05 were considered statistically significant.



4

RESULTS

4. Results

4.1 Gastrocolic reflex in healthy adults

For the first part of the study, 10 subjects, of whom 5 were women, were included, with a mean age of 22.9 ± 1.8 years and mean BMI of 22.8 ± 2.5 kg/m^2. Mean duration of fasting prior to the sEEnG procedure was 5.5 ± 1.1 hours for food and 5.3 ± 1.0 hours for drinks. Average time since last defecation was 9.0 ± 8.2 hours and defecation frequency was 8.5 ± 3.3 times per week with a mean Bristol stool scale of 3.4 ± 1.1. Meal duration between the fasting and postprandial recording was 12.8 ± 3.4 minutes and mean calorie intake was 568 ± 151 kcal. All subjects completed both pre- and postprandial sEEnG recordings. In Table 1, the differences between men and women regarding those characteristics are presented. No significant differences between both groups were found, although BMI and calorie consumption tended to be higher in men.

Table 1. Subject characteristics for men and women. No significant differences between men and women were found, but BMI and calorie consumption tended to be higher in men.

	Men (n = 5)	**Women (n = 5)**	***p*-value**
Age (years)	22.8 ± 2.5	23.0 ± 1.0	0.87
BMI (kg/m^2)	24.3 ± 2.0	21.4 ± 2.2	0.07
Defecation frequency (per week)	9.8 ± 4.2	7.2 ± 1.8	0.24
Bristol stool scale	3.4 ± 1.1	3.4 ± 1.1	1.00
Time since last defecation (hours)	8.1 ± 8.4	10.0 ± 8.8	0.73
Time of fasting (hours)	5.6 ± 1.2	5.4 ± 1.2	0.79
Meal duration (min)	11.8 ± 3.7	13.9 ± 3.1	0.36
Consumption between recordings (kcal)	656 ± 155	480 ± 88	0.06

BMI = body mass index.

Frequency, amplitude and power

Table 2 presents the dominant frequency, mean amplitude and MPD in the pre- and postprandial state and the PPD averaged over all electrode locations. Dominant frequency did not differ between pre- and postprandial measurements. In contrast, mean amplitude as well as MPD was significantly higher during postprandial measurements (p = 0.007 and p = 0.013 respectively). It is worth noticing that the interquartile range of postprandial MPD is approximately 140% of the median MPD, whereas this is just around 55% for mean amplitude.

Table 2. Dominant frequency, mean amplitude, mean power density and power percent difference averaged over all electrode locations. The dominant frequency does not differ between the two phases. Mean amplitude and mean power density are significantly larger in the postprandial phase and power percent difference is significantly larger than 0.

	Preprandial	**Postprandial**	***p*-value**
Dominant frequency (cpm)	3.4 (2.8 – 4.5)	2.9 (2.7 – 3.1)	0.114
Mean amplitude (μV)	61 (46 – 73)	89 (69 – 121)	0.007
MPD (μV^2/cpm)	307 (186 – 450)	704 (437 – 1,458)	0.013
PPD (%)	-	219 (98 – 421)	0.005*

*tested against PPD = 0.

Cpm = cycles per minute, MPD = mean power density.

Upon investigating the differences between pre- and postprandial sEEnG recordings per individual electrode location, we found that frequency only differed for electrode 5 (2.8 cpm (2.5 – 3.0) vs. 2.9 cpm (2.7 – 3.1), p = 0.008). In contrast, the postprandial mean amplitude and MPD were not different for electrode 5, whereas all other electrodes had significantly higher postprandial amplitudes and MPD's. It needs to be mentioned that electrode 5, targeted at the splenic flexure, often had to be placed more cranial and lateral when compared to electrode 3, targeted at the hepatic flexure, due to the proximity of the stomach. Additional figures and *p*-values regarding the results of individual electrode locations are presented in Appendix D.

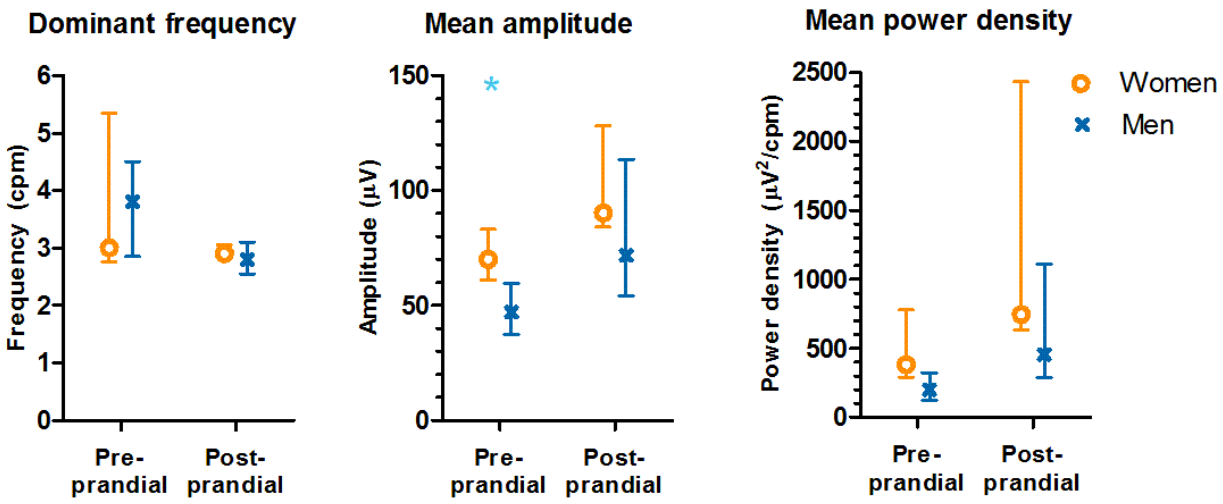


Figure 4. Dominant frequency, mean amplitude and mean power density in men and women. The dominant frequency does not differ between men and women in both pre- and postprandial states, but the mean amplitude and mean power density tend to be higher in women when compared to men in both pre- and postprandial states. The asterisk indicates a significant difference between men and women ($p < 0.05$).

Differences between men and women

Figure 4 shows the differences in dominant frequency, mean amplitude and MPD between men and women. The dominant frequency and MPD did not differ between both groups. Mean amplitude in the preprandial phase, however, was significantly higher in women, 70 µV (61 – 83) vs. 47 µV (38 – 60), $p = 0.032$. It is furthermore worth noticing that mean amplitude and MPD during both the pre- and postprandial measurements tend to be higher in women. This could possibly be explained by the tendency of women to have a lower BMI than men, but even after correction for BMI, mean amplitude in the fasting state remained significantly higher ($p = 0.042$).

Activity over time

As the gastrocolic reflex is a dynamic process, the power development over time was assessed. Figure 5 shows the spectrogram of a single electrode, clearly displaying the dynamic nature of colonic activity in the postprandial phase. We found that, on average, power reached its peak 19 ± 3 minutes postprandial and did not differ between the various electrode locations. However, intraindividual TTP's differed up to 17 minutes. These results demonstrate that colonic motility is variable in time as well as among the different electrode locations.

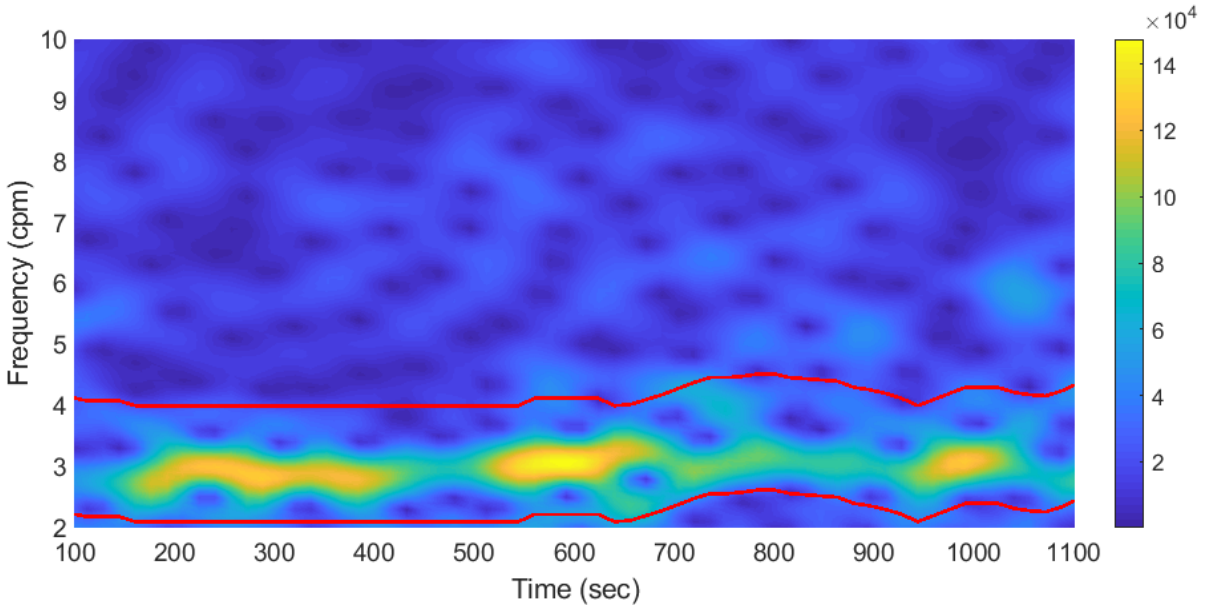


Figure 5. Spectrogram of a single sEEnG-electrode in the postprandial phase. The red lines indicate the 2-cpm bandwidth with the most power at each point in time. It clearly demonstrates that the magnitude of colonic activity is not constant over time.

4.2 Interference of gastric activity with sEEnG at the sigmoid in healthy adults

In this part of the study, 3 men and 3 women were included. Their median age was 23 years (22 – 24.5) and their median BMI was 21.9 kg/m^2 (20.9 – 23.8). They fasted 9 hours (5 – 13.5) prior to the measurement procedure and all subjects successfully completed the procedure. Meal duration was 10 minutes (7 – 17) and calorie intake was 300 kcal (283 – 479).

Frequency, amplitude and power

Table 3 shows the dominant frequency, mean amplitude and MPD for both the EGG and sEEnG signals during pre- and postprandial recordings. No significant differences between these variables were observed, meaning EGG and sEEnG signals are not distinguishable based on these characteristics.

Table 3. Dominant frequency, mean amplitude and mean power density in EGG and sEEnG during pre- and postprandial measurements. No significant differences between EGG and sEEnG were found.

		EGG	sEEnG	*p*-value
Pre-prandial	Frequency (cpm)	3.2 (2.6 – 4.1)	3.4 (2.6 – 4.7)	0.11
	Mean amplitude (μV)	42 (33 – 59)	30 (23 – 51)	0.35
	MPD (μV^2/cpm)	102 (62 – 361)	47 (37 – 190)	0.25
Post-prandial	Frequency (cpm)	4.1 (3.3 – 5.2)	4.0 (2.9 – 5.0)	0.50
	Mean amplitude (μV)	76 (48 – 128)	79 (34 – 119)	0.25
	MPD (μV^2/cpm)	453 (221 – 2,086)	675 (92 – 1,697)	0.25
	PPD (%)	448 (125 – 688)	1,052 (67 – 2,790)	0.35

EGG = electrogastrography, sEEnG = surface electroenterography, cpm = cycles per minute, MPD = mean power density and PPD = power percent difference.

Wavelet coherence

To gain insight in the time-dependent coherence of the stomach and sigmoid recordings, the wavelet coherence between these signals was determined. In the preprandial phase, wavelet coherence was significant during 38% (21 – 58) of total measured time. This did not differ from the postprandial phase, where wavelet coherence was significant for 54% (29 – 68) of the time. Visual inspection of the power over time for EGG and sEEnG revealed that most of the significant intervals were related to episodes of increased power. However, episodes of increased power in either one of the electrode locations was not necessarily accompanied by increased power in the other location, see Figure 6. Furthermore, no clear pattern of either the gastric or colonic activity continuously being higher than the other was observed. This means

that, even though EGG and sEEnG show significant correlations at several intervals, correlation is not continuously present and the activity of either the stomach or colon may fluctuate independently.

Temporal cross-correlation

Normalized temporal cross-correlations between EGG and sEEnG were computed to assess possible delayed correlations between both signals. In the preprandial state, maximum temporal cross-correlation was 0.59 (0.36 – 0.69) and was comparable to the postprandial state with a cross-correlation of 0.59 (0.33 – 0.70). The corresponding time delays were -0.07 seconds (-8.11 – 0.19) and 0.09 seconds (-0.04 – 55.82) respectively. It needs to be mentioned that there was one outlier with a cross-correlation of 0.19 and 0.33 and a delay of -31.86 and 222.70 seconds for the pre- and postprandial state, which explains the wide IQR's. Nevertheless, a relevant cross-correlation or delay between EGG and sEEnG does not seem very plausible based on these results.

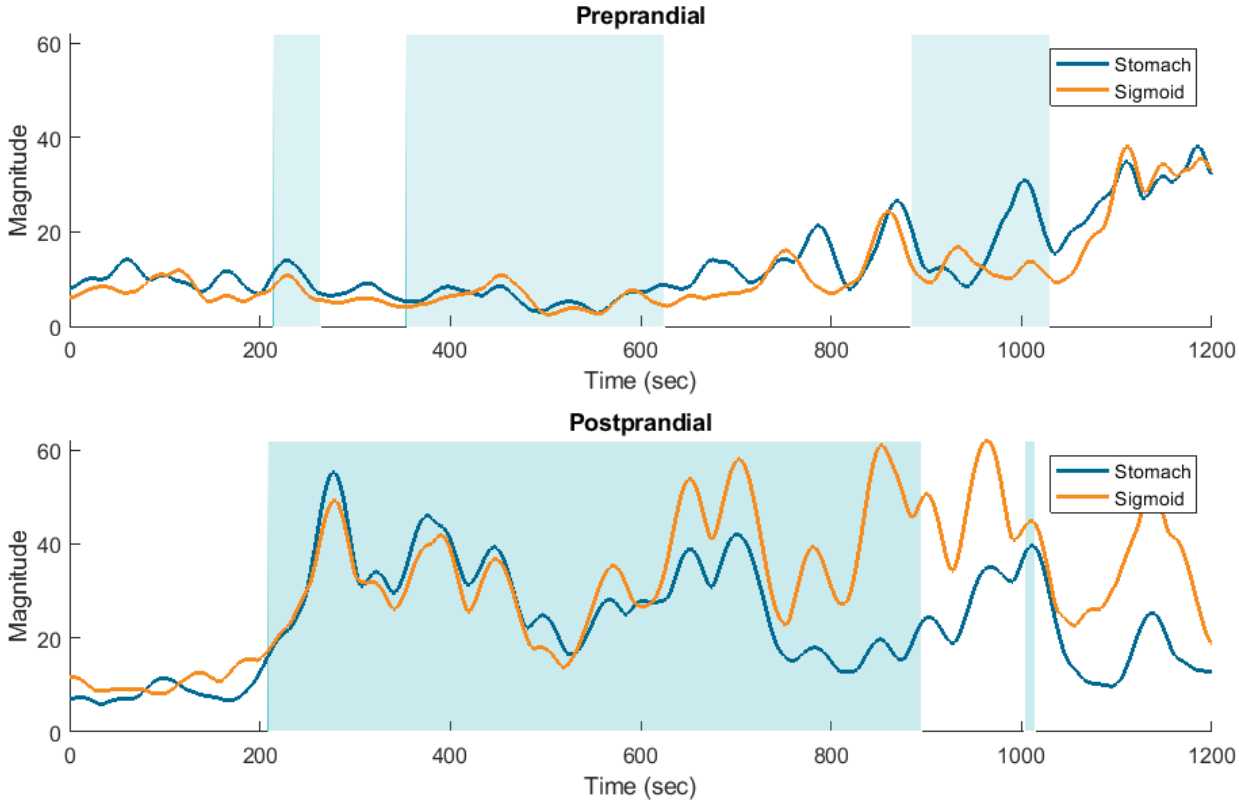


Figure 6. Power over time for the EGG and sEEnG at the sigmoid during pre- (top) and postprandial (bottom) recordings. The light blue area's indicate a statistically significant wavelet coherence between both signals. Note the increased sigmoidal power around 950 seconds in the bottom graph, whereas this power increase is not seen in the EGG signal. Wavelet coherence between the two signals is also not significant at that time.

4.3 Differences in sEEnG recordings between healthy and constipated subjects

In the final part of the study, 23 subjects were included, of whom twelve adults. However, because only two adult patients were included and the results of the healthy adults are already described in Chapter 4.1, the results of adult patients are only presented in Appendix E. Of the eleven children, four were healthy controls and seven were patients. Characteristics of these subjects can be found in the upper section of Table 4. Sex and mean z-score did not differ between both groups, but age was significantly higher in healthy controls. The lower section of Table 4 contains characteristics related to the sEEnG procedure. Calorie intake was significantly higher in the control group (p = 0.02), whereas all other characteristics did not differ between the groups. In two patients, the Bristol stool scale was not determined, because they only defecated following rectal washouts. All subjects completed the sEEnG procedure, even though in one subject, both pre- and postprandial recordings were shorter than 20 minutes due to logistic issues. In two other subjects, one from each group, one electrode was not attached well, which invalidated the measurements for that electrode.

Table 4. Subject characteristics (top) and sEEnG procedure characteristics (bottom) of controls and patients.

	Controls (n=4)	**Patients (n=7)**	***p*-value**
Subject characteristics			
Girls (#)	2	2	0.50
Age (years)	3.5 (1.6 – 9.5)	0.3 (0.3 – 0.5)	0.02
Z-score	0.69 (-1.3 – 1.3)	6.0 (4.5 – 6.0)	0.71
Hirschsprung's disease (#)	-	5	-
Awaiting rectal biopsy (#)	-	1	-
Idiopathic constipation (#)	-	1	-
sEEnG procedure			
Fasting (hours)	4.5 (4 – 5)	4 (3 – 4)	0.08
Last defecation (hours)	6 (1.5 – 20)	12 (4 – 18.0)	0.85
Defecation frequency (weekly)	8.5 (7 – 13)	14 (5.5 – 14)	0.43
Bristol stool scale	4 (4 – 5)	6 (4.5 – 6.5)	0.13
Meal content (kcal)	357 (222 – 542)	120 (75 – 160)	0.02

Gastrocolic reflex in healthy children

Earlier, we have shown that the gastrocolic reflex can be measured in adults, but for children, this has not yet been established. In the healthy children, dominant frequency did not differ between the pre- and postprandial recording, with frequencies of 3.8 cpm (3.1 – 4.6) and 3.2

cpm (3.0 – 3.8), p = 0.11. Mean amplitude and MPD did also not differ significantly between pre- and postprandial recordings, with values of 84 µV (63 – 160) vs. 141 µV (107 – 201), p = 0.07, and 494 μV^2/cpm (265 – 2,670) vs. 2,163 μV^2/cpm (1,372 – 3,686), p = 0.07, respectively. However, given the pronounced differences, the lack of statistical difference can potentially be attributed to the low number of subjects. Note that the values of healthy children (Table 4) are in the same order of magnitude as the values of healthy adults (Table 2).

Frequency, amplitude and power

Figure 7 presents the dominant frequency, amplitude and MPD averaged over all electrodes of healthy controls and patients. No significant differences between the groups were found. Nevertheless, it is remarkable that the values of the preprandial mean amplitude and MPD tend to be higher in the patient group when compared to the healthy controls, 151 µV (57 – 308) vs. 84 µV (63 – 160), p = 0.45, and 2270 μV^2/cpm (318 – 8356) vs. 494 μV^2/cpm (265 – 2670), p = 0.35, respectively. In contrast, PPD, the relative power increase between pre- and postprandial measurement, seemed to be lower in patients when compared to controls, with PPD's of 26% (-37 – 324) vs. 283% (104 – 699), although statistical significance was not reached (p = 0.13). Therefore, these results seem to indicate that patients have higher preprandial colonic activity when compared to healthy controls, but relative power increase after meal ingestion is lower.

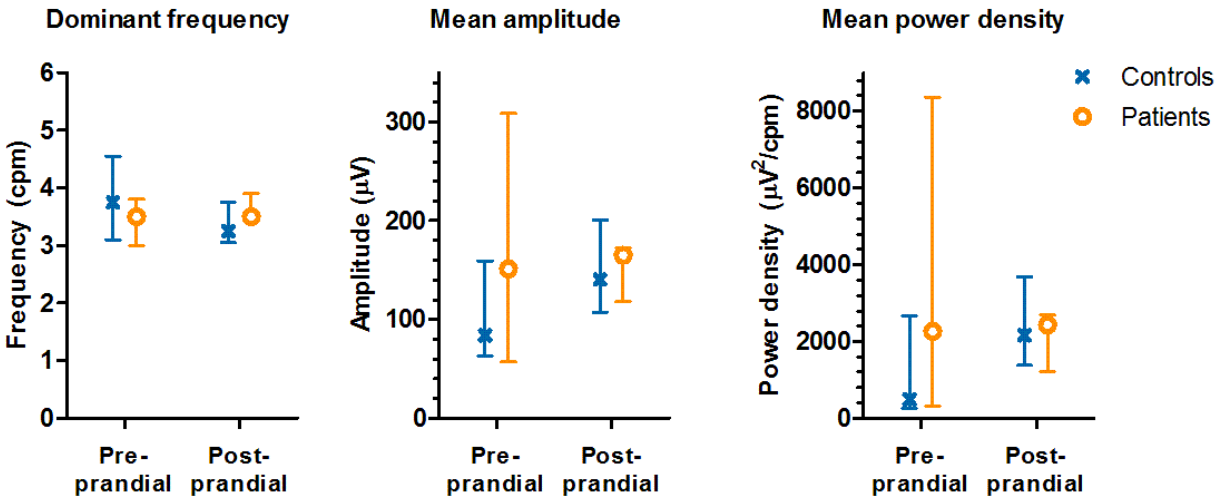


Figure 7. Dominant frequency, mean amplitude and mean power density for healthy controls and patients during pre- and postprandial measurements. During the preprandial phase, amplitude and MPD tend to be higher in patients, whereas relative increase in amplitude and MPD seem to be higher in the healthy subjects.

Electrode 8 for Hirschsprung's disease

As HSCR affects colonic motility in the distal colon, we performed a sub-analysis of electrode 8, the most distal electrode, in patients with confirmed HSCR. Because electrode 8 was not properly attached in one subject of each group, three healthy subjects and four patients could be included in this analysis. Significant differences between both groups in dominant frequency, mean amplitude, MPD and PPD were not found. However, mean amplitude and MPD tended to be higher in the patients in both pre- and postprandial recordings, whereas the PPD tends to be lower among patients. Nevertheless, these are preliminary results from a low number of subjects, so the results have to be interpreted with caution. More elaborate results regarding electrode 8 can be found in Appendix F.

Questionnaires regarding the experienced burden

Parents from all subjects completed the sEEnG questionnaire and six of the patients' parents also completed the questionnaire regarding the gold standard diagnostic procedure. Of the six patients, five were diagnosed using a rectal biopsy in the outpatient clinic and one was diagnosed using a rectal biopsy under general anesthesia. The sEEnG questionnaires were all completed on the same day as the sEEnG procedure, while the gold standard diagnostic procedure was performed 75.5 days (40 – 118.5) prior to completing the questionnaire regarding this procedure. On average, parents of patients rated the sEEnG procedure more positively than the gold standard procedure, 9 (8.5 – 10) vs. 6.5 (5 – 7.5), $p = 0.046$. Results of the individual questions are presented in Figure 8. It is clear from questions 1a and 1b that parents were significantly more anxious about the rectal biopsy than about the sEEnG procedure ($p = 0.042$ and $p = 0.043$). Regarding the understanding and duration of the procedure, questions 2 and 3 respectively, no differences were found. However, the pain, burden and general experience of the procedure, questions 4 – 6, were again all scored significantly more positively for the sEEnG procedure when compared to the gold standard procedure ($p = 0.039$, $p = 0.034$ and $p = 0.042$ respectively). Parents of healthy controls did not score the sEEnG procedure differently than parents of patients.

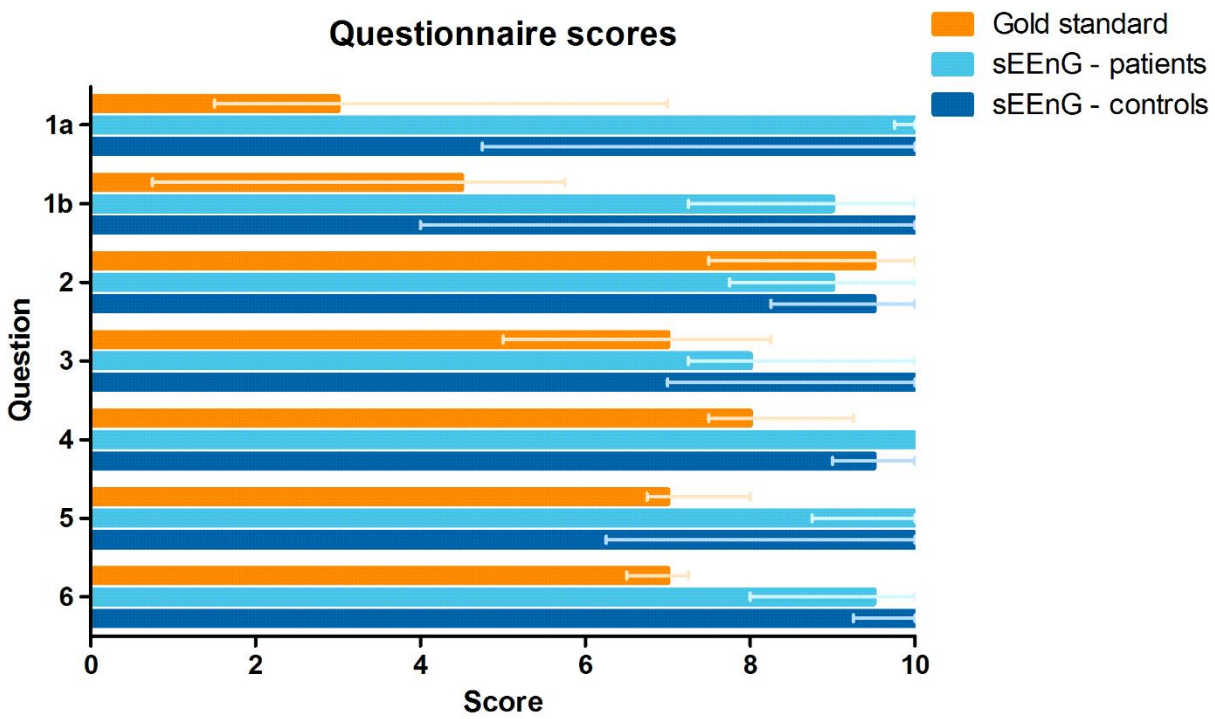
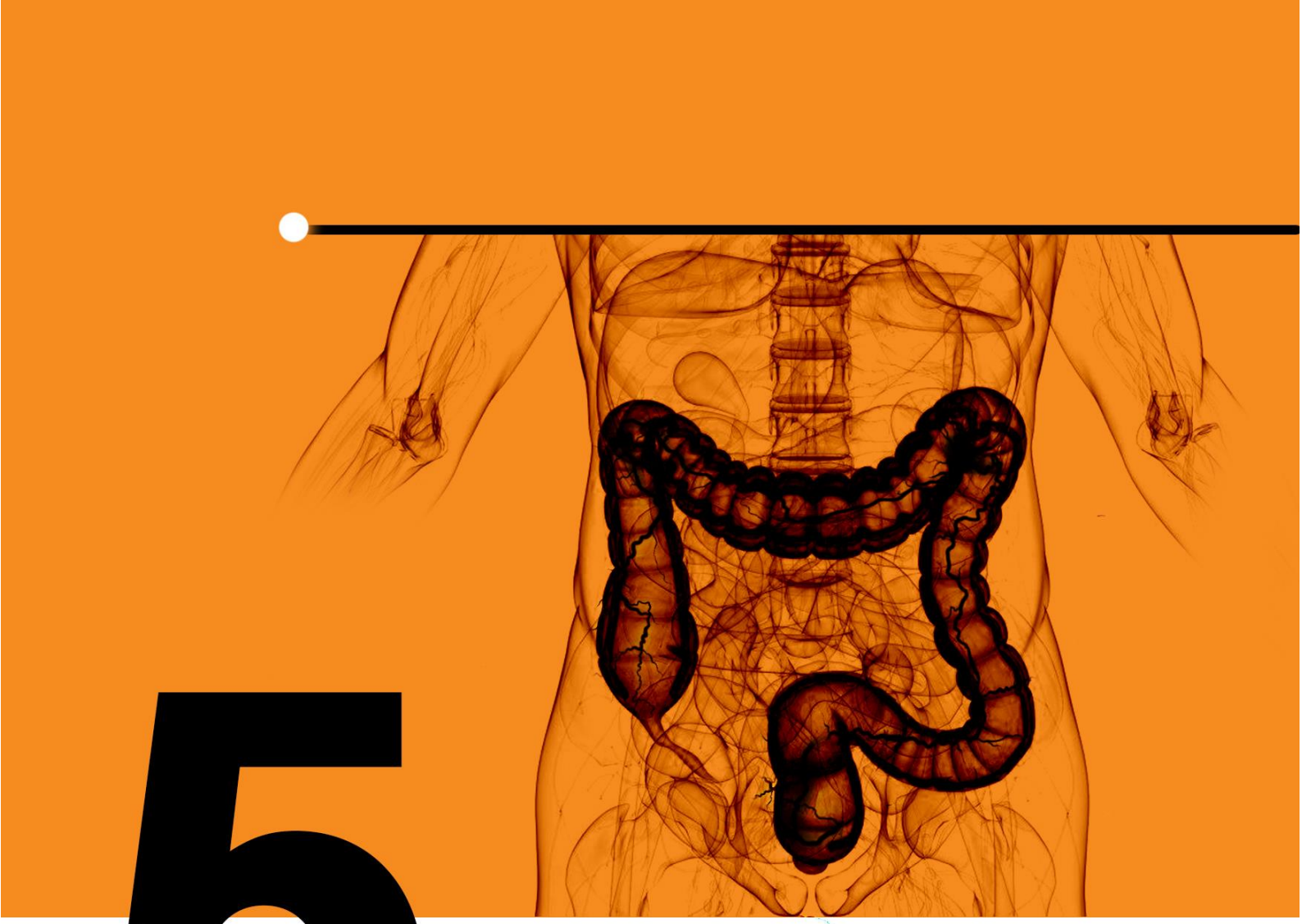


Figure 8. Questionnaire outcomes of the gold standard and sEEnG procedure. Questions regarding looking up against the procedure (questions 1a and 1b), pain, burden and general experience (questions 4 – 6) are scored more positively for the sEEnG procedure than the gold standard procedure. Questions regarding the understanding and duration of the procedures (questions 2 and 3) did not display significant differences. The sEEnG procedure was not scored differently by parents of patients when compared to parents of healthy subjects.



5

DISCUSSION

5. Discussion

To our knowledge, this is the first study aimed at measuring the gastrocolic reflex in healthy adults and children as well as in patients suffering from a colonic motility disorder using surface electrodes placed under ultrasound guidance. Main findings in healthy subjects were that the gastrocolic reflex could be adequately measured in adults and children and that gastric activity does not interfere with sEEnG measurements obtained at the sigmoid colon. Furthermore, comparing sEEnG recordings between healthy and constipated children demonstrated that the preprandial amplitude and MPD seemed higher in patients, whereas relative power increase between pre- and postprandial recordings tended to be lower in patients. Preliminary results of two adults with HSCR showed that relative postprandial power increase was lower in the distal electrodes when compared to the more proximal electrodes. Finally, patients rated the sEEnG procedure more positively than the rectal biopsy. Therefore, sEEnG shows to have potential to become a non-invasive, easy to use and readily available tool in the diagnostic process of colonic motility disorders in both adults and children.

Gastrocolic reflex in healthy adults and children

When measuring the gastrocolic reflex in healthy subjects, we found that signal amplitude as well as MPD significantly increased when comparing postprandial measurements to preprandial measurements. The dominant frequency, however, did not change between pre- and postprandial measurements and was in the range of colonic slow waves, around 3 cpm. These findings are in concordance with earlier studies using manometry [45], intraluminal EEnG measurements [46] and sEEnG measurements [35]. It is, however, remarkable that Erickson et al. reported a mean PPD of 38 ± 17%, whereas we found it to be 219% (98 – 421) [35]. This difference can probably be explained by the fact that Erickson et al. averaged the PPD over 30-32 electrodes placed all over the abdomen, whereas we positioned eight electrodes as close to the colon as possible. In addition, Erickson et al. measured up to 60-90 minutes postprandial as opposed to our 20-minute postprandial period. This may lead to time averaging effects, because the postprandial power peak is approximately 20 minutes after the start of the meal and then starts tapering off [24].

Another relevant finding regarding the gastrocolic reflex in healthy subjects is that the IQR of the MPD is around 140% of its median value, meaning interindividual differences are large. In contrast, IQR of the mean amplitude is approximately 55% of the mean value. It may therefore be better to use the relative increase in mean amplitude instead of MPD as representation of

the gastrocolic reflex. Nevertheless, mean amplitude is less specific in terms of frequency and may possibly result in an underestimation of colonic activity.

Furthermore, we found it remarkable that amplitude and MPD tended to be higher in women than in men, even after correction for BMI. This is contradictory to results previously found by Rao et al., who reported colonic activity to be lower in women than in men [27]. However, this conclusion was based on manometry measurements which records pressure waves instead of electrical activity, which possibly could explain the discrepancy. Other authors, to our knowledge, have never described sex related differences in sEEnG measurements, although it has been clearly established that functional gastrointestinal disorders show myriad differences between men and women in clinical presentation as well as in pathophysiologic characteristics [47]. It may therefore be interesting and even necessary to further investigate sex-related sEEnG differences in order to interpret the recordings correctly.

Regarding the individual electrode positions, mean amplitude and MPD of electrode 5, which is located at the splenic flexure, did not significantly differ between pre- and postprandial measurements. This could be caused by interference from electrical activity of the stomach, as the stomach also has a dominant frequency around 3 cpm [48]. However, as the stomach is also involved in the gastrocolic reflex, this explanation does not seem very likely. Another reason may be that electrode 5 often needed to be positioned further from the splenic flexure to avoid placing the electrode on the stomach. Hence, the distance between the electrode and the colon is increased, leading to decreased measurement accuracy. Other differences between electrodes in terms of either frequency, amplitude and MPD were not observed. Some studies, however, demonstrated differences in colonic activity in different parts of the colon [45, 49]. Jouet et al. [49] described the gastrocolic response to be of greater magnitude in the distal colon when compared to the proximal colon, which was recently reaffirmed by Pervez et al. [45], albeit both authors used manometry instead of surface electrography. This may indicate that sEEnG is not as accurate as manometry, so further study should determine whether sEEnG is sufficiently accurate to detect clinically relevant differences.

When comparing the sEEnG recordings in healthy with sEEnG recordings in healthy children, no relevant differences in terms of either dominant frequency, mean amplitude or MPD were found. This is in concordance with the studies performed by Shafik et al., who reported frequencies and amplitudes of intrarectal electrography recordings in both healthy children and

adults [14, 46]. Dominant frequency and mean amplitude for adults were 3.1 ± 0.8 cpm and 1.8 ± 0.6 mV respectively, whereas for children, these values were 2.9 ± 0.7 cpm and 2.2 ± 0.4 mV. Hence, there does not seem to be a vast difference in colonic electrical activity between adults and children and the gastrocolic reflex can be measured adequately in both groups.

Interference from gastric activity on sEEnG at the sigmoid in healthy adults

Analysis of EGG and sEEnG at the sigmoid colon recordings demonstrated that both signals are comparable regarding the dominant frequency, amplitude and power. However, no compelling evidence that gastric activity interferes with sEEnG signals obtained at the sigmoid was found. Interference between gastric and colonic activity has not been extensively studied, although in 1974, Taylor et al. performed intrarectal EEnG measurements and simultaneously obtained surface EGG recordings in two healthy adults [39]. Corresponding with our findings, they found that frequencies of both stomach and colon were around 3 cpm, but they were not identical. Furthermore, a close cross-correlation between gastric and colonic recordings was not observed, again in line with our results [39]. We can therefore conclude that the characteristics of EGG and sEEnG at the sigmoid are very comparable, but do not interfere with each other. However, given that electrical activity attenuates with distance, gastric interference may still pose a problem in sEEnG measurements around the splenic flexure [50].

Differences in sEEnG recordings between healthy and constipated subjects

The results from the comparison between healthy and constipated children show that there are no clear differences between both groups based on the dominant frequency, mean amplitude and MPD. Surprisingly, we found a tendency of preprandial amplitude and MPD to be greater in the patient group when compared to the healthy subjects. This is contrary to our expectations, given that the patients are suffering from hypermotility of (a part of) their colon. The age difference between both groups may be partially explanatory for this discrepancy. In babies, the distance between the electrode and colon is much smaller than in older children, meaning the signal is attenuated less by body tissue [50]. However, as the difference is only seen in the preprandial phase, this is deemed to be a less likely explanation. Another difference between both groups is that movement artefacts were much more present in the recordings of patients than of controls, probably affecting the results to a certain extent. The higher preprandial MPD could also be explanatory for the PPD being lower in patients, especially since the postprandial MPD differed much less between the healthy and constipated children.

Even though sEEnG measurements have never been performed in children suffering from HSCR, two authors have investigated intraluminal EEnG measurements in children suffering from surgically untreated HSCR [38, 46]. Marin et al. reported a remarkably flattening of the slow waves in HSCR when compared to healthy controls [38] and Shafik et al. even described HSCR patients to have a silent EEnG without any detectable slow waves [46]. In our sub analysis of children with HSCR, we found neither flattening nor total absence of slow waves in the rectosigmoid electrode (electrode 8). However, the periodograms of adults with HSCR clearly show attenuation of slow wave activity in the more distal electrodes when compared to the more proximal electrodes. Therefore, the results of this study implicate that there are certain differences in the sEEnG signal between healthy subjects and patients suffering from constipation, but especially in children, it is currently not clear whether these differences are representative of colonic motility.

The outcomes of the questionnaires clearly demonstrated that the sEEnG procedure was preferred over the rectal biopsy, especially due to the prior expectations of the procedure. In addition, retrospective pain and burden of the gold standard procedure was significantly scored more negatively for the rectal biopsy. Nevertheless, there are two factors that could have influenced these results. Firstly, because the outcome of the rectal biopsy had clinical implications for their child, parents may have experienced much more anxiety for that procedure than for the sEEnG procedure, which was used solely for research purposes. Also, the time between the rectal biopsy and completing the questionnaire was significantly longer than time between the sEEnG procedure and completing the questionnaire, which could have resulted in a recall bias. Nevertheless, because the results were evidently different and significant, even in a small group, this indicates that the sEEnG procedure is absolutely preferred over a rectal biopsy.

Strengths and limitations

The main strength of this study is that colonic motility is measured in both pre- and postprandial phases. As a result, an intraindividual comparison between the pre- and postprandial recordings could be performed instead of having to compare either only pre- or postprandial recordings between different subjects. Another strength is the use of ultrasound to guide electrode placement. This way it is ensured that the electrodes are positioned close to the colon and that electrode placement is comparable among different subjects. In the second part of the study, electrode placement was not performed due to logistical reasons. However, because electrical activity spreads diffusively and the electrodes were placed close to the

targeted structures, we do not think this will affect our findings drastically. A final strength of our study is that we mainly focused on HSCR, since this is one of the few colonic motility disorders with an organic etiology. This means we are sure colonic motility in these patients is altered, as opposed to other functional colonic motility disorders in which no clear cause can be found [2].

The most important limitation of our study, especially of part three, is the number of subjects included in our study. However, as recruitment is still ongoing, we hope to include 20 healthy controls and 20 patients for both adults and children. Especially when including more children, the aim will be to decrease the difference in average age between the two groups, presumably leading to improved comparison of the groups. The age difference posed a challenge in the interpretation of the current results. The main reason for this is that the healthy controls, aged between 2-11 years, were better capable of lying still than the patient group, aged under the age of one. This resulted in much less movement artefacts among the healthy subjects. Using a filter algorithm proven effective in electrogastrography in adults, we tried to eliminate movement artefacts as adequately as possible, but based on the results we do not think influence of movement artefacts is completely eliminated; this is therefore also a limitation of our study.

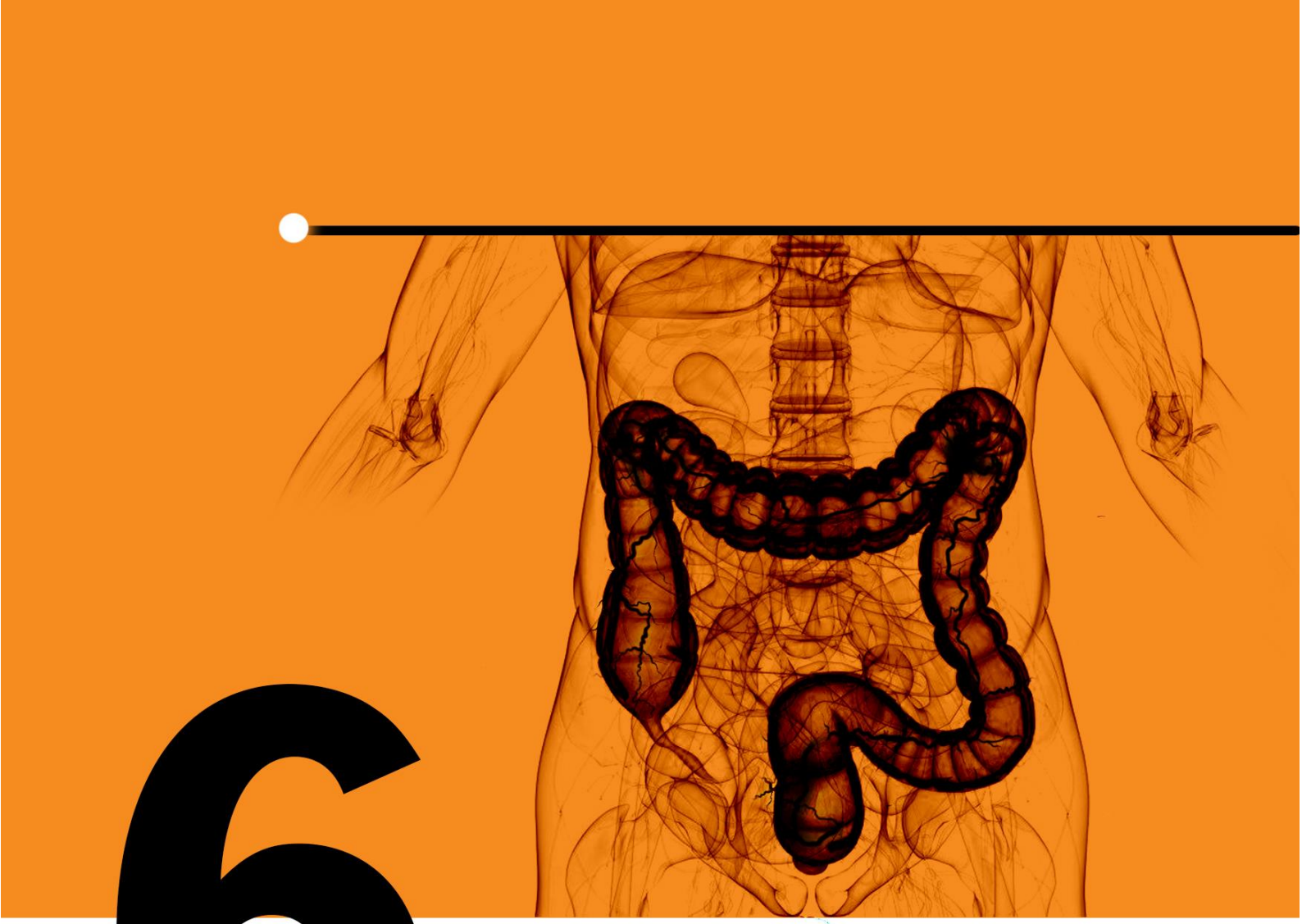
Clinical implications and future directions

Based on the current results, no explicit claim that sEEnG is able to differentiate between healthy subjects and subjects suffering from a colonic motility disorder can yet be made. Nevertheless, the preliminary results of adults with HSCR seem very promising. In addition, our study proved the feasibility, in terms of technical proceedings, of employing sEEnG measurements with ultrasound guidance in children and adults. Furthermore, we proved that the gastrocolic reflex could be measured in healthy children, meaning future research regarding sEEnG to measure colonic motility should also be continued in children.

Further research of sEEnG recordings should focus on improving artefact identification and filtering, especially in babies. It may be fruitful to use abdominal muscle activity as a measure for movement artefacts, as movement artefacts in sEEnG are almost always accompanied by increased abdominal muscle activity. This activity is already measured by the sEEnG electrodes and has a very different frequency range than colonic motility, approximately between 20-500 Hz [51]. Therefore, the amount of movement artefacts can be estimated by

determining the power in that frequency band and can be used to attenuate or discard the corresponding signal epochs. Another recommendation is to find factors influencing sEEnG recordings, so that these recordings can be normalized and properly compared between different subjects. In doing so, it is important to take the dynamic nature of colonic motility into account. Added to that, the repeatability of sEEnG recordings should be investigated extensively. Preliminary results regarding this subject already demonstrated that the repeatability greatly depends on the evaluated feature and may thus be important in choosing features to distinguish between healthy and constipated subjects [52].

If, in the future, sEEnG recordings can be interpreted adequately, its possibilities for further research and clinical applications are extensive. It could be used in the diagnostic process of other colonic motility disorders such as slow-transit constipation, chronic intestinal pseudo-obstruction and functional constipation. Moreover, inflammatory bowel diseases or certain types of irritable bowel syndromes may also exhibit altered motility, which could possibly be measured using sEEnG. Therefore, as sEEnG is non-invasive, easy to use and readily available, it may become a very important tool in the diagnostic process of several colonic diseases in both adults and children and hopefully will prevent situations like Sven's, as described in the introduction.

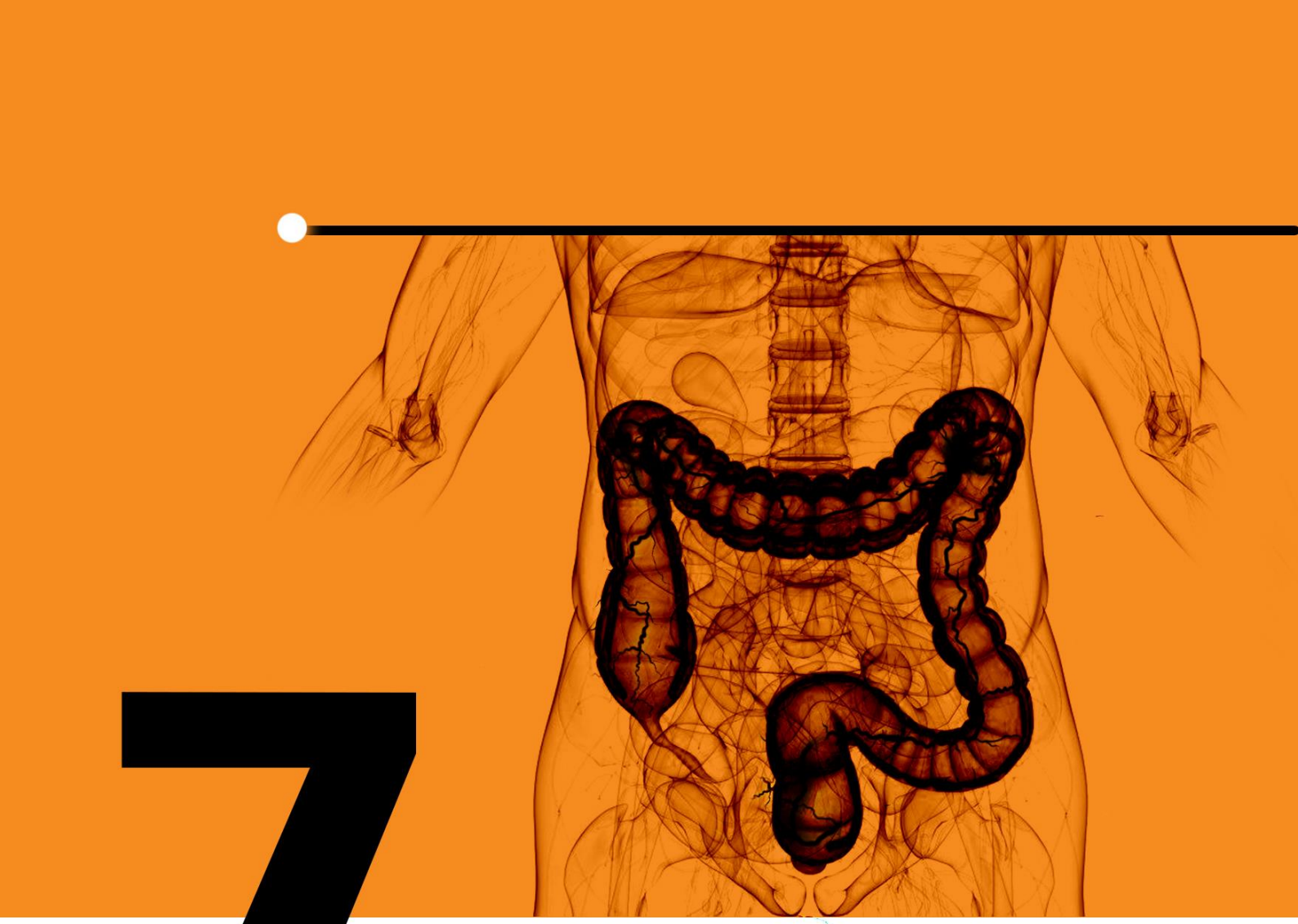


6

CONCLUSION

6. Conclusion

In this study, it was shown that sEEnG can be used to measure the gastrocolic reflex in healthy adults and children. In addition, interference of gastric activity with sEEnG measurements at the sigmoid does not seem to be a problem. Furthermore, comparing sEEnG recordings between healthy and constipated children seem to reveal certain differences, but the nature and implications of these differences remain uncertain as yet. Preliminary results from sEEnG recordings of adults with HSCR, however, seem very promising in detecting altered colonic motility. Finally, the sEEnG procedure is experienced much more positively than rectal biopsies. Still, further study is necessary to be able to interpret sEEnG recordings correctly.



7

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Appendix A: Dutch questionnaire to evaluate the experience of the procedures

Voor de vragen 1-6 kan een score van 0 tot en met 10 worden gegeven door een getal te omcirkelen. Bij elke vraag staat de betekenis van 0 en 10 beschreven.

1a. In welke mate zag u van tevoren <u>zelf</u> op tegen het onderzoek?

(0 = heel erg, 10 = helemaal niet)

0 1 2 3 4 5 6 7 8 9 10

1b. In welke mate zag u van tevoren op tegen het onderzoek <u>voor uw kind</u>?

(0 = heel erg; 10 = helemaal niet)

0 1 2 3 4 5 6 7 8 9 10

2. In hoeverre was het voor u van tevoren duidelijk wat het onderzoek precies inhield?

(0 = helemaal niet duidelijk; 10 = heel erg duidelijk)

0 1 2 3 4 5 6 7 8 9 10

3. In hoeverre vond u de duur van het onderzoek belastend voor uw kind?

(0 = heel erg belastend; 10 = helemaal niet belastend)

0 1 2 3 4 5 6 7 8 9 10

4. Hoeveel pijn heeft uw kind volgens u ervaren tijdens het onderzoek?

(0 = heel erg veel pijn; 10 = helemaal geen pijn)

0 1 2 3 4 5 6 7 8 9 10

5. In hoeverre heeft uw kind het onderzoek als belastend ervaren volgens u?

(0 = heel erg belastend; 10 = helemaal niet belastend)

0 1 2 3 4 5 6 7 8 9 10

6. **Hoe beoordeelt u het onderzoek als geheel?**

   (0 = heel erg vervelend; 10 = totaal niet vervelend)

   0 1 2 3 4 5 6 7 8 9 10

Note: for children, question 1b was skipped and all questions were rephrased for a better understanding.

Appendix B: Different methods of preprocessing sEEnG signals

Different methods to preprocess the sEEnG data were tried, but not all methods seemed fruitful. In this appendix, two of these methods will be described and compared to the method which was finally used in our study.

Offset removal by subtracting moving median

Paskaranandavadivel et al. provided a method to remove very low frequency offset from electrographic signals for the analysis of gastric slow wave activity [53]. They did this by subtracting a moving median from the signal, with a window length corresponding to the expected frequency of the signal (≈ 3 cpm). Erickson et al. employed this technique in the analysis of sEEnG signals and demonstrated that offset could be removed properly using this technique. However, filtering our data with a Butterworth bandpass between 1.5 – 20 cpm also removed the very low frequency offset adequately. Furthermore, removing the moving median before applying the Butterworth filter affected the power spectral density estimates, see figure

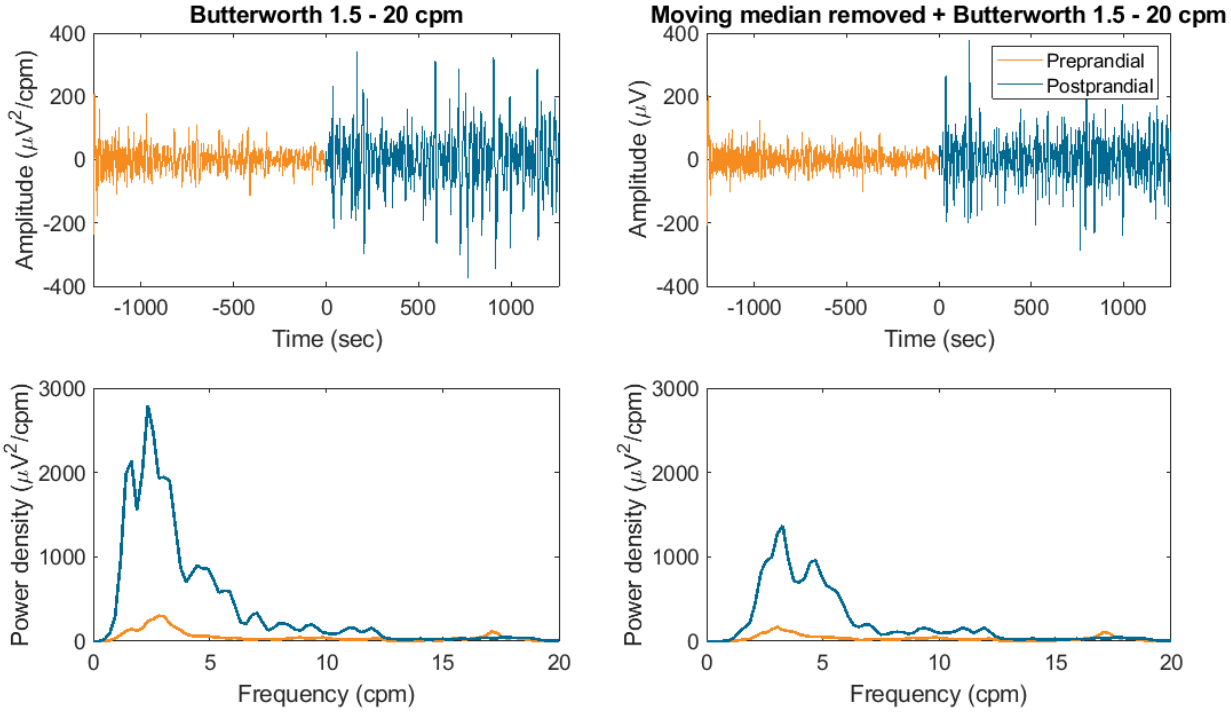

Figure B1. Top left: sEEnG signal after Butterworth filtering with bandpass frequencies of 1.5 and 20 cpm. Bottom left: Power spectral density estimate of this signal. Top right: sEEnG signal after subtracting the moving median with a window length of 20 seconds and Butterworth filtering with bandpass frequencies of 1.5 and 20 cpm. Note that this signal looks slightly different than the signal of which the moving average was not removed. Bottom right: Power spectral density estimate of this signal. Note that the power density is lower and different when compared to the signal on the bottom left.

B1. Therefore, bandpass filtering between 1.5 – 20 cpm was considered sufficient to remove very low frequency offset.

Empirical mode decomposition

A final method with which we tried to remove artefacts was empirical mode decomposition (EMD). EMD is a data-driven method in which the signal is decomposed in different intrinsic mode functions (IMF) [54]. After filtering using a 1.5 – 20 cpm Butterworth bandpass filter, we applied EMD and reconstructed the sEEnG signal by summation of the IMF's with at least 75 % of all power within the 2 – 6 cpm frequency band as determined using Welch's method. However, presumed movement artefacts were still present in the time-domain signal and power spectral density estimates did not differ much between the reconstructed IMF's signal and the original signal, see figure B3. Hence, EMD was not applied to our data.

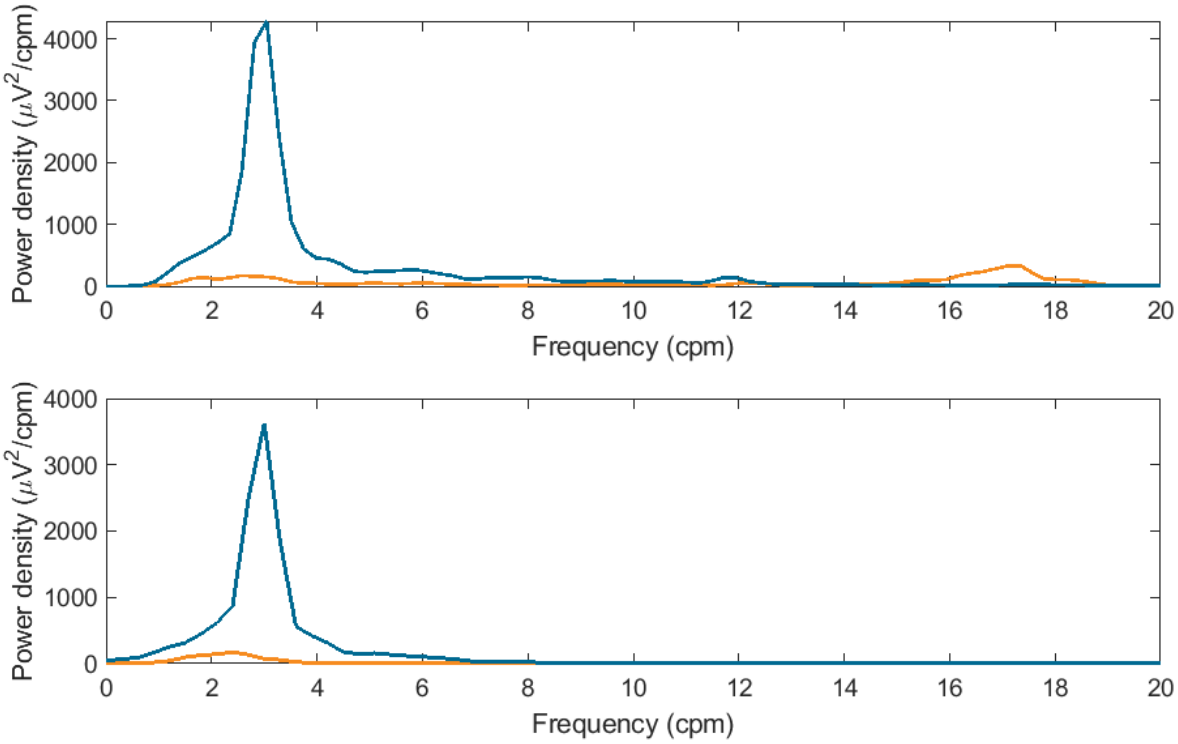

Figure B3. Power spectral density estimates after 1.5 – 20 cpm Butterworth filtering (top) and after selecting IMF's with dominant frequencies between 2 – 6 cpm. Note that estimates in both pre- and postprandial recordings are comparable.

Appendix C: Determining statistically significant wavelet coherence between EGG and sEEnG at the sigmoid

First, both the EGG and the EEnG at the sigmoid signals are put in the function *wco.m*, as described by Maraun et al. The result is the plot as shown in figure C1. The colors indicate the coherence and the thick black lines indicate patches of statistical significance after correction for area wise testing. The narrow black lines also indicate such patches, but these are not corrected for area wise testing. Subsequently, the mean, dominant frequency of the EGG and EEnG at the sigmoid is determined and the range of interest is defined as a 3-cpm band around the dominant frequency, see black dotted lines in figure C1. Next, for every point in time, if there was at least one point of statistical significance within the prespecified frequency range,

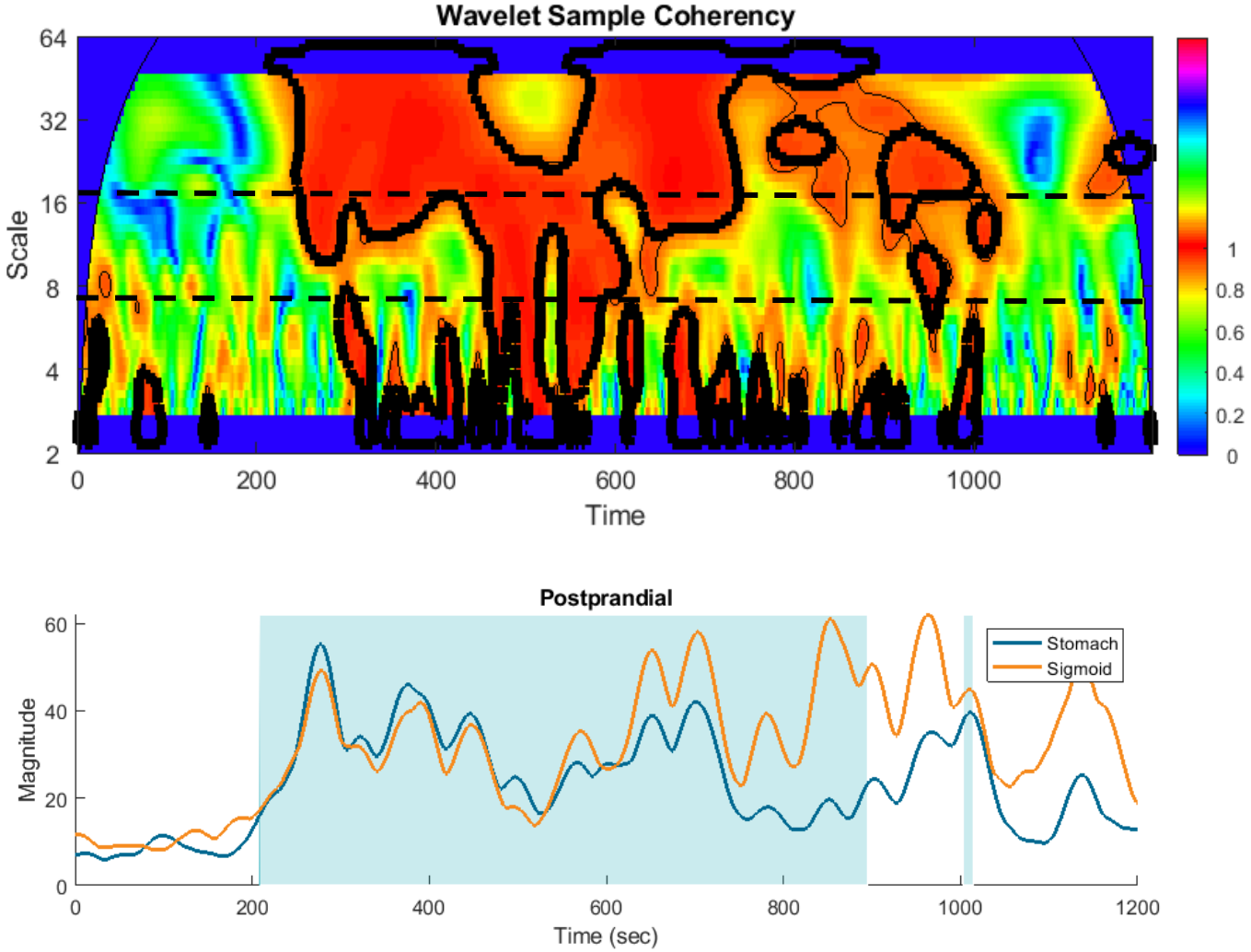

Figure C1. Top: coherence spectrum of the EGG and EEnG at the sigmoid signals. The scale on the y-axis denotes the time duration of one wave in seconds. Areas bound by thick black lines indicate statistically significant patches. The dotted black lines indicate the region of interest regarding frequency, i.e. a 3-cpm band around the mean dominant frequency of the EGG and sEEnG at the sigmoid signal. Bottom: power over time as determined by the continuous wavelet transform for the stomach and sigmoid. Light blue areas indicate statistically significant patches of coherence.

that timepoint was marked as having a significant coherence. Then, continuous wavelet transforms, with the same frequency range as for the coherence, of both signals were obtained and were averaged over the time dimension, i.e. the remaining signal was in the time domain. Finally, these signals were plotted and the timepoints with statistical significant coherence were indicated by light blue areas, see figure C1.

Appendix D: Comparing pre- and postprandial dominant frequency, amplitude and MPD of individual electrodes in healthy adults

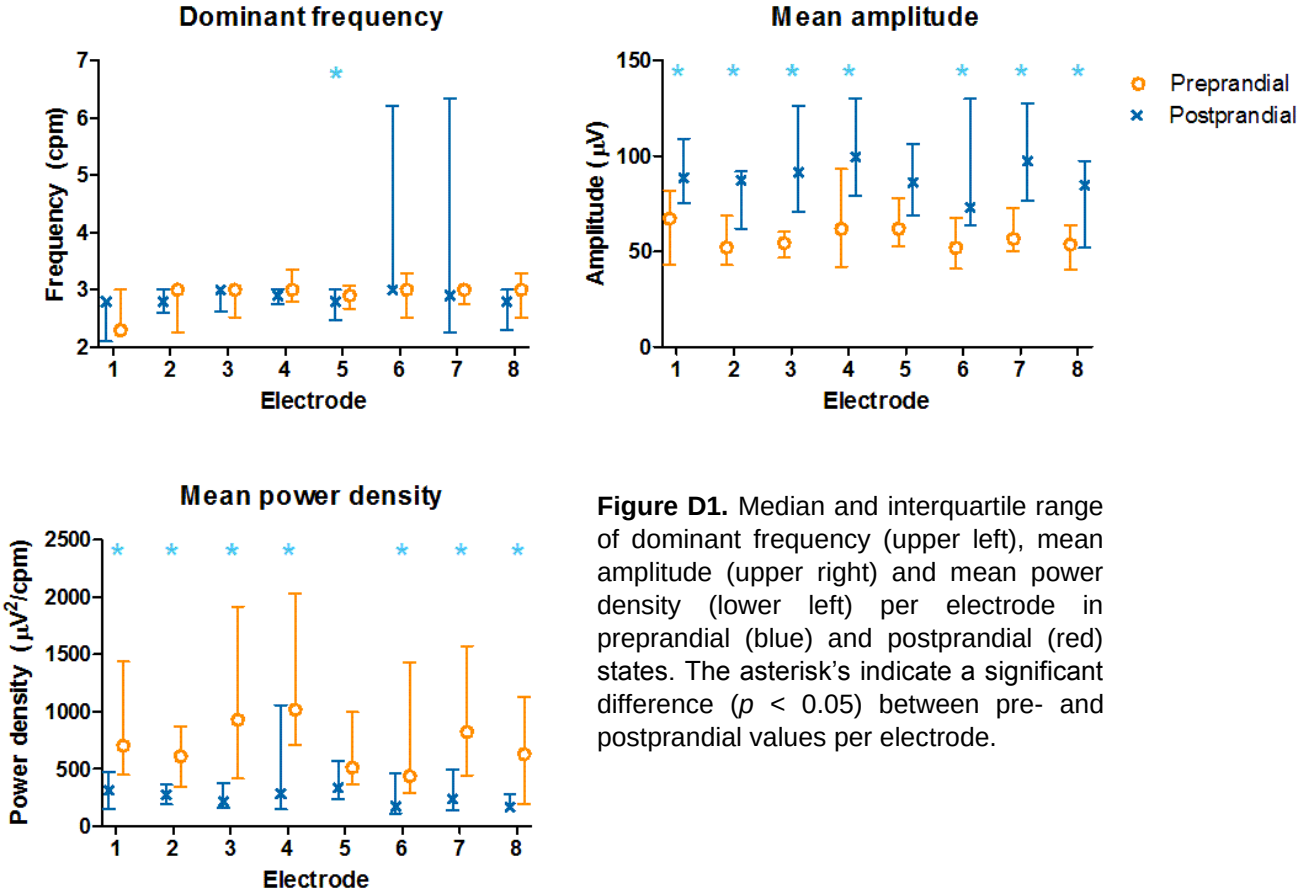

Figure D1. Median and interquartile range of dominant frequency (upper left), mean amplitude (upper right) and mean power density (lower left) per electrode in preprandial (blue) and postprandial (red) states. The asterisk's indicate a significant difference ($p < 0.05$) between pre- and postprandial values per electrode.

Table D1. *P*-values of differences between pre- and postprandial measurements per electrode.

Electrode	Frequency	Amplitude	MPD
1	0.726	0.037	0.028
2	0.670	0.022	0.013
3	0.888	0.005	0.005
4	0.086	0.017	0.047
5	0.008	0.074	0.114
6	0.109	0.013	0.017
7	0.506	0.005	0.005
8	0.858	0.007	0.005

Appendix E: Results of adults with surgically untreated HSCR

Figure E1 and E2 show the power spectral density estimates of two different adults with surgically untreated HSCR. These figures clearly demonstrate postprandial power density is lower in the more distal electrodes (7 and 8) when compared to the more proximal electrodes (1 – 6).

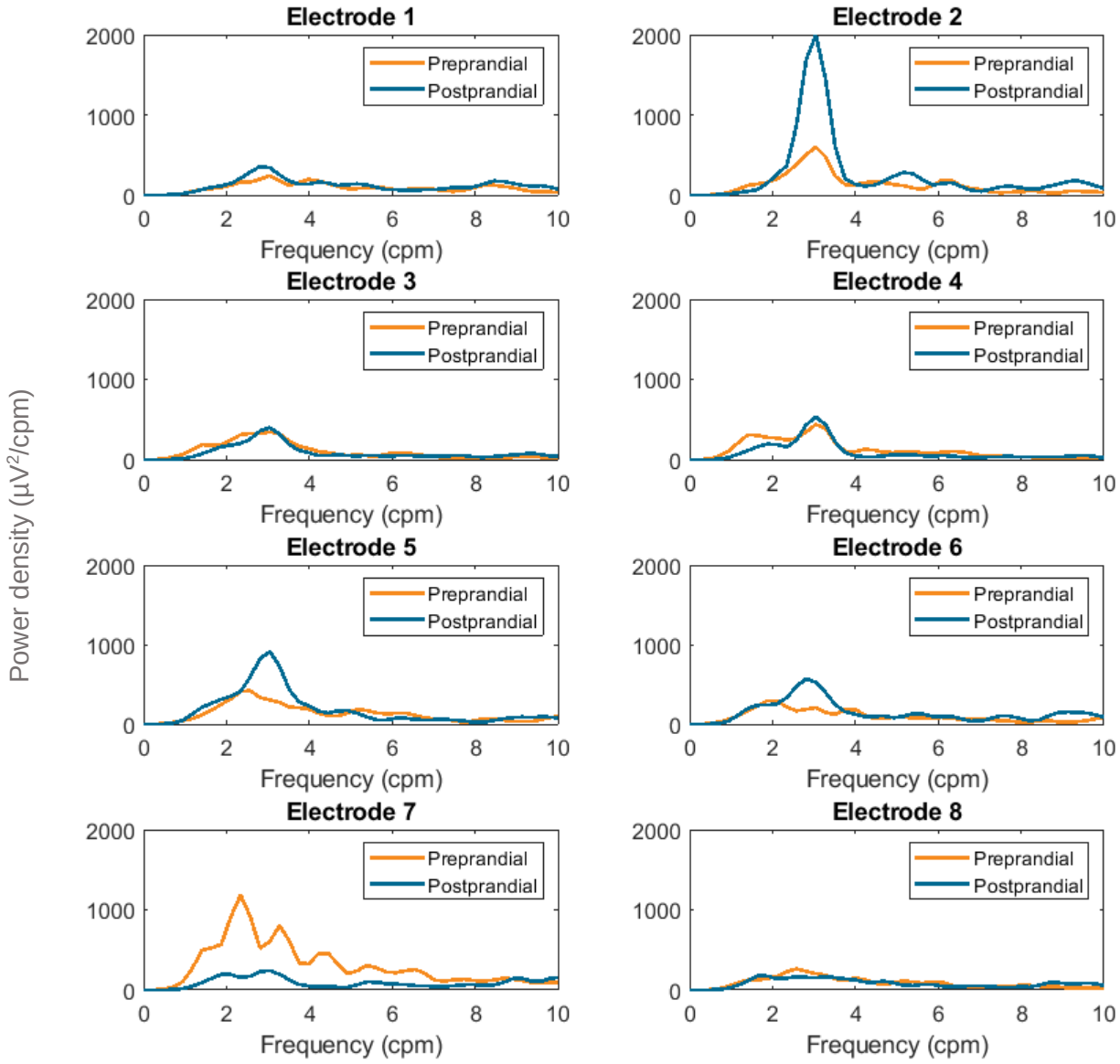

Figure E1. Power spectral density estimates of an adult with surgically untreated HSCR. In electrode 1-6, the postprandial power density around 3 cpm is higher than the preprandial power density. In electrode 7 and 8, the most distal electrodes, the postprandial power density is comparable or even lower than the preprandial power density.

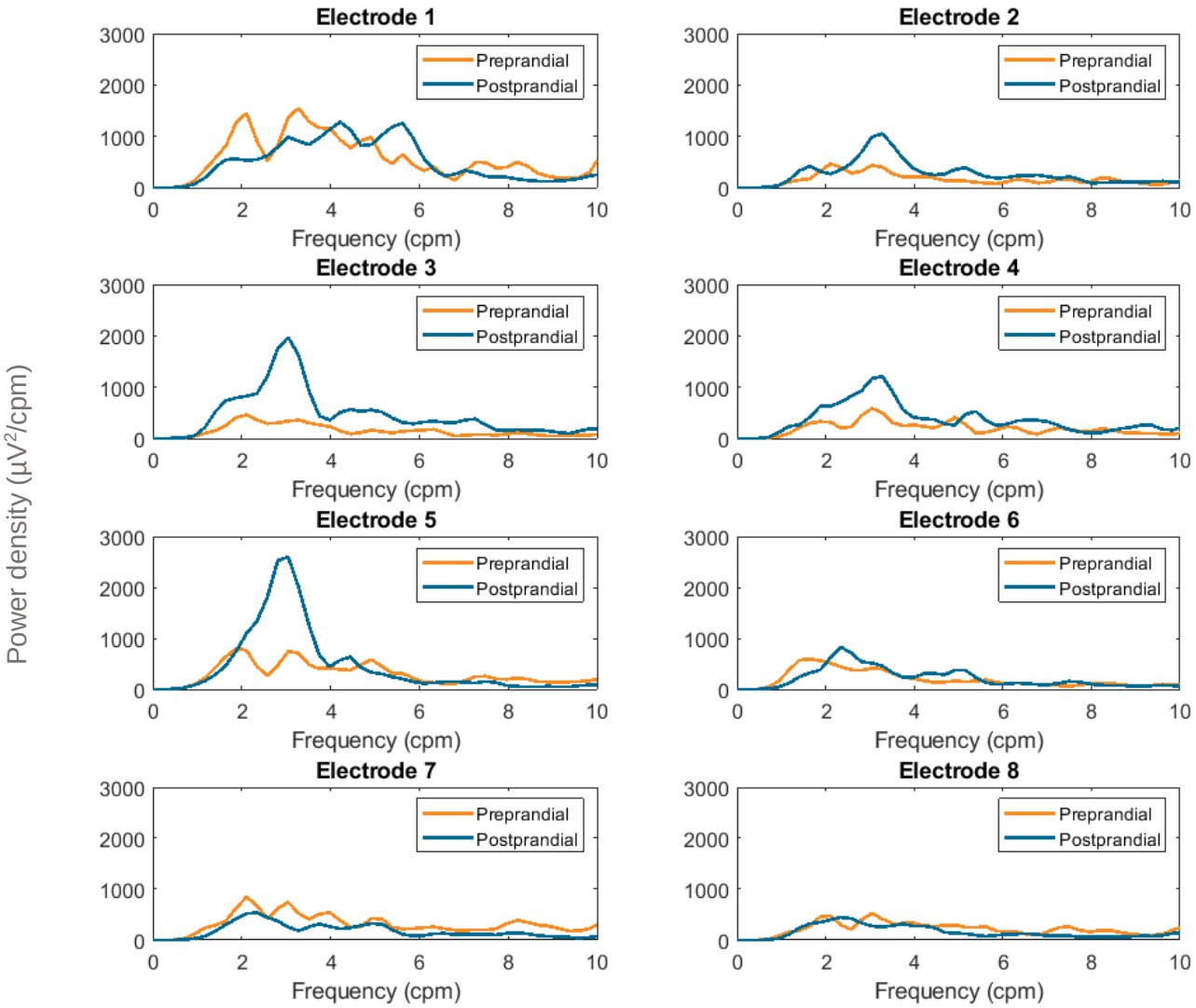

Figure E2. Power spectral density estimates of another adult with surgically untreated HCSR. In electrode 1-6, the postprandial power density around 3 cpm is higher than the preprandial power density. In electrode 7 and 8, the most distal electrodes, the postprandial power density is comparable or even lower than the preprandial power density.

Appendix F: Results of electrode 8 in healthy controls and patients with HSCR

Table F1 shows the dominant frequency, mean amplitude and MPD in healthy controls and subjects. No statistical significant differences are observed, even though mean amplitude and MPD tend to be higher in the patient group. The wide range of the PPD in patients can be explained by one outlier of 1,413%, drastically influencing the results. Omitting this subject from the analysis results in a PPD of -86% – -56% for the patient group.

Table F1. Dominant frequency, mean amplitude and mean power density for electrode 8 in controls and HSCR patients.

	Controls (n=3)	**Patients (n=4)**	***p*-value**
Preprandial			
Dominant frequency (cpm)	2.4 – 3.3	4.2 (3.2 – 4.8)	0.229
Mean amplitude (μV)	57 -74	263 (73 – 372)	0.400
MPD (μV^2/cpm)	161 – 233	6,746 (879 – 11,456)	0.400
Postprandial			
Dominant frequency (cpm)	3.9 – 4.2	3.6 (3.4 – 3.6)	0.057
Mean amplitude (μV)	65 – 107	183 (127 – 253)	0.400
MPD (μV^2/cpm)	293 – 673	3,531 (1,589 – 5,214)	0.229
PPD	26 – 44	4 (-78 – 1,076)	0.857

Cpm = cycles per minute, MPD = mean power density and PPD = power percent difference.