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Research Project

INFLUENCE OF VISCERAL TECHNIQUES ON SEROTONIN LEVEL IN THE BLOOD ANALYSIS: A PHASE 0 PLACEBO CONTROLLED BLINDED STUDY

Scientific paper to acquire the title „D.O. IN OSTEOPATHY" granted by the F.I.C.O

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Declaration

I hereby declare that this thesis has been composed by me and is based on my own work and has not been submitted elsewhere for examination/review. Foreign sources are identified and labelled with information about their origin.

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1. Abstract

TITLE: Influence of visceral techniques on serotonin secretion due to enteric nervous system stimulation and its relevance in osteopathic treatment.

RESEARCH TITLE: Influence of visceral techniques on serotonin level in the blood analysis: a phase 0 placebo controlled blinded study

TUTOR: De Dene Pascal D.O.

METHODOLOGICAL TUTOR: Quaghebeur Jörgen Ph.D Med. Sc.

YEAR: 2018

BACKGROUND: The purpose of this study was to investigate the influence of visceral techniques on serotonin secretion due to enteric nervous system stimulation and its relevance in osteopathic treatment. There is limited evidence that osteopathic visceral treatment cannot only influence the musculoskeletal system of the body but also the autonomic nervous system and the endocrine system. This study could give further information about the connection between the neuro-endocrine system and visceral manipulation.

OBJECTIVE: The aim of this study is to confirm this neuroendocrine mechanism as a consequence of visceral manipulation and answer the specific question: Can osteopathic treatment affect the endocrine changes in the concentration of serotonin in the blood? Possible changes in the level of serotonin in the blood due to osteopathic intervention will suggest whether further research should be developed.

METHOD: Level of serotonin in blood was measured by serotonin lab test of blood sample delivered in phial. Twenty volunteers participated in this study. Ten of them were distributed to the experimental group, the remaining ten were distributed to the control group. Patients in the first group received a specific set of visceral techniques. Patients in the second group received a placebo electrical stimulation.

RESULTS: In both groups changes in the concentration of serotonin are observed. An increase of the level of serotonin was measured in eight out of ten subjects from the experimental group. A decrease of the level of serotonin was measured in seven out of ten from the control group.

DISCUSSION: This study has shown that the visceral manipulations are likely to increase the level of serotonin as a consequence of their execution. As mentioned by several authors in scientific research this may be due to mechanic stimulation of the enteric nervous system. Future studies with a larger population can prove the validity of the thesis in this study and support the research of other authors on the effect of visceral manipulation on neuroendocrine secretion.

CONCLUSION: This study provides preliminary evidence for the influence of visceral manipulations on serotonin secretion due to enteric nervous system stimulation.

KEYWORDS: visceral treatment, serotonin secretion, osteopathy, blood analysis

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5. Introduction

The purpose of this study was to investigate the influence of visceral manipulation on serotonin secretion due to autonomic and enteric nervous system (ENS) stimulation and its relevance in osteopathic treatment.

There is a significant evidence support the hypothesis that osteopathic visceral techniques can influence the musculoskeletal system of the body. Howere for the influence of the autonomic nervous system, the ENS and the neuroendocrine system not much evidence exists. This study could give us some insight about the possible connection between the neuroendocrine system and visceral manipulation (Panagopoulos, 2013).

It is widely known that visceral manipulations are very useful in osteopathic treatment. It is especially useful in biomechanical disorders such as hip pain, lower back pain, and visceral disorders such as dyspepsia, infertility, concussion recovery. It can be very helpful to understand the mechanism of action (Panagopoulos, 2013), (Kramp, 2012), (Christine S. Martin i Eileen V. Johnson), (Harrow), (Wetzler).

6. Background

6.1 Anatomy and physiology

The anatomical and physiological parts of this dissertation are limited only to the key components. As the whole study is directed towards individuals, who have an in-depth knowledge of anatomy and physiology of the human body therefore there is no need to explain all in details. If any topic would be, in the reader's consideration, insufficiently explained, please refer to any book of anatomy and physiology listed in the references section.

6.1.1 Anatomy of the abdominal cavity

The abdominal cavity is a large body cavity in humans and many other animals that contains many organs. It is a part of the abdominopelvic cavity. It is located below

the thoracic cavity, and above the pelvic cavity. Its dome-shaped roof is the thoracic diaphragm, is composed of a thin sheet of muscle under the lungs, and its floor is the pelvic inlet, opening into the pelvis (Wingerd, 1994).

6.1.2 Anatomy of gastro intestinal tract

The human gastrointestinal tract (GIT) consists of the esophagus, stomach, and intestines, and is divided into the upper and lower tract. The GIT includes all structures between the mouth and the anus, forming a continuous passageway that includes the main organs of digestion, namely, the stomach, small intestine, and large intestine. However, the complete human digestive system comprises the GIT plus the accessory organs of digestion (the tongue, salivary glands, pancreas, liver, and gallbladder). The tract may also be divided into foregut, midgut, and hindgut, reflecting the embryological origin of each segment (Rhodes, 2001).

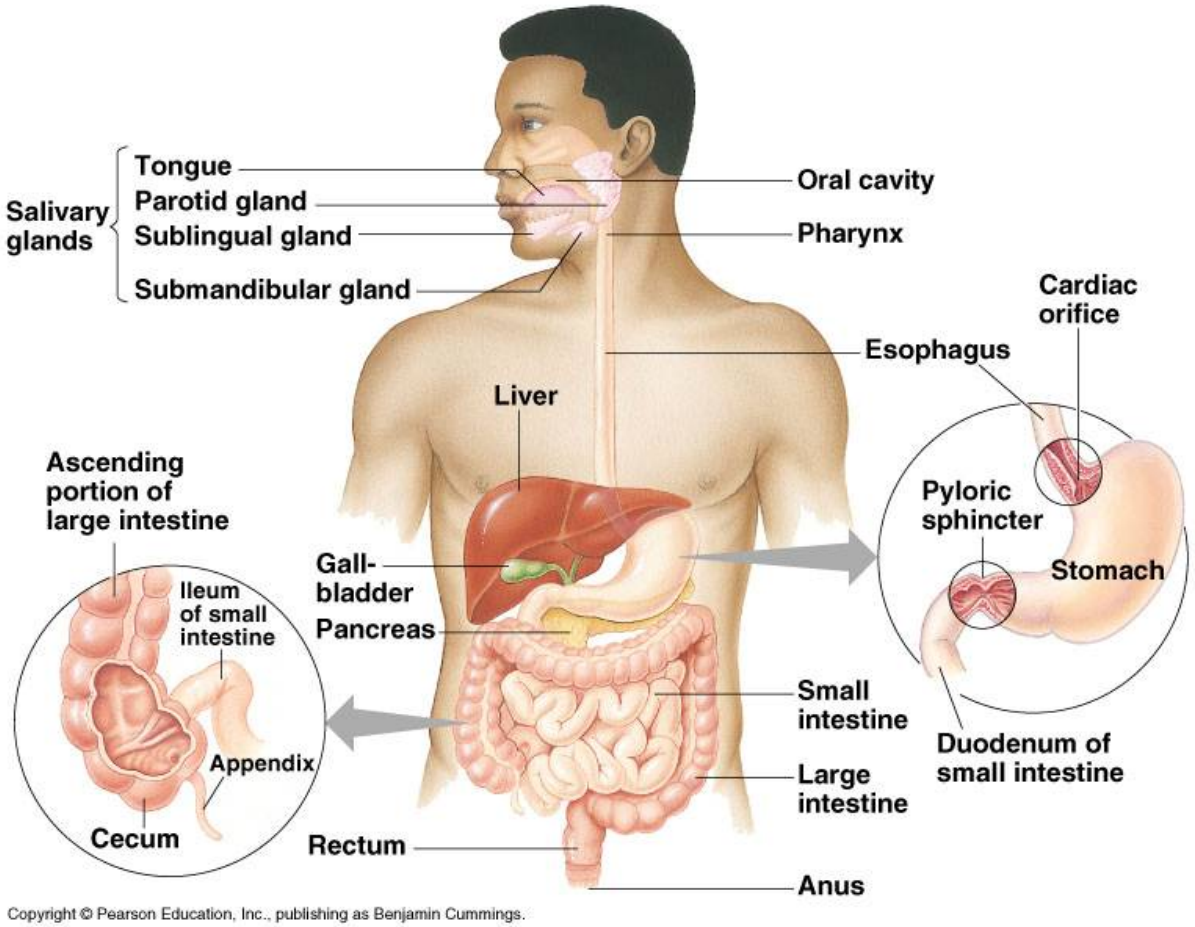

Figure 1: Gastrointestinal tract

6.1.2.1 Upper gastrointestinal tract

The upper GIT consists of the buccal cavity, pharynx, esophagus, stomach, and duodenum. The exact demarcation between the upper and lower tracts is the suspensory muscle of the duodenum. This delineates the embryonic borders between the foregut and midgut, and is also the division commonly used by clinicians to describe gastrointestinal bleeding as being of either "upper" or "lower" origin. Upon dissection, the duodenum may appear to be a unified organ, but it is divided into four segments based upon function, location, and internal anatomy. The four segments of the duodenum are as follows (starting at the stomach, and moving toward the jejunum): bulb, descending, horizontal, and ascending. The suspensory muscle attaches the superior border of the ascending duodenum to the diaphragm (Warrell, 2005).

The suspensory muscle is an important anatomical landmark which shows the formal division between the duodenum and the jejunum, the first and second parts of the small intestine, respectively. This is a thin muscle which is derived from the embryonic mesoderm (Warrell, 2005).

6.1.2.1.1 Anatomy of the stomach

The stomach lies between the oesophagus and the duodenum (the first part of the small intestine). It is in the left upper part of the abdominal cavity. The top of the stomach lies against the diaphragm. Lying behind the stomach is the pancreas. There is also a large double fold of visceral peritoneum called the greater omentum which hangs down from the greater curvature of the stomach. Two sphincters keep the contents of the stomach contained:

- the inferior esophageal sphincter (IES, found in the cardiac region), at the junction of the oesophagus and stomach,
- the pyloric sphincter at the junction of the stomach with the duodenum. (Sherwood, 1997).

The stomach is surrounded by parasympathetic (stimulant) and sympathetic (inhibitor) plexuses (networks of blood vessels and nerves in the anterior gastric, posterior, superior and inferior, celiac and myenteric), which regulate both the secretory activity of the stomach and the motor (motion) activity of its muscles (Sherwood, 1997).

In adult humans, the stomach has a relaxed, near empty volume of about 75 milliliters. Because it is a distensible organ, it normally expands to hold about one liter of food. The

stomach of a newborn human baby will only be able to retain about 30 milliliters (Sherwood, 1997).

In classical anatomy, the human stomach is divided into four sections, beginning at the gastric cardia, each of which has different cells and functions:

- The cardia is where the contents of the oesophagus empty into the stomach. The cardia is defined as the region following the "z-line" of the gastroesophageal junction, the point at which the epithelium changes from stratified squamous to columnar. Near the cardia is the IES.
- The fundus (from Latin, "bottom") is formed by the upper curvature of the organ.
- The body is the main, central region.
- The pylorus (from Greek, "gatekeeper") is the lower section of the organ that facilitates emptying the contents into the small intestine (Brunicardi, Andersen, i al., 2010).

6.1.2.1.2 Anatomy of the duodenum

The duodenum is a 25–30 cm (10-12 inch) C-shaped structure lying adjacent to the stomach. It is divided anatomically into four sections. The first part of the duodenum lies within the peritoneum but its other parts are retroperitoneal (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005), (J i JP, 2011).

The first part, or superior part, of the duodenum is a continuation from the pylorus to transpyloric plane. It is superior to the rest of the segments, at the vertebral level of L1. The duodenal bulb about 2 cm long, is the very first part of the duodenum and is slightly dilated. The duodenal bulb is a remnant of the mesoduodenum, a mesentery which suspend the organ from the posterior abdominal wall in fetal life (Singh i Pal, 2012).

The first part of the duodenum is mobile, and connected to the liver by the hepatoduodenal ligament of the lesser omentum. The first part of the duodenum ends at the corner, the superior duodenal flexure (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

The second part, or descending part, of the duodenum begins at the superior duodenal flexure. It goes inferior to the lower border of vertebral body L3, before making a sharp turn medially into the inferior duodenal flexure, the end of the descending part. The pancreatic duct and common bile duct enter the descending duodenum, through the major duodenal papilla. The second part of the duodenum also contains the minor duodenal papilla, the entrance for the accessory pancreatic duct. The junction between the embryological foregut and midgut lies just below the major duodenal papilla (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

The third part, or horizontal part or inferior part of the duodenum begins at the inferior duodenal flexure and passes transversely to the left, passing in front of the inferior vena cava, abdominal aorta and the vertebral column. The superior mesenteric artery and vein are anterior to the third part of duodenum. This part may be compressed between the aorta and SMA causing superior mesenteric artery syndrome (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

The fourth part, or ascending part, of the duodenum passes upward, joining with the jejunum at the duodenojejunal (DJ) flexure. The fourth part of the duodenum is at the vertebral level L2, and may pass directly on top of, or slightly left to, the aorta (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

Under microscopy, the duodenum has a villous mucosa. This is distinct from the mucosa of the pylorus, which directly joins to the duodenum. Like other structures of the GIT, the duodenum has a mucosa, submucosa, muscularis externa, and adventitia. Glands line the duodenum, known as Brunner's glands, which secrete mucus and bicarbonate in order to neutralise stomach acids. These are distinct glands not found in the ileum or jejunum, the other parts of the small intestine (Deakin i J., 2006).

6.1.2.2 Lower gastrointestinal tract

The lower GI tract includes most of the small intestine and all of the large intestine. In human anatomy, the intestine is the segment of the GIT extending from the pyloric sphincter of the stomach to the anus and, as in other mammals, consists of two segments, the small intestine and the large intestine. In humans, the small intestine is further subdivided into the duodenum, jejunum and ileum while the large intestine is subdivided into the cecum, colon, rectum, and anal canal (Kapoor, 13 Jul 2011), (Gray, 1918).

6.1.2.2.1 Anatomy of the intestine

The small intestine or small bowel is the part of the GIT tract between the stomach and the large intestine, and is where most of the end absorption of food takes place. The small intestine has three distinct regions – the duodenum, jejunum, and ileum. The duodenum is the shortest part of the small intestine and is where preparation for absorption begins. The primary function of the small intestine is the absorption of nutrients and minerals from food, using small finger-like protrusions called villi (Britannica, 2018).

The jejunum is the midsection of the small intestine, connecting the duodenum to the ileum. It is about 2.5 m long, and contains the plicae circulares, and villi that increase its surface area. Products of digestion (sugars, amino acids, and fatty acids) are absorbed into the bloodstream here. The suspensory muscle of duodenum marks the division between the duodenum and the jejunum (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

The ileum: the final section of the small intestine. It is about 3 m long, and contains villi similar to the jejunum. It absorbs mainly vitamin B12 and bile acids, as well as any other remaining nutrients. The ileum joins to the cecum of the large intestine at the ileocecal junction (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

The jejunum and ileum are suspended in the abdominal cavity by mesentery. The mesentery is part of the peritoneum. Arteries, veins, lymph vessels and nerves travel within the mesentery (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

6.1.2.3 Anatomy of the peritoneum

The peritoneum is one continuous sheet of tissue, forming two layers and a potential space between them: the peritoneal cavity.

The outer layer, the parietal peritoneum, is attached to the abdominal wall and the pelvic walls. The tunica vaginalis, the serous membrane covering the male testis, is derived from the vaginal process, an outpouching of the parietal peritoneum.

The inner layer, the visceral peritoneum, is wrapped around the visceral organs, located inside the intraperitoneal space for protection. It is thinner than the parietal peritoneum. The mesentery is a double layer of visceral peritoneum that attaches to the GIT. There are often blood vessels, nerves, and other structures between these layers. The space between these two layers is technically outside of the peritoneal sac, and thus not in the peritoneal cavity.

The potential space between these two layers is the peritoneal cavity, filled with a small amount (about 50 mL) of slippery serous fluid that allows the two layers to slide freely over each other (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005), (Tank, 2013).

6.1.2.4 Anatomy of the mesentery

The mesentery is a continuous set of tissues now recognized as an organ and entered as such in the 2017 edition of Gray's Anatomy. The mesentery is a set of tissues which is formed by the double fold of peritoneum that attaches the intestines to the wall of the abdomen, supplying blood vessels, lymphatics and nerves and storing fat. It has been proposed for reclassification as an organ due to research findings at the University of Limerick in 2010 (Coffey i O'Leary, 2016), (Guarino, 4 January 2017).

Conventional teaching has described the mesocolon as a fragmented structure with all the named parts—the ascending, transverse, descending, and
sigmoid mesocolons, mesoappendix, and mesorectum as separately terminating their insertion into the posterior abdominal wall (Coffey J. , August 2013).

In 2012, following detailed microscopic and electron microscopic examinations, the mesocolon was shown to be a single, continuous structure that commenced from the DJ flexure and extended to the level of the distal mesorectum. This simpler concept has enabled substantial advances to be made in different aspects of surgery on the colon and rectum. It has also had implications for sciences related to surgery, anatomy, and development (Coffey JC, June 2014).

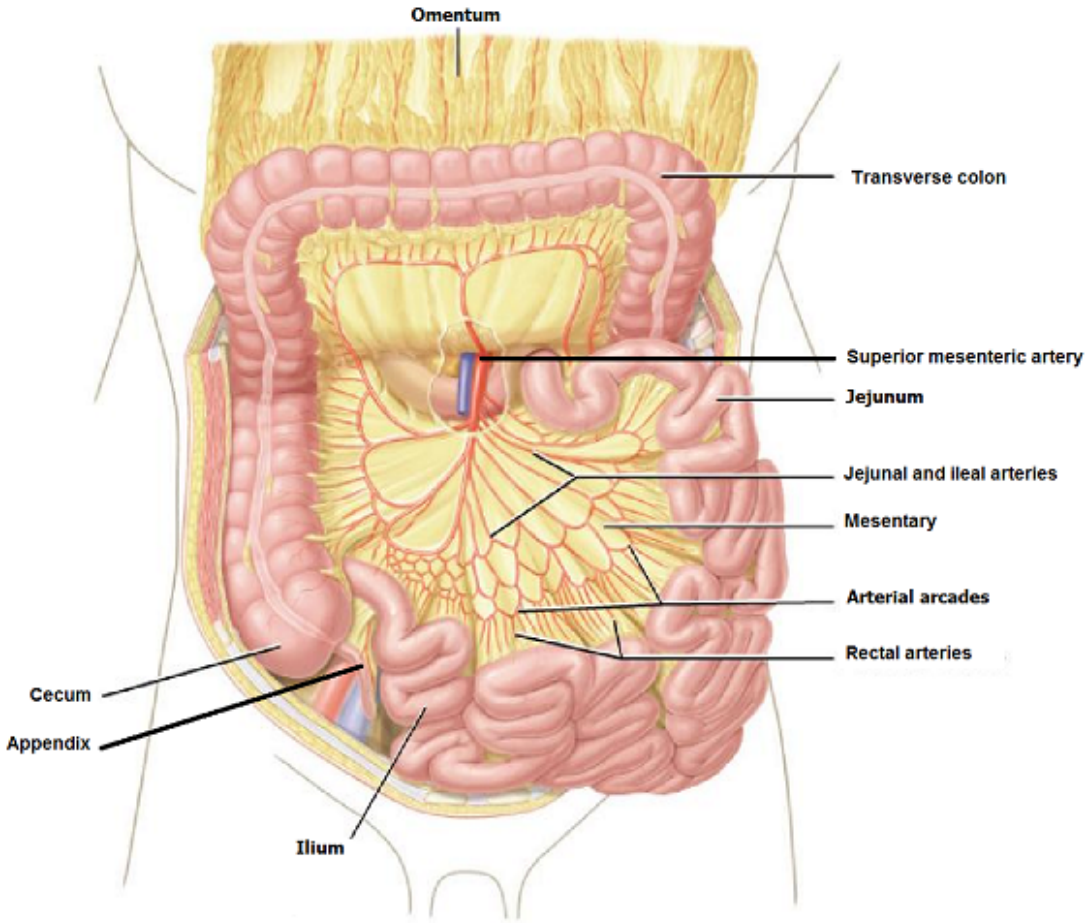

Figure 2: Mesentery (from www.quora.com)

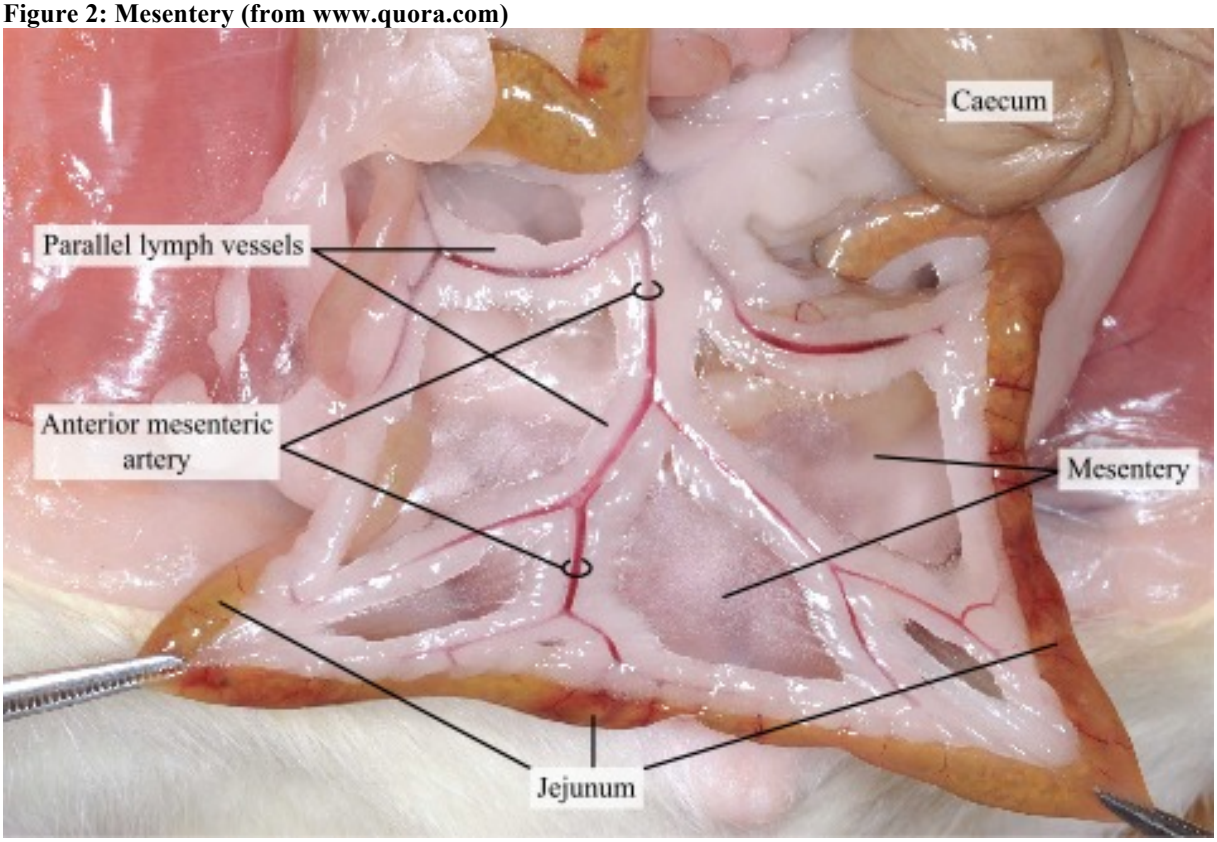

Figure 3: Mesentery 2 (from www.bszm.elte.hu)

6.1.2.5 Arterial supply

The small intestine receives a blood supply from the coeliac trunk and the superior mesenteric artery. These are both branches of the aorta. The duodenum receives blood from the coeliac trunk via the superior pancreaticoduodenal artery and from the superior mesenteric artery via the inferior pancreaticoduodenal artery. These two arteries both have anterior and posterior branches that meet in the midline and anastomose. The jejunum and ileum receive blood from the superior mesenteric artery. Branches of the superior mesenteric artery form a series of arches within the mesentery known as arterial arcades, which may be several layers deep. Straight blood vessels known as vasa recta travel from the arcades closest to the ileum and jejunum to the organs themselves (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

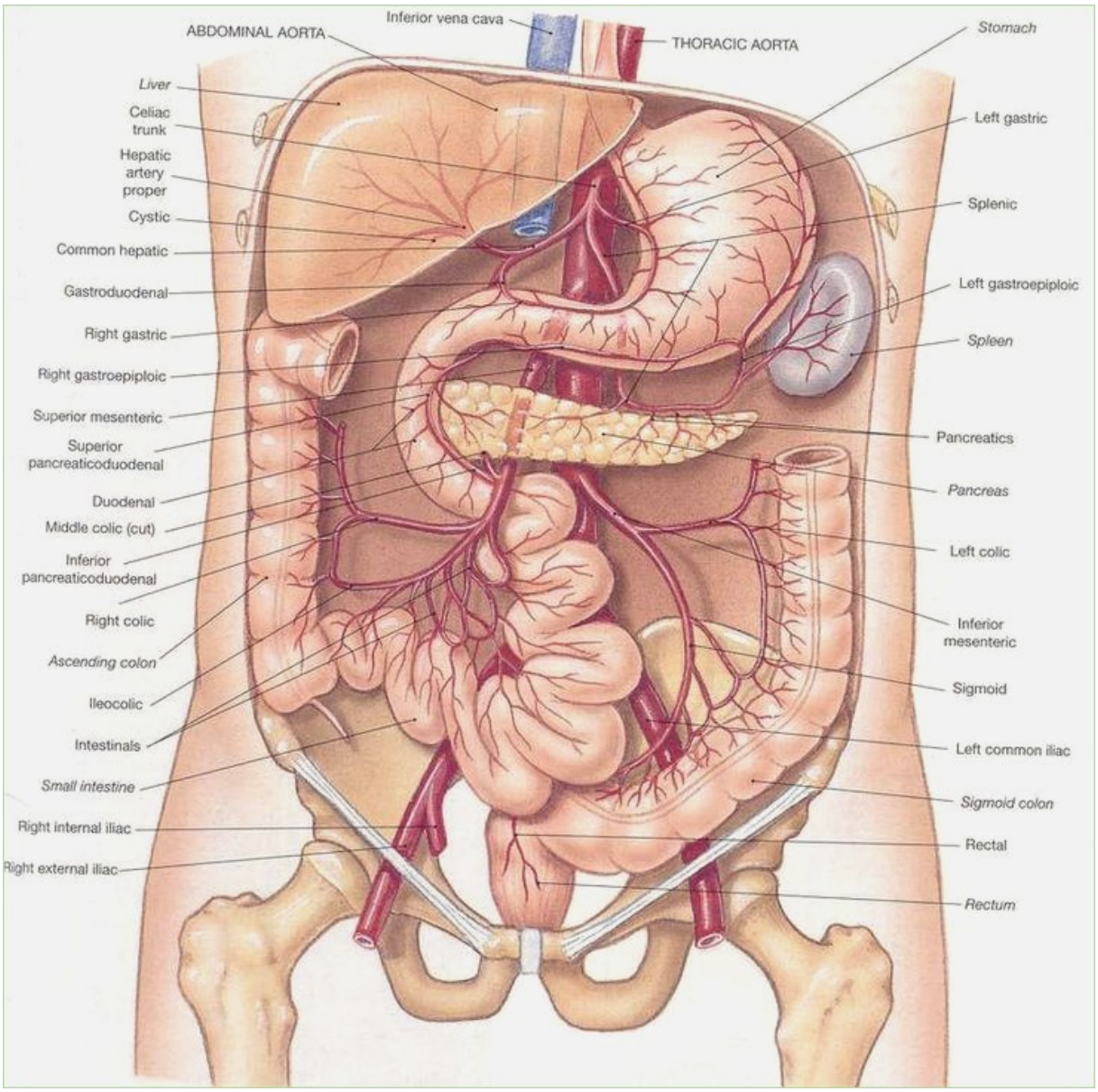

Figure 4: Arterial supply of the abdomen (from www.nanoprom.info)

6.1.2.6 Hepatic portal system

The portal venous system is responsible for directing blood from parts of the GIT to the liver. Substances absorbed in the small intestine travel first to the liver for processing before continuing to the heart. Not all of the GIT is part of this system. The system extends from about the lower portion of the esophagus to the upper part of the anal canal. It also includes venous drainage from the spleen and pancreas.

Blood flow to the liver is unique in that it receives both oxygenated and (partially) deoxygenated blood. As a result, the partial gas pressure of oxygen (pO_2) and perfusion pressure of portal blood are lower than in other organs of the body. Blood passes from branches of the portal vein through cavities between "plates" of hepatocytes called sinusoids. Blood also flows from branches of the hepatic artery and mixes in the sinusoids to supply the hepatocytes with oxygen. This mixture percolates through the sinusoids and collects in a central vein which drains into the hepatic vein. The hepatic vein subsequently drains into the inferior vena cava. The hepatic artery provides 30 to 40% of the oxygen to the liver, while only accounting for 25% of the total liver blood flow. The rest comes from the partially deoxygenated blood from the portal vein. The liver consumes about 20% of the total body oxygen when at rest. That is why the total liver blood flow is quite high, at about one liter a minute and up to two liters a minute. That is on average one fourth of the average cardiac output at rest.

Large veins that are considered part of the *portal venous system* are the:

- Hepatic portal vein
- Splenic vein
- Superior mesenteric vein
- Inferior mesenteric vein

The superior mesenteric vein and the splenic vein come together to form the actual hepatic portal vein. The inferior mesenteric vein connects in the majority of people on the splenic vein, but in some people, it is known to connect on the portal vein or the superior mesenteric vein.

Roughly, the portal venous system corresponds to areas supplied by the celiac trunk, the superior mesenteric artery, and the inferior mesenteric artery (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

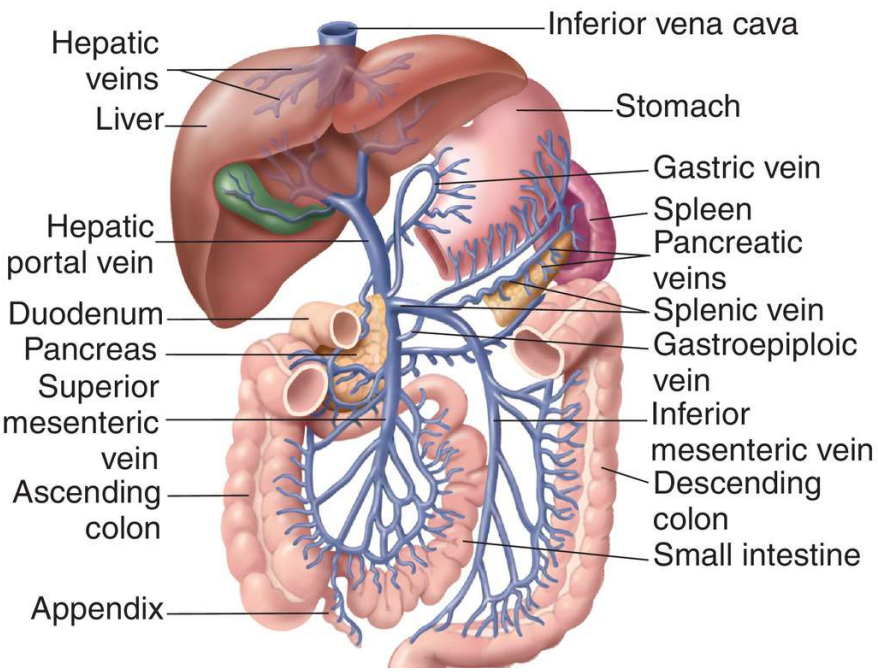

Figure 5: hepatic portal system (from www.medical-dictionary.thefreedictionary.com

6.1.2.7 Innervation of the GI

Parasympathetic innervation to the ascending colon is supplied by the vagus nerve. Sympathetic innervation is supplied by the splanchnic nerves that join the celiac ganglia. Most of the digestive tract is innervated by the two large celiac ganglia, with the upper part of each ganglion joined by the greater splanchnic nerve and the lower parts joined by the lesser splanchnic nerve. It is from these ganglia that many of the gastric plexuses arise (Hall, 2011).

6.1.2.7.1 Sympathetic

There are two kinds of neurons involved in the transmission of any signal through the sympathetic system: pre-ganglionic and post-ganglionic. The shorter preganglionic neurons originate from the thoracolumbar region of the spinal cord specifically at T1 to L2~L3, and travel to a ganglion, often one of the paravertebral ganglia, where they synapse with a postganglionic neuron. From there, the long postganglionic neurons extend across most of the body (Drake, Vogl, Tibbitts, Richard, i Richardson, 2005).

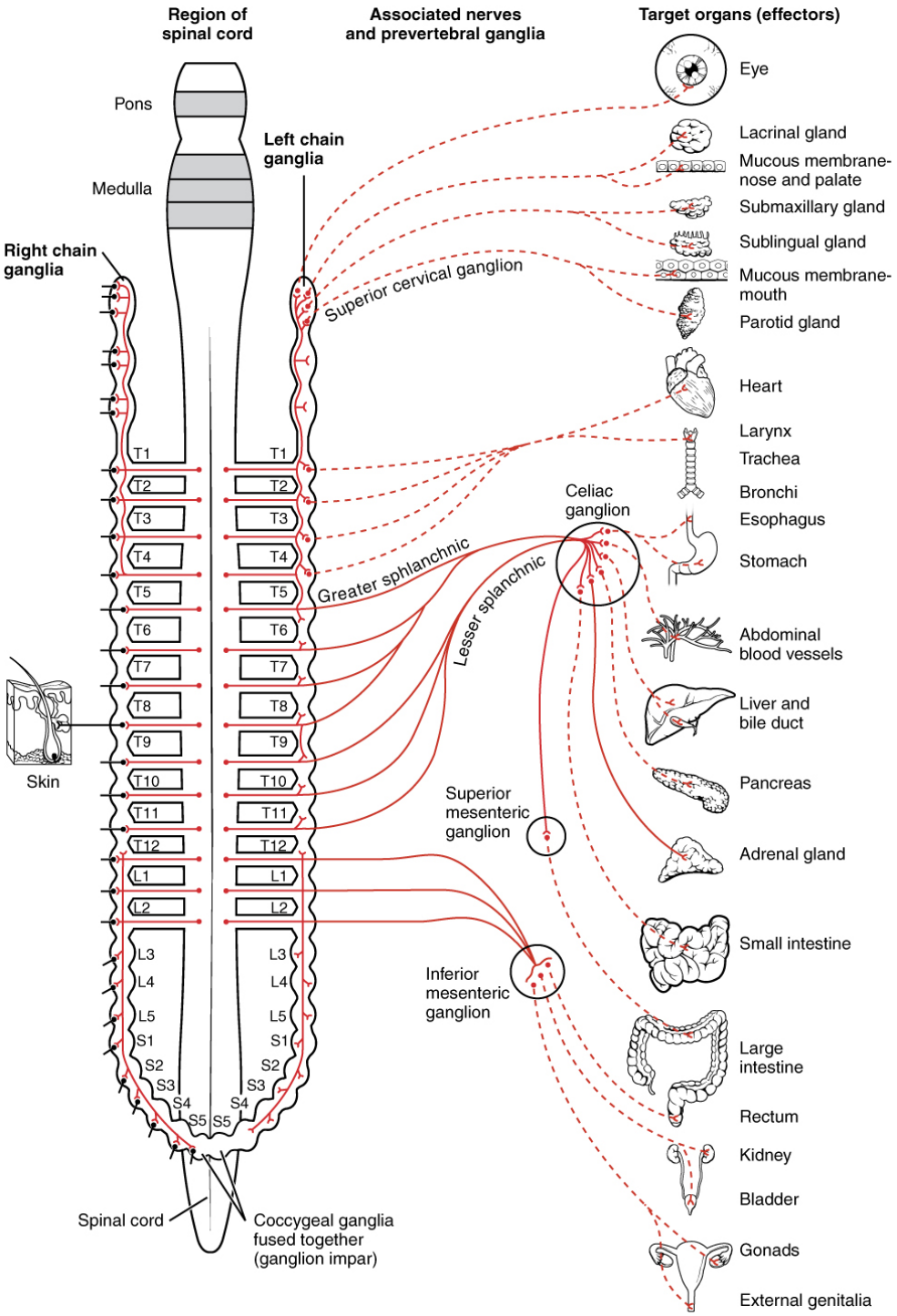

Figure 6: Sympatethic nervous system (from https://en.wikipedia.org)

6.1.2.7.2 Parasympatethic

The parasympathetic nerves are autonomic or visceral branches of the peripheral nervous system (PNS). Parasympathetic nerve supply arises through three primary areas:

- Some of the cranial nerves (CN) in the cranium, namely the preganglionic parasympathetic nerves (CN III, CN VII, and CN IX) usually arise from specific nuclei in the central nervous system (CNS) and synapse at one of four

parasympathetic ganglia: ciliary, pterygopalatine, otic, or submandibular. From these four ganglia, the parasympathetic nerves complete their journey to target tissues via trigeminal branches (ophthalmic nerve, maxillary nerve, mandibular nerve).

- The vagus nerve does not participate in these cranial ganglia as most of its parasympathetic fibers are destined for a broad array of ganglia on or near thoracic viscera (esophagus, trachea, heart, lungs) and abdominal viscera (stomach, pancreas, liver, kidneys, small intestine, and about half of the large intestine). The vagus innervation ends at the junction between the midgut and hindgut, just before the splenic flexure of the transverse colon.
- The pelvic splanchnic efferent preganglionic nerve cell bodies reside in the lateral gray horn of the spinal cord at the T12-L1 vertebral levels (the spinal cord terminates at the L1-L2 vertebrae with the conus medullaris), and their axons exit the vertebral column as S2-S4 spinal nerves through the sacral foramina. Their axons continue away from the CNS to synapse at an autonomic ganglion. The parasympathetic ganglion where these preganglionic neurons synapse will be close to the organ of innervation. This differs from the sympathetic nervous system, where synapses between pre- and post-ganglionic efferent nerves in general occur at ganglia that are farther away from the target organ.

As in the sympathetic nervous system, efferent parasympathetic nerve signals are carried from the CNS to their targets by a system of two neurons. The first neuron in this pathway is referred to as the preganglionic or presynaptic neuron. Its cell body sits in the CNS and its axon usually extends to synapse with the dendrites of a postganglionic neuron somewhere else in the body. The axons of presynaptic parasympathetic neurons are usually long, extending from the CNS into a ganglion that is either very close to or embedded in their target organ. As a result, the postsynaptic parasympathetic nerve fibers are very short (Encyclopedia), (Moore i Agur, 2007).

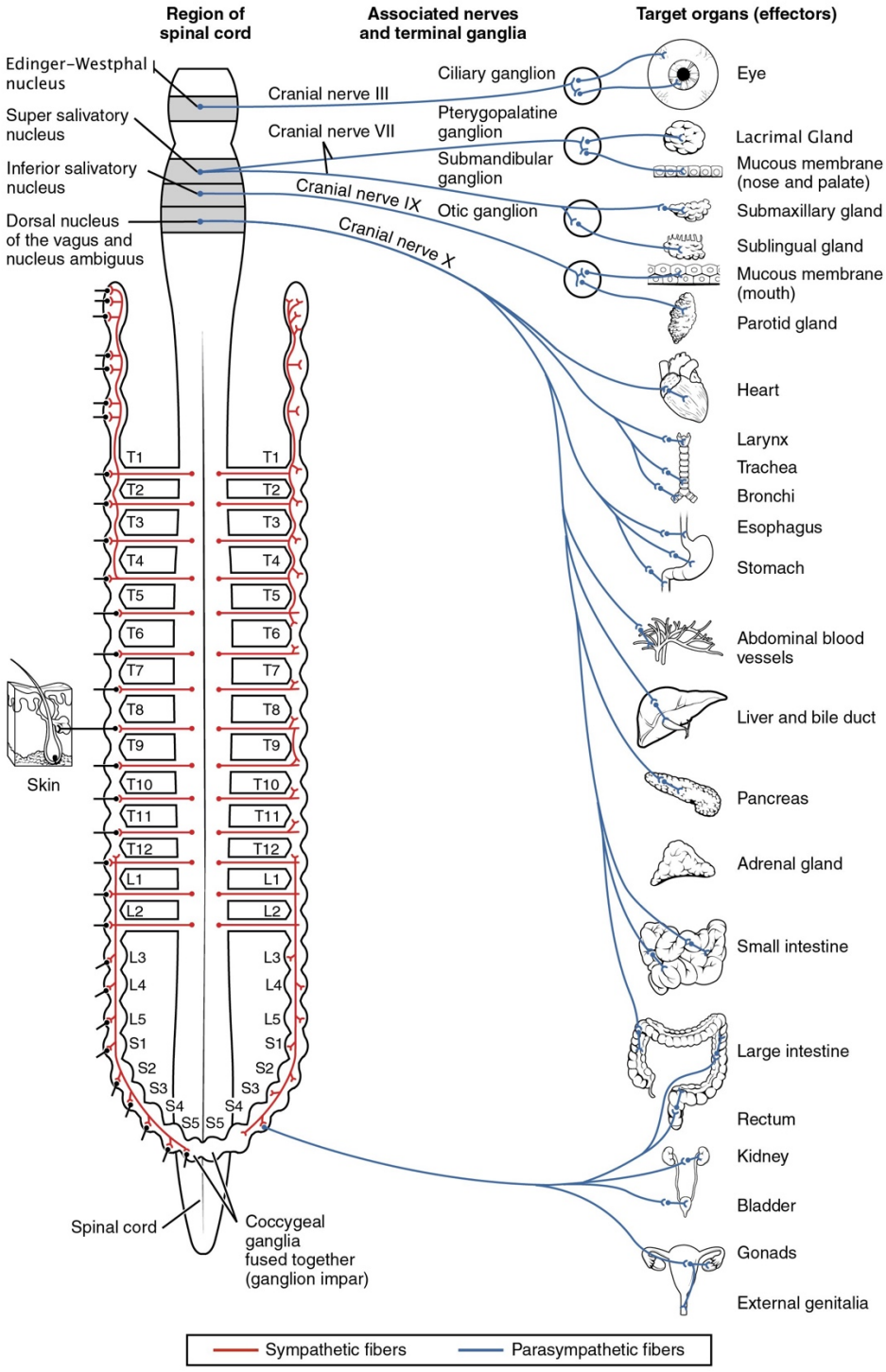

Figure 7: Parasympathetic nervous system (from https://en.wikipedia.org)

6.1.2.7.3 Enteric nervous system

The ENS consists of some one hundred million neurons that are embedded in the peritoneum, the lining of the GIT extending from the esophagus to the anus. These neurons are collected into two plexuses - the myenteric (or Auerbach's) plexus that lies between the longitudinal and the smooth muscle layers, and the submucosal (or Meissner's)

plexus that lies between the circular smooth muscle layer and the mucosa (Boron i Boulpaep, 2005), (Hall, 2011), (Costa, Brookes, i Hennig, 2000).

Control of the digestive system is also maintained by ENS, which can be thought of as a digestive brain that can help to regulate motility, secretion and growth. Sensory information from the digestive system can be received, integrated and acted upon by the enteric system alone. When this occurs, the reflex is called a short reflex.[3] Although this may be the case in several situations, the ENS can also work in conjunction with the CNS; vagal afferents from the viscera are received by the medulla, efferents are affected by the vagus nerve. When this occurs, the reflex is called vago-vagal reflex. The myenteric plexus and submucosal plexus are both located in the gut wall and receive sensory signals from the lumen of the gut or the CNS (Silverthorn Ph. D, April 2, 2006), (Bowen DVM PhD, July 5, 2006).

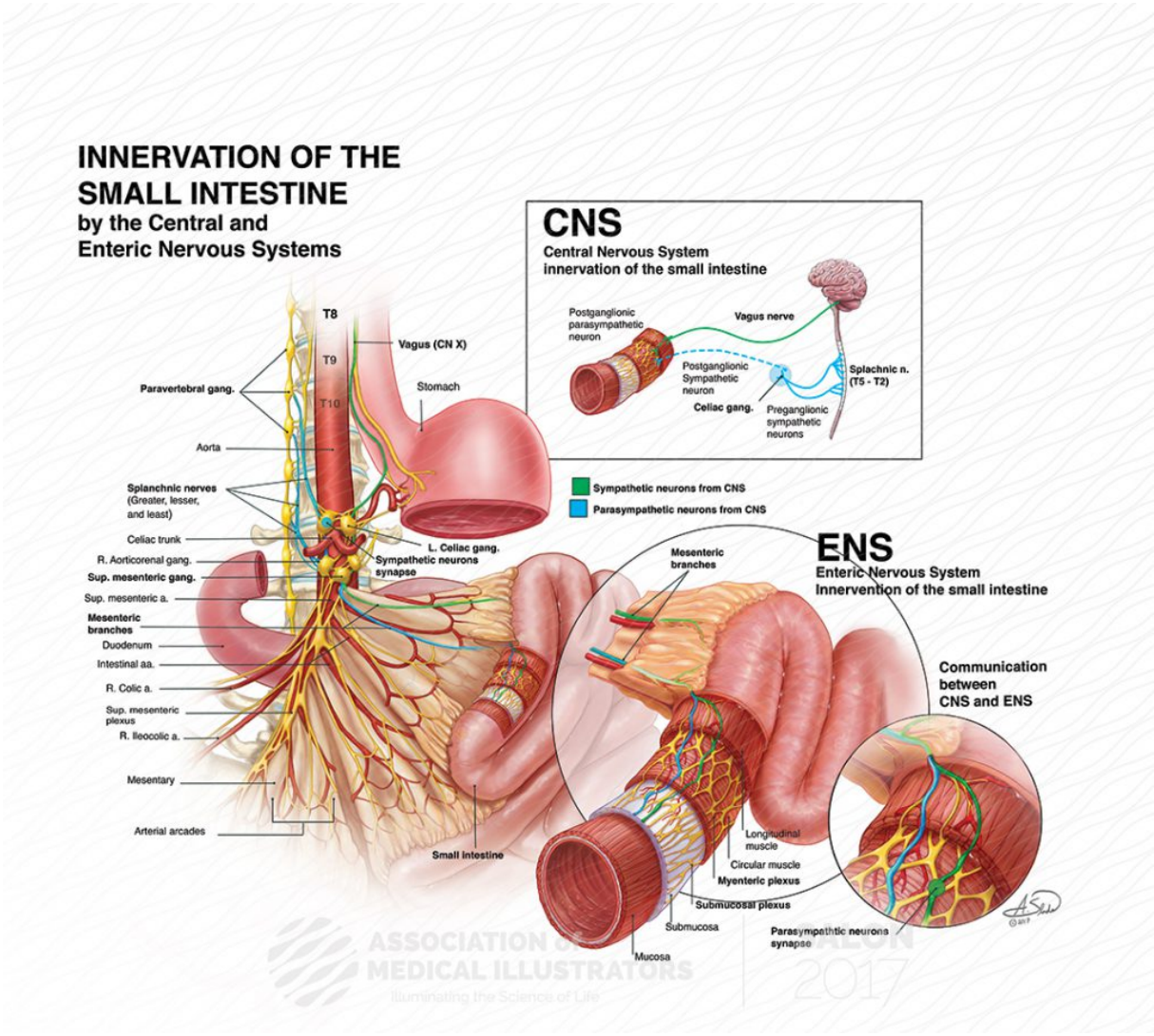

Figure 8: Enteric Nervous System (from http://meetings.ami.org)

6.1.3 Physiology of serotonin

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan, (González-Flores, 2011). Serotonin is primarily found in the GI tract, blood platelets, and the CNS of animals, including humans. It is popularly thought to be a contributor to feelings of well-being and happiness (Young, 2007).

Approximately 90% of the human body's total serotonin is located in the enterochromaffin (EC) cells in the GI tract, where it is used to regulate intestinal movements (King, 2009), (Berger M, 2009). The serotonin is secreted luminally and basolaterally which leads to increased serotonin uptake by circulating platelets and activation after stimulation, which gives increased stimulation of myenteric neurons and gastrointestinal motility (Yano JM, 2015). The remainder is synthesized in serotonergic neurons of the CNS, where it has various functions. These include the regulation of mood, appetite, and sleep. Serotonin also has some cognitive functions, including memory and learning. Modulation of serotonin at synapses is thought to be a major action of several classes of pharmacological antidepressants.

Serotonin secreted from the EC cells eventually finds its way out of tissues into the blood. There, it is actively taken up by blood platelets, which store it. When the platelets bind to a clot, they release serotonin, where it can serve as a vasoconstrictor and/or a vasodilator while regulating hemostasis and blood clotting. In high concentrations, serotonin acts as a vasoconstrictor by contracting endothelial smooth muscle directly or by potentiating the effects of other vasoconstrictors (e.g. angiotensin II, norepinephrine). The vasoconstrictive property is mostly seen in pathologic states affecting the endothelium such as atherosclerosis or chronic hypertension. In physiologic states, vasodilation occurs through the serotonin mediated release of nitric oxide from endothelial cells. Additionally, it inhibits the release of norepinephrine from adrenergic nerves (PM, February 1987). Serotonin is also a growth factor for some types of cells, which may give it a role in wound healing. There are various serotonin receptors.

Serotonin is metabolized mainly to 5-HIAA, chiefly by the liver. Metabolism involves first oxidation by monoamine oxidase to the corresponding aldehyde. This is followed by oxidation by aldehyde dehydrogenaseto 5-HIAA, the indole acetic acid derivative. The latter is then excreted by the kidneys.

Aside from mammals it is found in all bilateral animals including worms and insects, as well as in fungi and plants. Serotonin's presence in insect venoms and plant spines serves to cause pain, which is a side-effect of serotonin injection. Serotonin is produced by pathogenic

amoebae, and its effect in the human gut is diarrhea. Its widespread presence in many seeds and fruits may serve to stimulate the digestive tract into expelling the seeds(Chen J, 2010), (McGowan K, August 1983), (Feldman JM, October 1985).

6.2 Enterochromaffin cells

EC cells (also known as Kulchitsky cells), discovered by Nikolai Kulchitsky of Karazin Kharkiv National University (Drozdov, Modlin, Kidd, i Goloubinov, 2009). They are a type of enteroendocrine and neuroendocrine cell. They reside alongside the epithelium lining the lumen of the digestive tract and play a crucial role in gastrointestinal regulation, particularly intestinal motility and secretion. EC cells modulate neuron signaling in the ENS via the secretion of the neurotransmitter serotonin and other peptides. As enteric afferent and efferent nerves do not protrude into the intestinal lumen, EC cells act as a form of sensory transduction (Bertrand i Bertrand, 2010). Serotonin in the ENS acts in synergy with other digestive hormones to regulate sensory and motor gastrointestinal reflexes. EC cells respond to both chemical and neurological stimuli. They are also reactive to mechanosensation and can be stimulated by a bolus moving through the bowel. Upon activation, EC cells release serotonin to act upon serotonin receptors on ENS neurons. Dependent on concentration, serotonin can then modulate peristaltic contraction and secretion through activation of smooth muscle and glands respectively (Mawe i Hoffman, 2013).

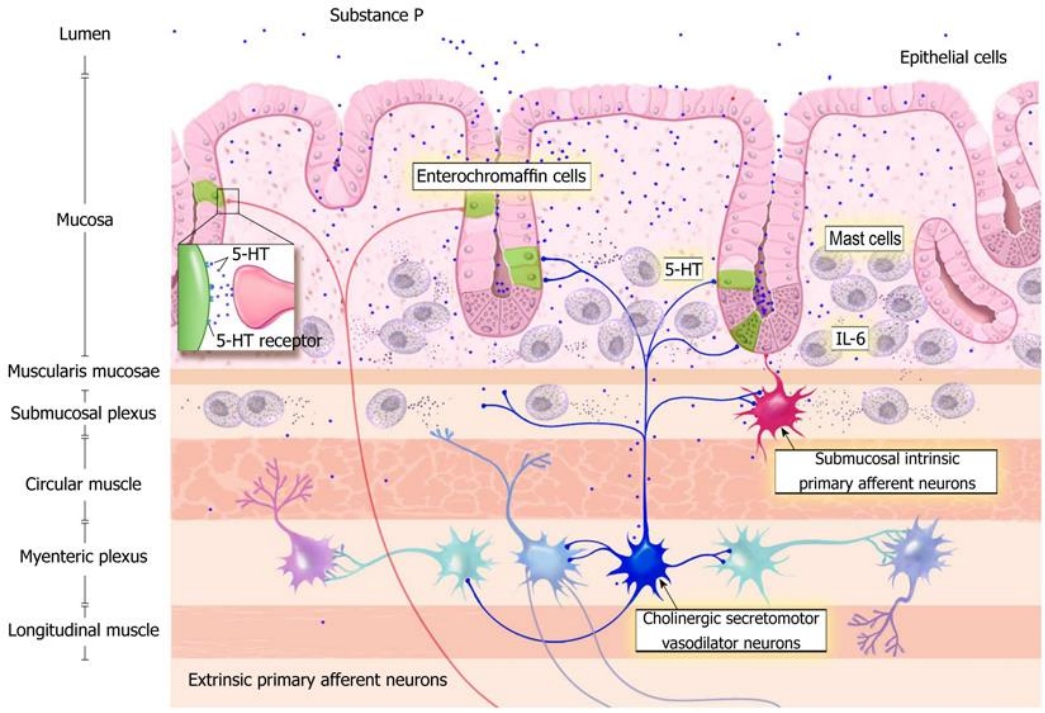

Figure 9: Enterochromaffin cell (from https://www.wjgnet.com)

6.3 Osteopathic approach to Visceral response

Osteopathy is a characteristic health care profession that emphases the role of the musculoskeletal, visceral and cranio – sacral system in health in order to promote optimal function of the tissues of the body. This is achieved by using a variety of manual techniques to improve the function of the body (DiGiovanna EL, 2005).

For the osteopath, everything can be manipulated. Osteopathy was wanted to be locked up in vertebral manipulations, but everything can be stimulated, solicited, inhibited. Thus, the visceral system can be manipulated, it requires as much dexterity and precision as the spine, limbs or the skull (Barral, 2004).

The influence of osteopathic techniques on the vegetative nervous system has been studied extensively (Charles E Henley, 2008), (Celander E, May 1968), (Sergueef N, Nov 2002). Although it has been shown that the techniques have an effect on the nervous system, the exact mechanism of this influence is still not fully understood.

The explanation of this phenomenon may be “mechanotransduction”, it is the ability of cells to process mechanical information into biochemical (J.BurgerE.H., 1995) and bioelectrical (Biswas, Manivannan, i Srinivasan, 2014) information. For example, mechanical stimulation can affect gene expression (DANIEL J. TSCHUMPERLIN, 2001).

External Forces are increasingly recognized as major regulators of cell structure and function. The mechanical properties of cells are essential to the mechanisms by which cells sense forces, transmit them to the cell interior or to other cells, and transduce them into chemical signals that impact a spectrum of cellular responses. (Janmey PA)

Durotaxis is a form of cell migration in which cells are guided by rigidity gradients, which arise from differential structural properties of the extracellular matrix (ECM). Most normal cells migrate up rigidity gradients (in the direction of greater stiffness). (Plotnikov, Pasapera, Sabass, i Waterman, Dec 2012)

Based on these studies, we can assume that by working manually with tissue stiffness, we can influence the migration of cells and their activity.

6.3.1 Neuroendocrine effect of Visceral manipulation

Sympathetic stress or mechanical tension can have a negative effect on the functioning of EC cells and their endocrine secretion of serotonin. Visceral manipulations are aimed at:

- restoring the elasticity of tissues
- normalizing the activity of the vegetative system
- normalizing smooth muscle tension caused by hyperactivity of the ENS
- improving blood supply through vasodilatation of arteries
- improving venous drainage by changing the pressure distribution within the venous system.

7. Methodology and Measurements

7.1Measurements

The level of serotonin concentration is measured in peripheral blood samples by lab test. The procedure is done as follows:

- The site is cleaned with germ-killing gel (antiseptic)
- The health care provider wraps an elastic band around the upper arm of the subject to apply pressure to the area and makes the vein swell with blood.
- The health care provider gently inserts a needle into the vein.
- The blood collects into an airtight vial or tube attached to the needle.
- The needle is removed.
- The puncture site is covered to stop any bleeding.

7.2 Research

This study assesses the effect of a specific set of visceral techniques on serotonin secretion due to stimulation of ENS situated inside de intestine wall.

The main research problem:
Does the use of a set of visceral techniques affect the level of serotonin in blood?

In order to verify the above research problem, the following hypotheses were formulated:

7.3 Hypotheses

- **Hypothesis 1**

There is no difference in the level of serotonin in the blood before and after the use of a set of visceral techniques in the experimental group.

- **Alternative hypothesis 1**

There is a difference in the level of serotonin in the blood before and after the use of a set of visceral techniques in the experimental group.

- **Hypothesis 2**

There is no difference in the level of serotonin in the blood before and after the use of a set of visceral techniques in the placebo group.

- **Alternative hypothesis 2**

There is a difference in the level of serotonin in the blood before and after the use of a set of visceral techniques in the placebo group.

7.4Material and methods

7.4.1 Study type

This study was a phase 0 placebo controlled study.

7.4.2 Study progress

All participants gave written informed consent before the start of the study. Two days before examination the subjects were informed of the time and date of the procedure. They received a list of recommendations according to products they needed to omit 24 hours before the testing. Food and drugs substances, which can increase catecholamine levels, include:

- Coffee
- Tea
- Cacao
- Alcohol
- Cocaine
- Banana
- Citrus fruit

- Nuts
- Vanilla
- Grapes
- Amphetamines
- Antidepressants
- Chocolates

Test subjects were also informed they should avoid stressful situations and vigorous exercise since both can affect the accuracy of the test results. They were asked to draw a number between 1 and 20.

On the test day, patients arrived to the clinic in order from 1 to 20 and sat in waiting room for 10 minutes. One by one they went for their first blood testing performed by a registered nurse, blinded to the patient distribution. After the first blood draw, all test subjects were asked to enter the treatment room one by one. Patients numbers were randomly draw. Numbers 2.4.6.8.10.12.14.16.18.20 were assigned to experimental group. Numbers 1.3. 5.7.9.11.13.15.17.19 were assigned to placebo group. Test subjects from experimental group, where given a specific set of visceral manipulations. Test subjects from the placebo group where given micro-electrostimulation with the device turned off. Each subject, from both groups, was after the treatment immediately sent for their second blood draw. All together the blood testing and the treatment took approximatively 20 minutes. The treatment itself took 15 minutes with both the visceral manipulation and preparation of the patient. The second blood draw also taken peripherally from the right arm was performed immediately after the treatment. After the blood test, patients from the placebo group where informed that they can have visceral treatment afterwards in term that suits them best. Blood phials were determined as follows: patient 1. phial nr. 1-1 for first blood testing, phial nr. 1-2 for second etc. All phials were transported to a registered medical laboratory for analysis.

7.4.3 Population

The research group consisted of 20 volunteers in the age range from 20 to 52 years old. Unexpectedly, the majority of the volunteers were females (14 on 20) and the majority of volunteers (12 on 20) were between 22-27 years old.

7.4.4 Inclusion criteria

- Over 18 years old
- No medical history of intestine diseases
- No medical history of cancer
- Fixed nutrition
- Signed an informed consent prior to study start

7.4.5 Exclusion criteria

- Under 18 years old
- Metabolic diseases (pancreas, kidneys, adrenal glands, thyroid glands)
- GIT diseases
- Any pre-existent form of cancer
- Trauma in medical history, causing internal damage

7.4.6 Investigation

This study examines the effect of a specific set of visceral techniques on the secretion of serotonin in the blood.

7.4.7 Procedure

Test subjects were given a specific set of visceral techniques. Control group subjects were given electrotherapy treatment using micro – current. The electrotherapy machine gave the start and end sounds but the intensity was set to 0 amperes.

7.4.8 Placebo intervention

Test subjects from placebo group received a 15-minute micro-electrotherapy treatment.

Patient position output:

The patient will lie on the supine position, the electrodes will be placed on the abdomen of the patient.

Therapist position output:

The therapist left the patient alone in the room during the procedure

Execution:

The electrotherapy device will be turned on and will sound when setting parameters. The parameters will be set on zero, so there will be no electrical impact on the tissues. The treatment period last 15 minutes and after the end of the period the device will sound.

7.4.9 Experimental technique

Test subject where given a specific set of 12 visceral techniques as describes below.

1. Technique on the coeliac plexus according to Kuchera (Eric U. Hebgen, 2008)

Starting position:

The patient is in the supine position. The practitioner stands next to the patient.

Procedure:

At the height of the projection of the pre-aortic plexus on the abdominal wall (T12-L1), place the fingers of both hands next to each other in the center line and let them sink into the depth of the abdomen until you reach the plexus. It may be necessary to stop repeatedly on the way in and await fascial release.

Treatment:

When you have arrived at the plexus, maintain pressure until you have obtained fascial release. Then stimulate the pre-aortic plexus by means of repeated rebounds.

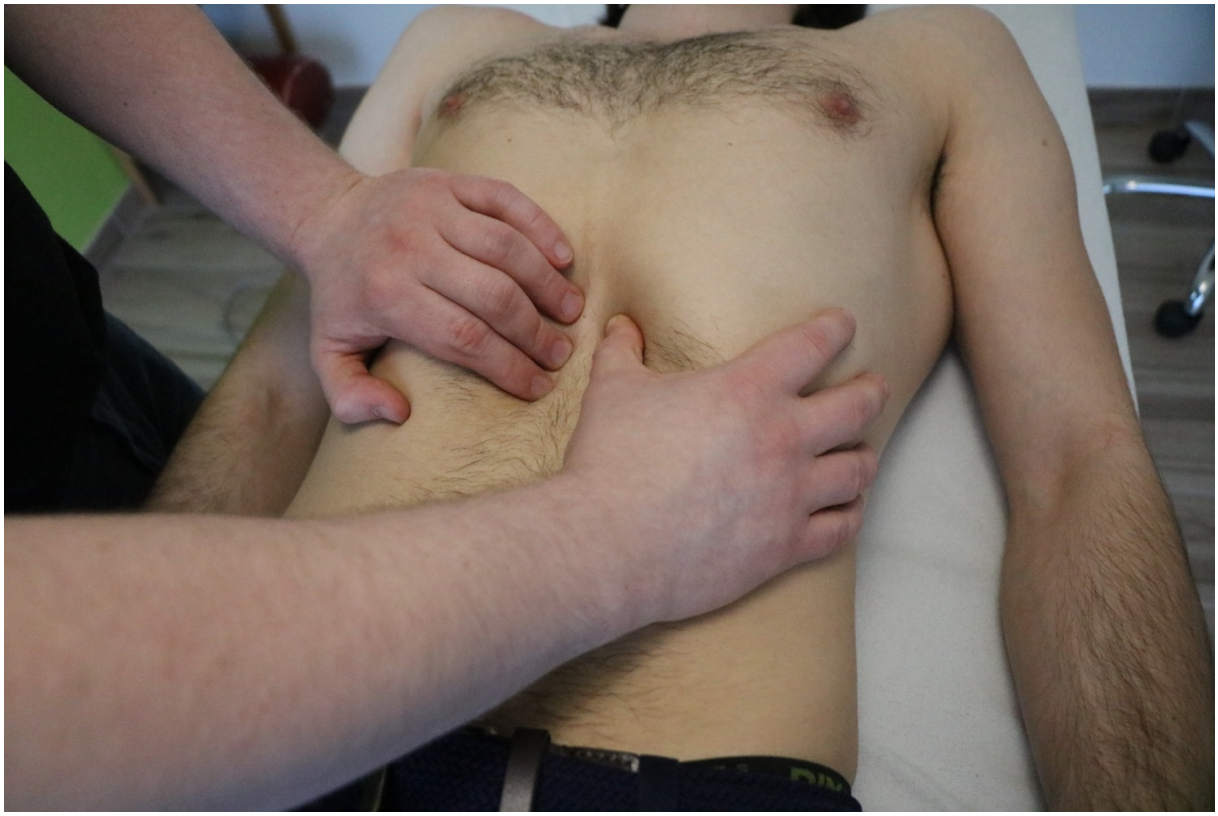

Figure 10: Technique on the coeliac plexus

2. Technique on the superior mesenteric plexus according to Kuchera (Eric U. Hebgen, 2008)

Starting position:

The patient is in the supine position. The practitioner stands next to the patient.

Procedure:

At the height of the projection of the pre-aortic plexus on the abdominal wall (L1-L2), place the fingers of both hands next to each other in the center line and let them sink into the depth of the abdomen until you reach the plexus. It may be necessary to stop repeatedly on the way in and await fascial release.

Treatment:

When you have arrived at the plexus, maintain pressure until you have obtained fascial release. Then stimulate the pre-aortic plexus by means of repeated rebounds.

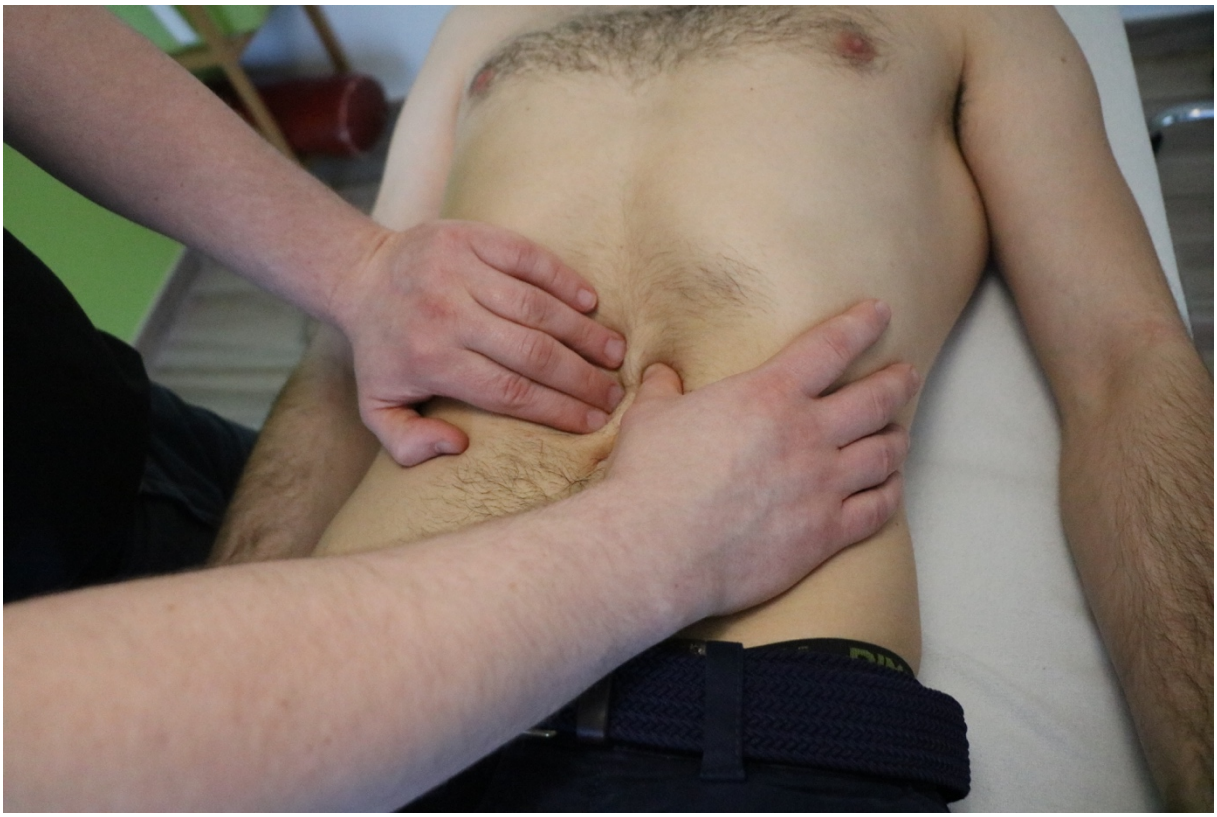

Figure 11: Technique on the superior mesenteric plexus

3. Technique on the cardia: Cardia induction according to Barral (Barral J. , 2004)

Starting Position:

The patient is in the supine position, legs bent. The practitioner stands on the patient's le side.

Procedure:

The patient is in the supine position, placing one hand under the patient's back at the left 11th costo-verebral joint, and the other on the 7th left chondro-costal cartilage. Initially, the anterior hand rests on the chest in the direction of the posterior hand.

Treatment:

The two hands will work together and gradually, the previous support is relaxed until it becomes null. The manipulation is then finished. This induction is active-passive and seems to play mainly on the spasms of the IES.

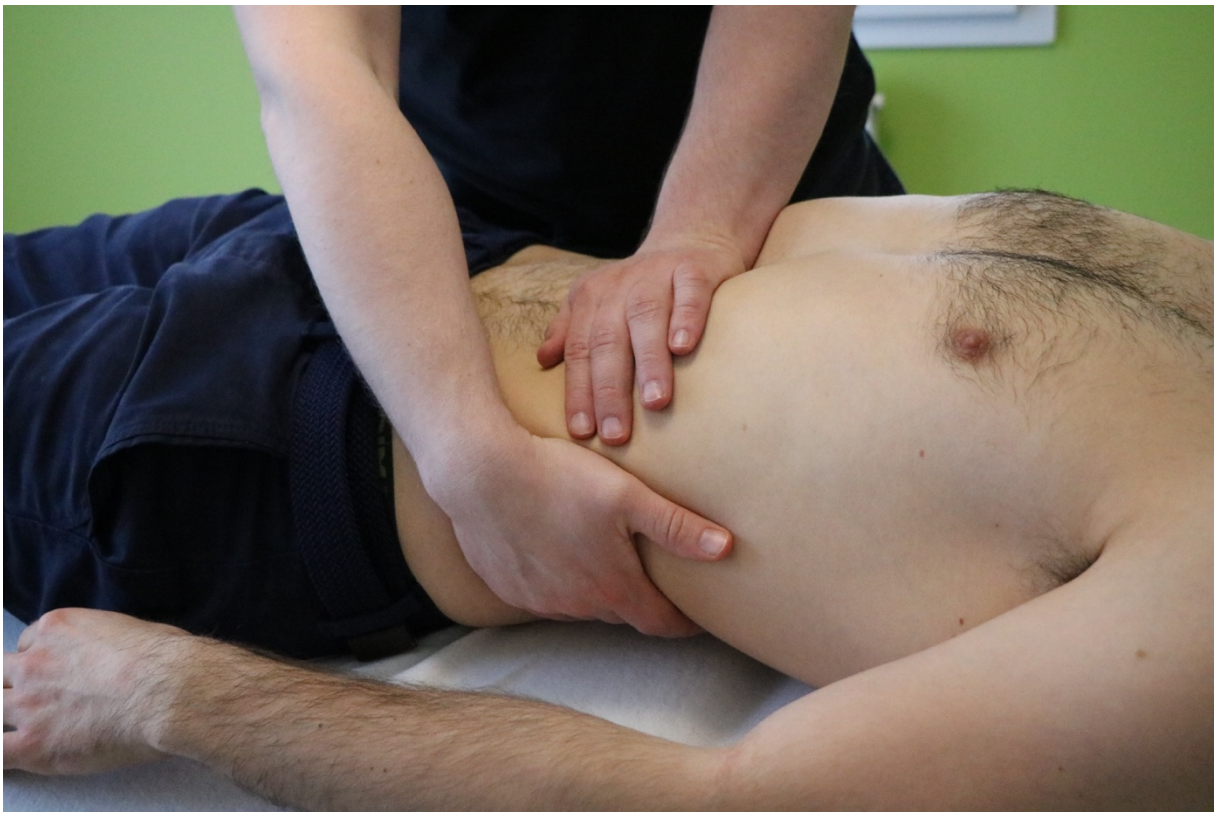

Figure 12: Technique on the cardia

4. Technique on the pylorus according to Barral (Eric U. Hebgen, 2008)

Starting Position:

The patient is in the supine position, legs bent. The practitioner stands on the patient's le side.

Procedure:

To find the pylorus, look for its approximate projection on the stomach wall. For this purpose, move from the navel about five finger-widths cranially. From there, place your fingers slightly to the right, next to the median line. At this point, slowly slide posteriorly into the abdomen. It is important to proceed slowly, to give the superficial structures time to move out of the way and allow the fascia to relax.

Once you have advanced deeply enough in this palpation, you will usually find a supple, roughly hazelnut- sized solidification within 0.5-1em of this palpation point. In most cases, the pylorus is sensitive to palpation.

Treatment:

You can now carry out small circulations, vibrations, or inhibitions on this point, until the tonus and sensitivity are clearly reduced.

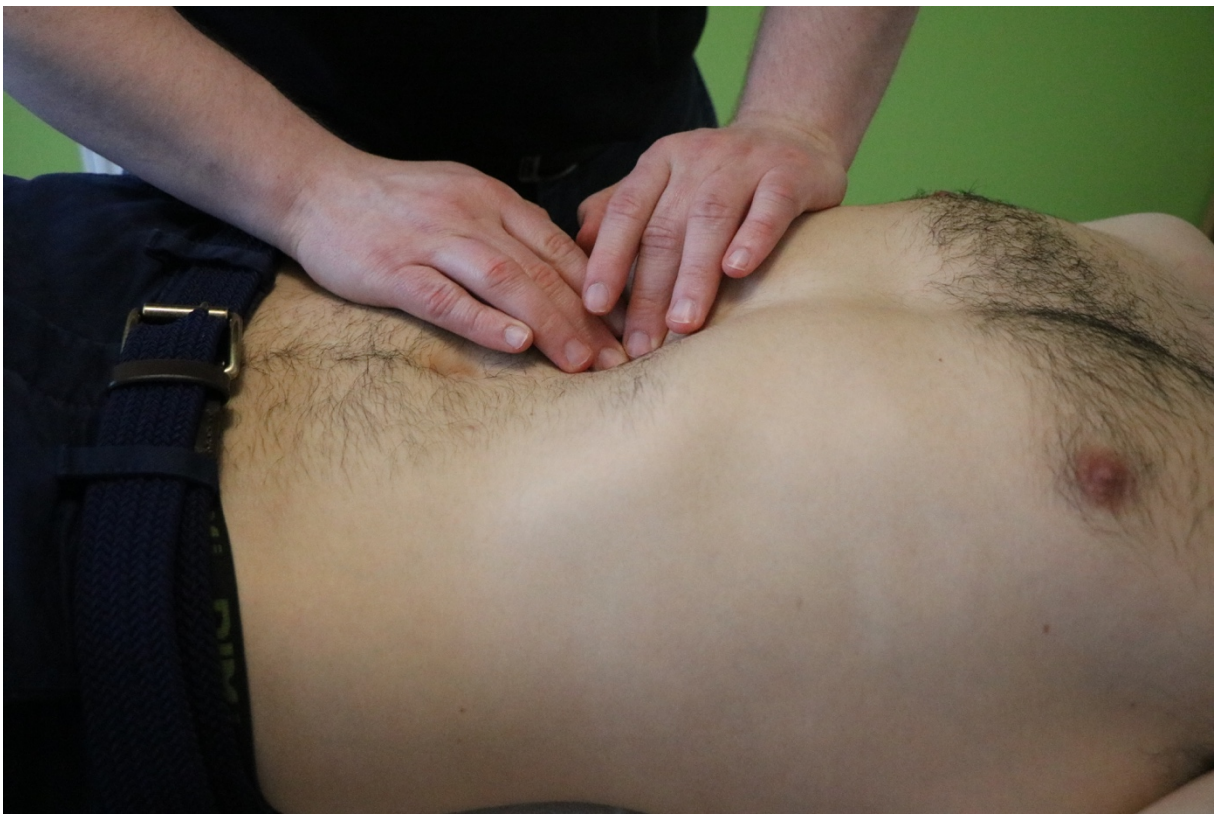

Figure 13: Technique on the pylorus

5. Technique on the stomach: Fascial treatment according to Finet and Williame (Eric U. Hebgen, 2008)

Starting position

The patient is in the supine position, legs stretched out. The practitioner stands by the patient's left side.

Procedure:

Place your right hand with the side of the little finger below the left costal arch, fingers pointing toward the right shoulder. Place the left hand with the side of the little finger to the left of the median line, with the fingertips pointing toward the patient's left shoulder and lying slightly below the right hand.

With both hands, apply the right amount of pressure posteriorly to reach the fascial plane.

Treatment:

During inhalation, both hands simultaneously pull caudally. As an additional result, the right hand rotates in a clockwise and the left in a counterclockwise direction. A longitudinal stretch of the stomach results.

During expiration, maintain the position reached. Repeat this procedure until you have reached the end of the fascial movement. In the next exhalation, release the pull.

Repeat the whole procedure four or five times.

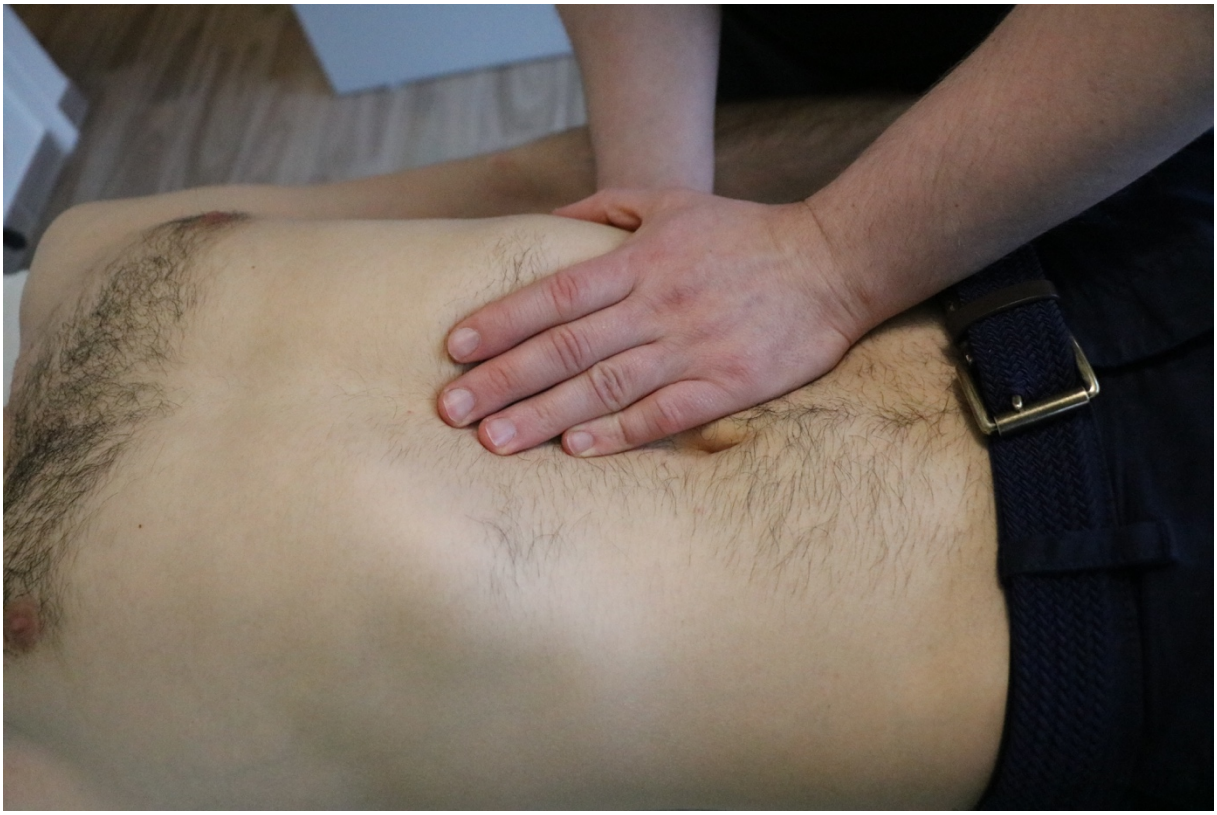

Figure 14: Technique on the stomach

6. Technique on the duodenum according to Finet and Williame (Eric U. Hebgen, 2008)

Starting position:

The patient is in the supine position, legs stretched out. The practitioner stands by the patient's right side.

Procedure:

Place both hands on the abdomen on both sides of the median line. The fingers point cranially, the fingertips lying below the costal arch. With both hands, apply the right amount of pressure posteriorly so that you reach the fascial plane.

Treatment:

During inhalation, both hands simultaneously pull caudally and medially, and rotate clockwise. During exhalation, maintain the position reached. Repeat this procedure until you have reached the end of the fascial movement. In the next exhalation, release the pull. Repeat the whole procedure four or five times.

Figure 15: Technique on the duodenum

7. Technique on the sphincter of Oddie according to Barral (Eric U. Hebgen, 2008)

To improve the flow of bile, begin by treating the sphincter of Oddi, which frees drainage.

Starting Position:

The patient is in the supine position, legs bent. The practitioner stands on the patient's right side.

Procedure:

To find the sphincter of Oddi, you have to determine its approximate projection onto the abdominal wall. For this purpose, move from the navel about three finger-widths cranially. From there, move horizontally in a lateral direction until you cross a line that connects the navel and the right nipple (or the navel and the intersection between the right medio - clavicular line and the right costal arch). At this point, slowly slide posteriorly into the abdomen. It is important here to proceed slowly, so that the superficial intestinal loops and the transverse colon have enough time to move away from the pressure and the fascia can relax.

Treatment:

Once you have advanced deeply enough in this palpation, you will usually find a supple, roughly pea-sized solidification within 0.5-1 cm of this palpation point. In most cases, the sphincter is sensitive to palpation.

You can now carry out small circulations, vibrations, or inhibitions on this point, until the tonus or soreness is clearly reduced.

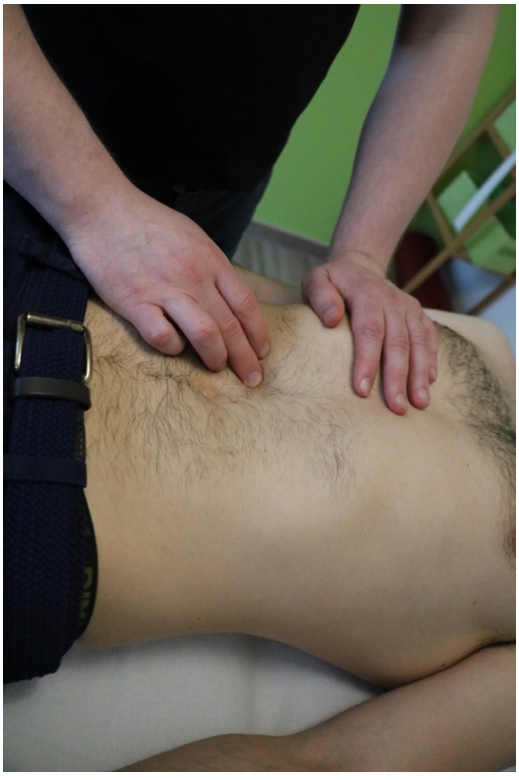

Figure 16: Technique on the sphincter of Oddie

8. Technique on the duodeno – jejunal junction according to Barral (Eric U. Hebgen, 2008)

Starting Position

The patient is in the supine position, legs bent. The practitioner stands on the patient's left side.

Procedure

To palpate the DJ flexure, proceed in a mirror image to the sphincter of Oddi: from the navel, pal- pate about three finger-widths cranially. From there, move horizontally in a lateral direction until you cross a line that connects the navel and the left nipple (or the navel and the intersection between the left medio-clavicular line and the left costal arch). At this point, slowly slide

posteriorly into the abdomen. It is important here to proceed slowly, so that the superficial intestinal loops or the transverse colon have a chance to move out of the way and the fascia can relax.

Treatment:

Once you have advanced deeply enough in this palpation, you will usually find a pressure-sensitive spot within 0.5-1 cm of this palpation point. You can now apply small circulations, vibrations, or inhibitions on this point, until the tonus and sensitivity are clearly reduced. The treatment of these two reflex points (sphincter of Oddi and DJ flexure) causes a reduction in duodenal tonus and in addition general relaxation in the abdomen. These treatments can also be performed independently from duodenal indications as a general visceral treatment.

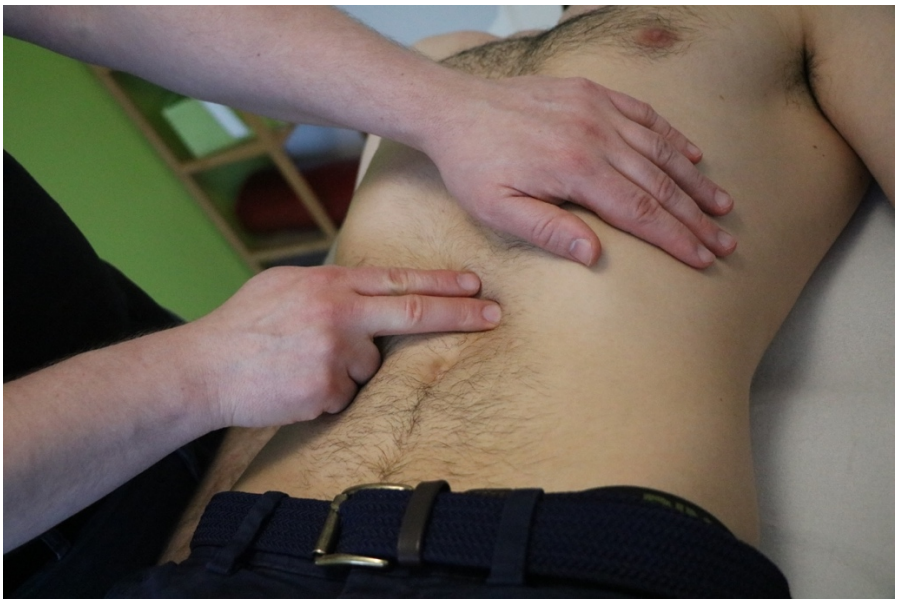

Figure 17: Technique on the duodeno – jejunal junction

9. Technique on the superior mesenteric artery according to Barral (Jean-Pierre Barral, 2009)

Starting Position:

The patient is supine with arms around the body, a soft cushion placed under the thoracolumbar junction. You sit to the right of the patient.

Procedure:

First look for the DJ angle through the finger above the umbilicus, on the left umbilical-medio-clavicular line. Slide a finger along the medial edge of the DJ junction until you feel the pulse of the superior mesenteric artery. This pulse will be used as reference

Treatment:

On the right side of your patient, you place an inch where you felt the pulsation the superior mesenteric artery; it will serve as a reference and a fixed point. The other inch is located at the ileo-caecal junction, on the lateral third of the umbilical line iliac spine ant upper right. Direct the ileo-caecal thumb caudally and laterally, drawing a convex line between the two thumbs. Maintain this stretch for about twenty seconds, and appreciate the difference in pulsations of the superior mesenteric artery after manipulation.

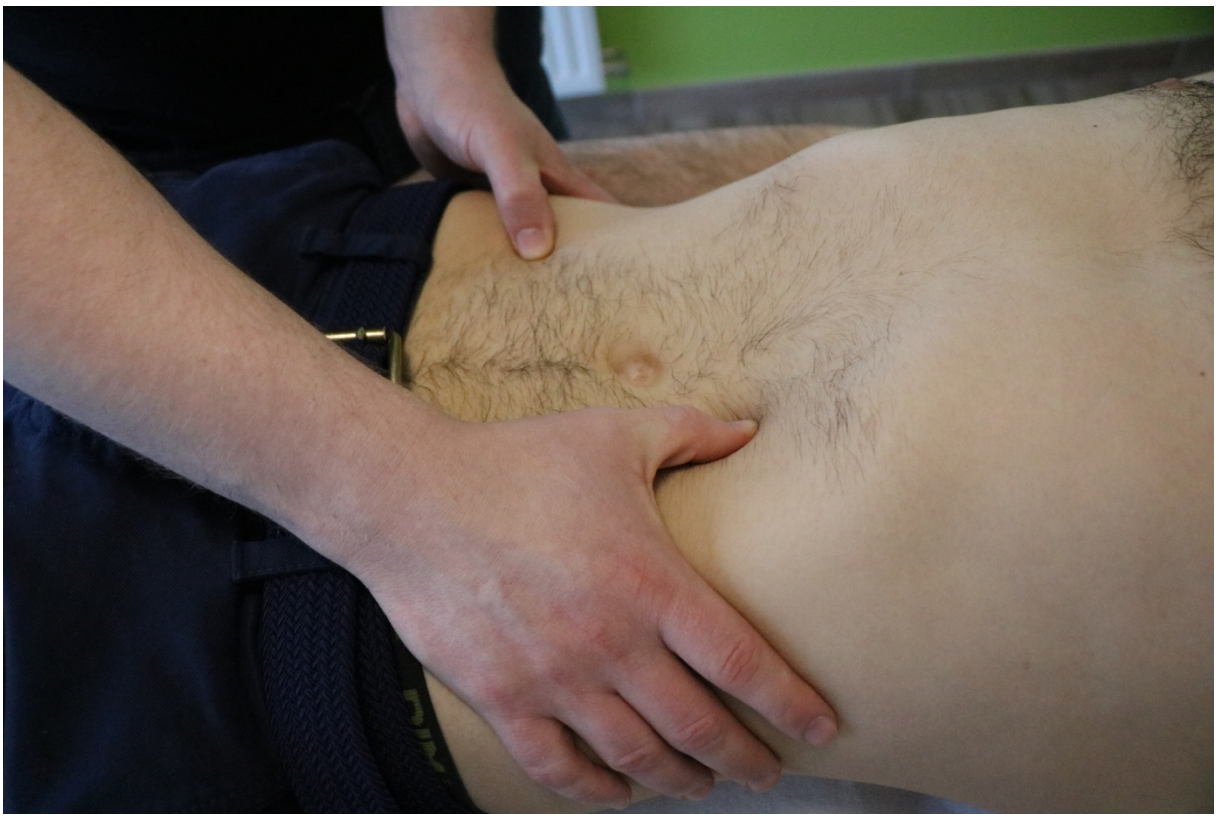

Figure 18: Technique on the superior mesenteric artery

10. Technique on the radix mesentery according to Barral (Eric U. Hebgen, 2008)

Starting Position:

The patient is in the lateral position facing left, legs bent. The practitioner stands behind the patient.

Procedure:

With both hands next to each other, reach into the abdomen lateral to the small intestinal loops and medial to the descending colon. The loops now lie in your palms, the direction of palpation being in a posteromedial direction. In this way, reach the root of the mesentery deep down, in its slanted course from left top to right bottom. Now palpate its entire length for differences in tonicity and sensitivities, thereby stretching it in the direction of the patient's right shoulder.

Treatment:

If differences in tonicity or sensitivities are found. stretch the root in the direction of the patient's right shoulder with a constant pull until the symptoms are clearly reduced or disappear completely. You can apply this treatment over the entire length of the root or at isolated spots.

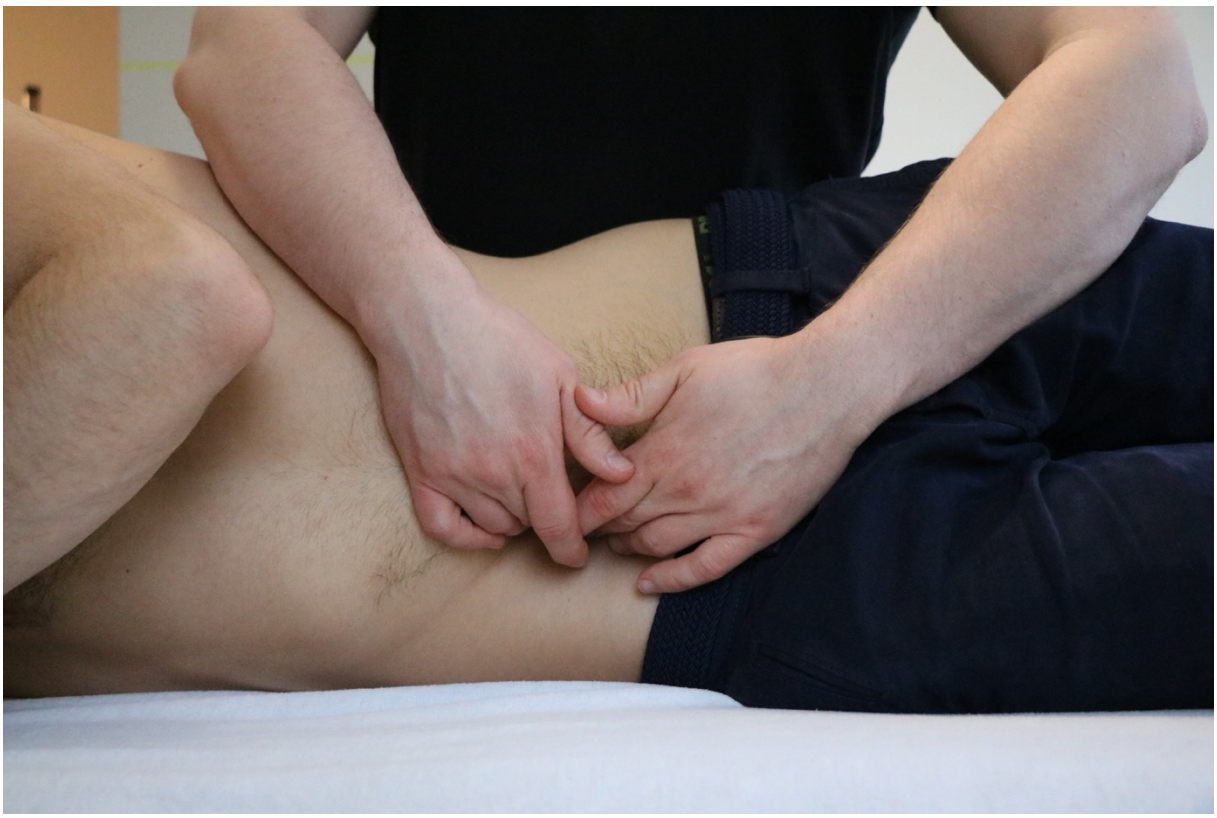

Figure 19: Technique on the radix mesentery

11. Technique on the liver: liver pump according to Barral (Eric U. Hebgen, 2008)

Starting Position

The patient is in the supine position, legs bent. The practitioner stands on the patient's left side.

Procedure

With the cranial hand, enfold the right costal arch in such a way that the fingers end up lying posteriorly and the thenar laterally. Place the caudal hand with the side of the little finger below the costal arch.

During the patient's exhalation, the caudal hand pushes in the direction of the right shoulder while at the same time the cranial hand pulls the costal arch toward the caudal hand. In this way, the liver is compressed. During inhalation, maintain the position reached, and increase it further during the next exhalation.

Repeat this through two or three breaths. Then ask the patient to inhale deeply and, during the start of this inhalation, release the pressure with both hands simultaneously and abruptly.

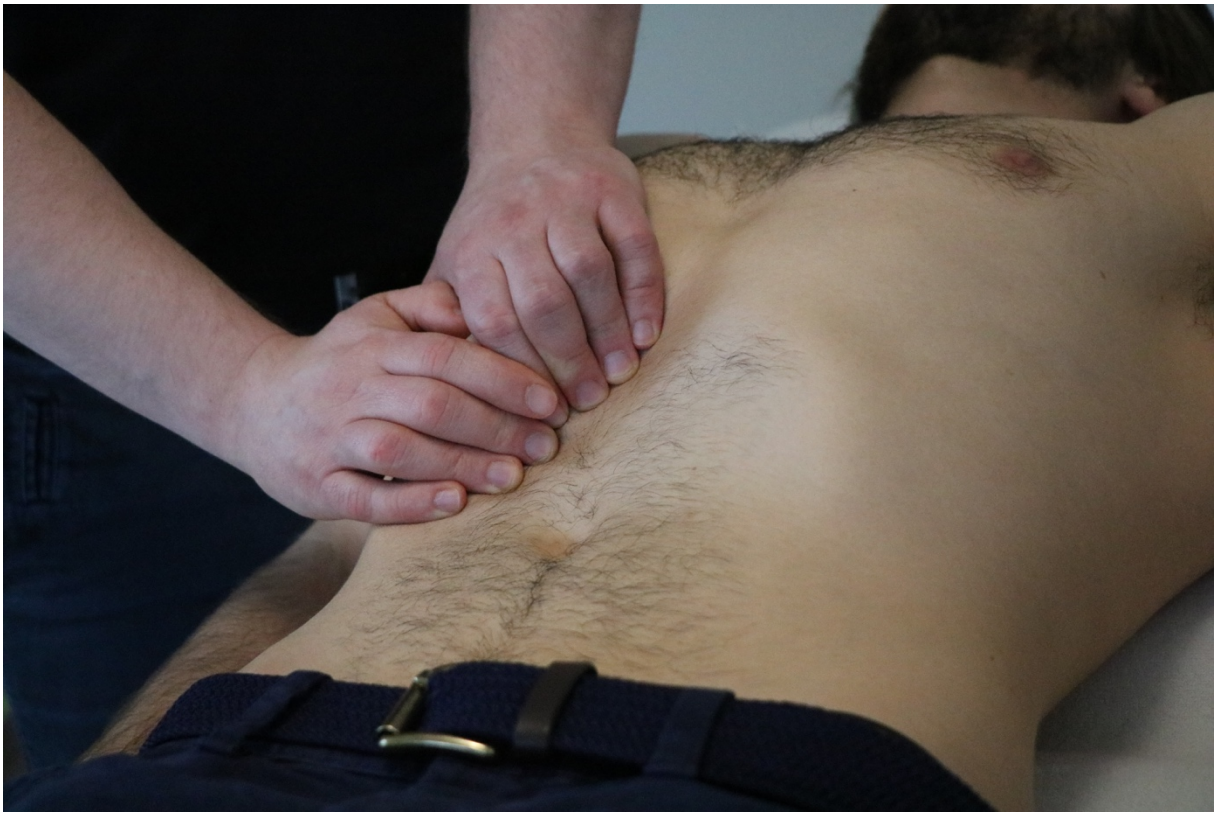

Figure 20: Technique on the liver

12. Technique on the sternum: Energetic liberation of the diaphragm (A. Chantepie, 2013)

Starting position:

The patient is lying supine, the practitioner places himself behind the patient's head and contacts with the heel of his hands the upper edge of the rib just below the collarbone.

Procedure:

The practitioner asks the patient for ample breathing and accompanies the patient mouvments during expiratory time. It maintains the amplitude gained during the inspiration. After three or four breaths, he suddenly relaxes his pressure at the beginning of the inspiratory phase.

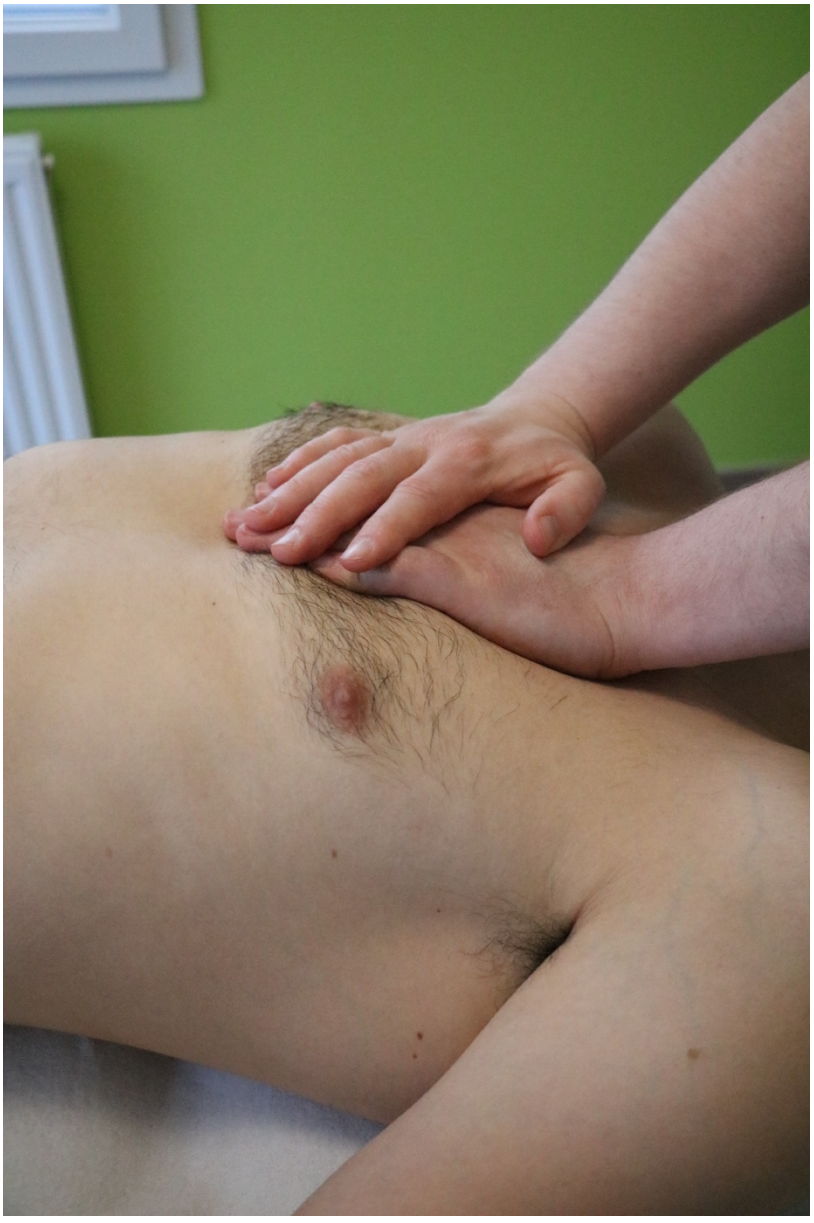

Figure 21: Technique on the sternum

7.4.10 Blinding

The registered nurse was completely blinded to the distribution of the study subjects to both the placebo and test groups.

7.4.11 Processing

Blood analysis was done in BPR ANALYSES SPECIALISEES in Pannes, the vials were sent to Pannes by Laboratoire Medibio La Source in Orleans. The lab results measured the level of serotonin.

8. Results

Statistical analysis was performed using the IBM SPSS Statistics 24 computer program. In order to verify the hypotheses about the influence of visceral techniques on the level of serotonin in the blood, significance tests for dependent groups were used, the aim of which is to show whether the statistical communities we are interested in are the same in terms of the examined feature or are different from each other. These tests are used, among others to compare the mean before and after the given stimulus - in the case of these studies - osteopathic therapy.

First of all, the Shapiro-Wilk test was used to examine the normality of the distribution of variables, while the parametric Student's t-test for dependent samples was used to examine the intergroup differences in the analysis. To avoid the first type of error, the significance level $\alpha = 0.05$ was assumed. When determining the alpha error, the confidence interval $(1- \alpha)$ was determined at the same time, if the calculated value of the test statistic is in this area, the verified zero hypothesis about the independence of variables should be rejected. At the same time, in order to demonstrate the statistical power (test power), which defines the error of the second type (β), consisting in not rejecting the null hypothesis, which is in fact false, assumed:

• if the value of $p < 0.05$ (significance level), the null hypothesis should be rejected and the alternative hypothesis accepted. This means that the results from the sample are statistically significant and reflect the value of the examined feature in the population.

• if the value of $p \geq 0.05$ should be considered that there are no grounds to reject the null hypothesis, the results cannot be generalized to the population.

Due to the above, intergroup differences were considered statistically significant if the asymptotic significance level was lower than the assumed level of significance ($p < 0.05$). The presented results also show mean values and standard deviations (SD).

To decide whether to use a parametric or non-parametric test, the Shapiro-Wilk test was used, which is based on the following null (H0) and alternative (H1) hypotheses:

H0 - distribution of the examined trait in the population is a normal distribution;

H1 - distribution of the examined trait in the population is different from the normal distribution.

If the significance level is higher than or equal to $\alpha = 0.05$, there is no reason to reject H0, so it is assumed that the distribution is normal. The table presents the results of the Shapiro-Wilk test for the variables from this study.

	Shapiro-Wilk	
Samples	Test levels	p-value
A study for patients from the experimental group before therapy	0.878	0.124
A study for patients from the experimental group after the application of therapy	0.976	0.938
A study for patients in the placebo group before therapy	0.930	0.448
A study for patients in the placebo group after treatment	0.878	0.124

Table 1: Shapiro-Wilk test results

The significance in all cases is higher than 0.05 and so we assume that the distribution is normal, thus rejecting the alternative hypothesis (H1). In the case of normal distribution, parametric tests are used. Therefore, the Student's t-test for dependent tests was applied. The t test for dependent samples is used to compare means between groups from the same population or from related populations. The tables below present the results of the Student's t test and statistics for dependent samples.

Basic Statistics					
		Average	N	Standart deviation	Standard error of the mean
Para 1	A study for patients from the experimental group before therapy	92,2340	10	51,36229	16,24218
	A study for patients from the experimental group after the application of therapy	113,4740	10	39,51762	12,49657
Para 2	A study for patients in the placebo group before therapy	118,8460	10	54,62721	17,27464
	A study for patients in the placebo group after treatment	110,4130	10	58,12490	18,38071

Table 2: Basic Statistics

The table below presents the correlation of R Pearson. The significance of this correlation is an important interpretive element. If the correlation would be irrelevant the analysis of the t-test would be unjustified.

Correlation				
		N	Korelacja	Istotność
Couple 1	A study for patients from the experimental group before therapy & A study for patients from the experimental group after the application of therapy	10	,865	,001
Couple 2	A study for patients in the placebo group before therapy & A study for patients in the placebo group after treatment	10	,965	,000

Table 3: Correlation

As the table shows, the significance in Pearson's R correlation is statistically significant, which is why you can go on to interpret the t test.

	A study for patients from the experimental group after the application of therapy - A study for patients from the experimental group before therapy	**A study for patients in the placebo group after treatment - A study for patients in the placebo group before therapy**
t	-2.563	1.753
p-value (two-tailed)	**0.031**	**0.113**

Table 4: t Paired Sample T-Test results

The main hypotheses set in the work have been verified. The test result for the experimental group is t = -2.563. This result is statistically significant, p <0.05, so it can be assumed that there is a difference in the level of serotonin in the blood before and after the use of a set of visceral techniques in the experimental group (alternative hypothesis 1 is confirmed). However, the test result for the placebo group is t = 1.753. This result is not statistically significant, p> 0.05, so it should be assumed that there is no difference in the level of serotonin

in the blood before and after the use of the micro current treatment in the placebo group (the null hypothesis 2 is confirmed).

In the light of the above analyzes, one can verify the main research problem posed at work and confirm the assumption that the use of a set of visceral techniques affects the level of serotonin in the blood.

The results of 20 volunteers are listed below in the table. The standard for this test is 30-200 ng / mL. The mean percentage increase of serotonin in the blood in the experimental group was 38.69%. The mean percent decrease in serotonin in the placebo group was 9.29%.
The highest increase in serotonin levels was observed in the experimental group and accounted for 101%. The highest decrease was observed in the placebo group and it was -43%.

Patient number	Before treatment (ng/mL)	After treatment (ng/mL)	Difference (ng/mL)	Difference in %	Treatment / Placebo
1	72,23	91,91	19,68	27,24629655	Treatment
2	221,6	197,2	-24,4	-11,0108303	Placebo
3	59,81	103,26	43,45	72,6467146	Treatment
4	128,51	111,6	-16,91	-13,1585090	Placebo
5	25,89	52,11	26,22	101,2746234	Treatment
6	74,47	59,28	-15,19	-20,3974754	Placebo
7	61,1	110,1	49	80,19639935	Treatment
8	164,99	176,98	11,99	7,267107097	Placebo
9	76,18	89,88	13,7	17,98372276	Treatment
10	175,27	184,68	9,41	5,368859474	Placebo
11	73,72	77,79	4,07	5,520889853	Treatment
12	125,3	111,28	-14,02	-11,1891460	Placebo
13	78,75	145,31	66,56	84,52063492	Treatment
14	70,83	61,2	-9,63	-13,5959339	Placebo
15	126,37	143,92	17,55	13,88778982	Treatment
16	76,51	43,44	-33,07	-43,2231080	Placebo
17	202,02	188,21	-13,81	-6,83595683	Treatment
18	98,12	106,25	8,13	8,285772523	Placebo
19	146,27	132,25	-14,02	-9,58501401	Treatment
20	52,86	52,22	-0,64	-1,21074536	Placebo

Table 5: Results

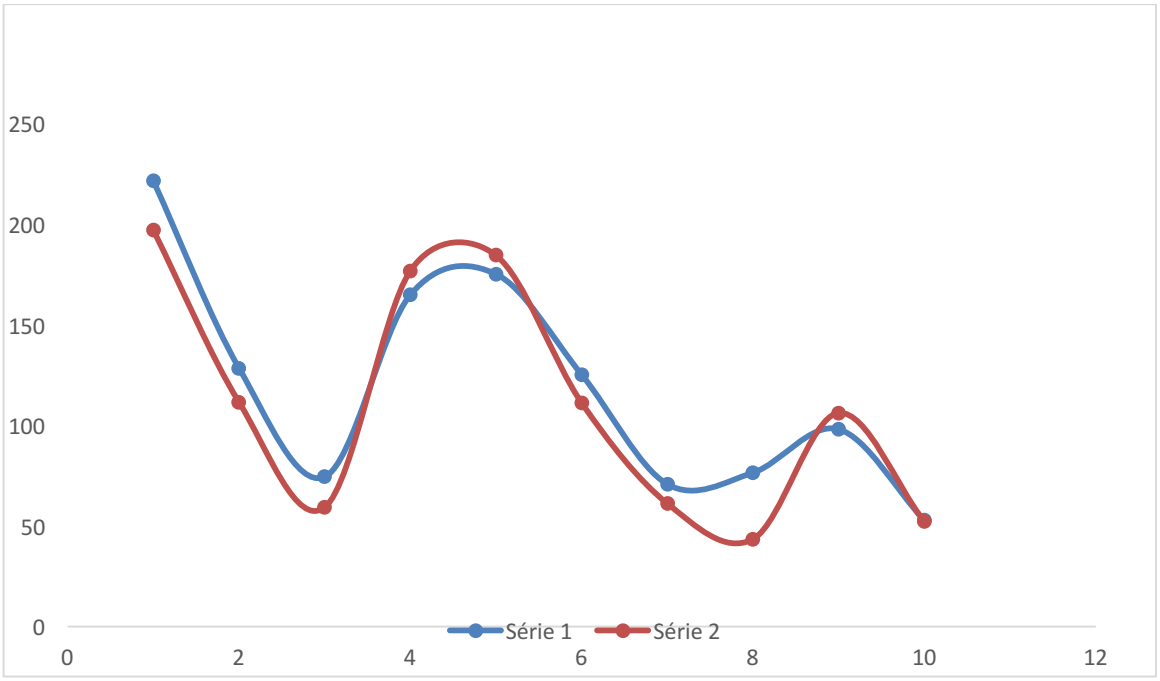

Table 6: Control group

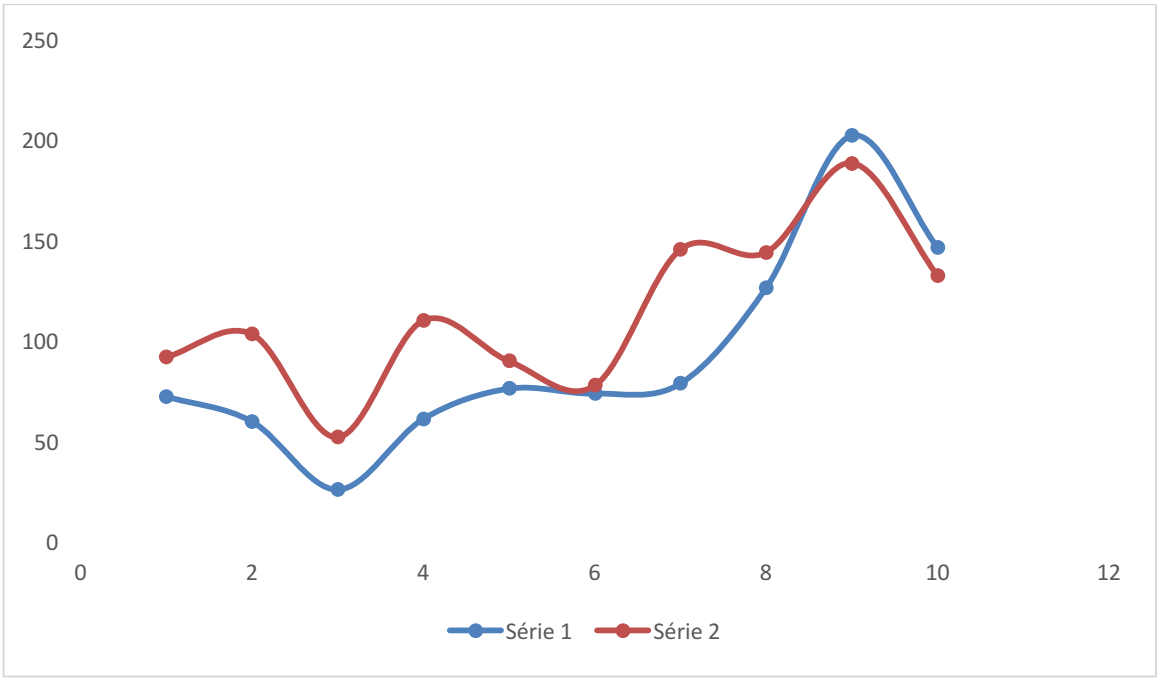

Table 7: Experimental group

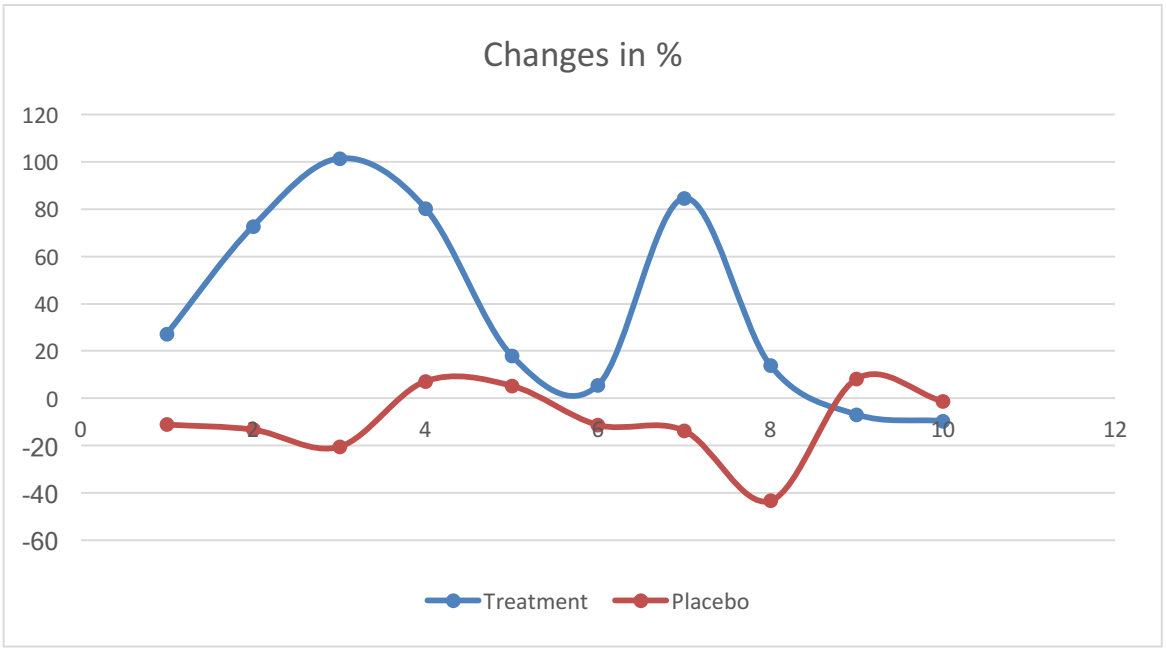

Table 8: Changes in %

9. Discussion

In the light of the above analyzes, one can verify the main research problem posed at work and confirm the assumption that the use of a set of visceral techniques affects the level of serotonin in the blood.

Although the level of serotonin seems not to be dependent on sex or age, one of the bias may be that the surveyed groups consist mostly of women (14/20) and most volunteers are aged 22-27 (12/20). The groups do not represent objectively the cross-section of the society.

Some of the volunteers were afraid of blood collection procedures. In some cases, due to the condition of the venous system, the nurse had to do more than one puncture to take blood. Pain and fear associated with blood collection could affect the results of the study.

This study shows only a short-term effect on the level of serotonin, it cannot be determined whether this change is clinically significant. Future studies with a larger number of people in the experiment can prove the validity of the thesis in this study and research of other authors on the effect of visceral manipulation on neuroendocrine secretion.

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11. List of abbreviations

EC – Enterochromaffin cell

ENS – Enteric nervous system

ECM – Extracellular matrix

IES – Inferior Esophageal sphincter

GIT – Gastrointestinal tract

PNS – Peripheral nervous system

CNS – Central nervous system

CN – Cranial nerve

DJ – Duodeno-jejunal

12. Addendum

12.1 Informed consent

Le consentement du patient à participer à la recherche de fin d'études d'ostéopathie FICO POLSKA

Titre de la recherche : « **L'influence des techniques viscérales sur le niveau de sérotonine dans le sang**. »

J'accepte de suivre les étapes diagnostic médical à des fins scientifiques. J'ai été informé (e) des contre-indications pour effectuer la méthode de diagnostique / traitement, des procédures nécessaires pour mener à bien la recherche, de toutes les conséquences prévisibles et des effets à long terme de la manœuvre.

On m'a précisément informé, d'une manière compréhensible la façon de conduite requise pour participer au traitement, y compris la nécessité d'éliminer certains comportements, des restrictions de la nourriture, des médicaments et d'autres activités.

Avant la procédure diagnostique et thérapeutique j'ai répondu aux questions que le thérapeute m'a posé lors de l'entrevue sur l'état de ma santé, les traitements en cours, trauma et opération chirurgical passe. J'ai été informé que le fait de dissimuler ou de fournir de fausses informations sur l'état de santé, la prise des médicaments, les blessures et les traitements subit dans le passe, est considéré comme contribuant aux erreurs dans le processus d'examen.

L'information fournie par le thérapeute avant d'exécuter la procédure de diagnostic invasive était entièrement claire et compréhensible pour moi. Pendant la conversation avec le thérapeute, j'ai eu l'occasion de poser des questions sur la recherche menée. Je déclare avoir lu le texte ci-dessus et donner mon consentement pour effectuer des procédures diagnostiques invasives.

Date et signature du patient :

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