CHARLES UNIVERSITY IN PRAGUE

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DEPARTMENT OF BIOLOGICAL AND MEDICAL SCIENCES

DIPLOMA THESIS

EFFECTS OF OBESITY AND SLEEVE GASTRECTOMY ON FUNGIFORM PAPILLAE IN A MODEL OF WISTAR RAT

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HRADEC KRÁLOVÉ, 2015 JANA ZADRAŽILOVÁ

UNIVERZITA KARLOVA V PRAZE

FARMACEUTICKÁ FAKULTA V HRADCI KRÁLOVÉ

KATEDRA BIOLOGICKÝCH A LÉKAŘSKÝCH VĚD

DIPLOMOVÁ PRÁCE

VLIV OBEZITY A TUBULIZACE ŽALUDKU NA HOUBOVITÉ PAPILY U POTKANŮ WISTAR

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Prohlášení

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Abstract

Jana Zadražilová

Effects of obesity and sleeve gastrectomy on fungiform papillae in a model of Wistar rat

Diploma thesis

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Pharmacy

Obesity has become a worldwide issue in which taste sensitivity and food preferences play crucial roles. The most effective treatment of morbidly obese patients is bariatric surgery. In our preliminary study, we tried to determine the effect of diet induced obesity and sleeve gastrectomy (SG) on the histology of rat tongue, especially how they affect the number of fungiform papillae (FP).

Male Wistar rats were used in the experiment. After inducing obesity with a high-fat diet (HFD), some of the rats underwent SG, some had sham surgery and others stayed surgery-free to be considered as a control group, additionally, one group stayed on normal diet (ND) the entire duration of the experiment. After 16 weeks, all rats were sacrificed and histological examination of rat tongue was performed.

The highest mean number of FP (5.11 ± 1.45) was counted in diet induced obese rats that were fed a ND the last four weeks of the experiment. The lowest number of FP (3.38 ± 1.45) was found in the experimental group of rats fed a HFD throughout the experiment, but underwent SG after 12 weeks of fattening. There was no difference in either the number of taste buds (TB) or in ghrelin staining in taste cells among the groups.

The difference in FP number in rats from different experimental groups is noteworthy, even though the results were only close to statistical significance level (p-value = 0.0710). As this is a preliminary study, it would be interesting to increase the number of animals in each group in order to count FP in higher number of slides.

Keywords: obesity, sleeve gastrectomy, fungiform papillae, ghrelin

Abstrakt

Jana Zadražilová

Vliv obezity a tubulizace žaludku na houbovité papily u potkanů Wistar

Diplomová práce

Univerzita Karlova v Praze, Farmaceutická fakulta v Hradci Králové

Farmacie

Obezita se stala celosvětovým problémem, v jejímž rozvoji hrají významnou roli vnímání chutí a výběr potravy. Nejúčinnější léčbou morbidně obézních pacientů je bariatrická operace. V naší pilotní studii jsme se snažili stanovit vliv obezity, vyvolané vysokokalorickou stravou, a tubulizace žaludku (SG) na histologickou stavbu jazyka potkanů a zvláště pak jejich efekt na změny v počtu houbovitých papil (FP).

V pokusu byli použiti samci potkanů kmene Wistar. Obezita byla navozena podáváním vysokotukové stravy. Poté vybrané skupiny zvířat podstoupily SG nebo sham operaci. Jako kontrolní skupiny byli použiti potkani jak na vysokotukové dietě tak potkani na standardní laboratorní dietě. Po šestnácti týdnech byla zvířata usmrcena a bylo provedeno histologické hodnocení jazyka.

Největší počet FP (průměr: 5.11 ± 1.45) byl u vysokotukovou stravou indukovaných obézních potkanů, kteří poslední 4 týdny experimentu dostávali stravu vyváženou. Nejméně FP (průměr: 3.38 ± 1.45) bylo nalezeno u potkanů, kteří byli krmeni stravou s vysokým obsahem tuku po celou dobu pokusu a kteří ve třináctém týdnu podstoupili SG. Mezi skupinami nebyl pozorován žádný rozdíl v počtu chuťových pohárků ani v přítomnosti grelinu v chuťových buňkách.

Rozdíl v počtu FP u potkanů z různých pokusných skupin je pozoruhodný, přestože výsledky se pouze blíží statistické významnosti (hodnota $p = 0,0710$). Jelikož se jedná o pilotní studii, bylo by zajímavé zvýšit počet zvířat v každé skupině a detailněji prozkoumat počty FP na větším množství vzorků.

Klíčová slova: obezita, tubulizace žaludku, houbovité papily, grelin

1 Introduction

Obesity has become a worldwide issue. In 2014, there were more than 1.9 billion overweight adults (representing 39% of the adult population) of which 600 million were obese (WHO, 2015). In 2013, 42 million children under the age of five were overweight or obese (WHO, 2015).

Conventional, non-invasive, therapy is insufficient in treating severe or morbid obese patients, as bariatric surgery has proved to be the most efficient treatment of such states of obesity (WHO, 2015). Gastric surgery is the only treatment resulting in more than 15% weight loss lasting for more than 10 years. Additionally, it decreases most cardiovascular risk factors and positively affects type-2 diabetes (Sjöström, 2008).

One of the reasons, why gastric surgery is such successful treatment of obesity, may be its subsequent effect on food intake and preferences. Some studies have found that after gastric surgery, patients show significant difference in taste perception and taste preferences, coupled with lower appetite (Karamanakos et al., 2008).

Taste cells located within TB of taste papillae in the tongue are responsible for the taste perception. We tried to determine whether different taste perception after SG can be caused and explained by different tongue histology, and whether we could observe correlations between different types of diet, bariatric surgery and tongue histology, particularly in the characteristic of taste papillae.

2 Theoretical part

2.1 General histological Traits

The tongue is a long and quite flexible muscular organ projecting into the oral cavity. The posterior surface is somewhat elevated; the anterior surface of the tongue is divided longitudinally by a shallow groove which terminates at the tongue's tip (Fish et al., 1944). It has an essential role in the process of tasting, digesting and swallowing (Gray, 2000). Macroscopic structure of the rat tongue is shown in Figure 1.

The exterior layer of a tongue is the mucosa. In rats, it consists of keratinized stratified squamous epithelium, beneath which lays a connective tissue called the lamina propria, (Figure 2a). The dorsal surface, unlike the ventral, is covered with mucosal irregularities and elevations, lingual papillae of different shapes and types: filiform, fungiform, foliate and circumvallate (Gray, 2000).

The core of the tongue is formed by skeletal muscle that is sustained by blood vessels, nerves and connective tissue, (Figure 2b). The striated muscle is arranged in bundles that generally run in three different planes transverse, longitudinal and oblique, thus enabling enormous flexibility and precision in the movements of the tongue (Gray, 2000).

Blood vessels supply the muscle with oxygen and nutrients and carry away waste and carbon dioxide, (Figure 2b). It is possible to distinguish arteries that contain much thicker, smooth muscular layer, tunica media, and veins with very thin musculature.

2.1.1 Papillae

Rats, as well as other mammals, possess four different types of papillae: filiform, fungiform, foliate and circumvallate, (Figure 3b) (Fish et al., 1944; Ross and Pawlina, 2011).

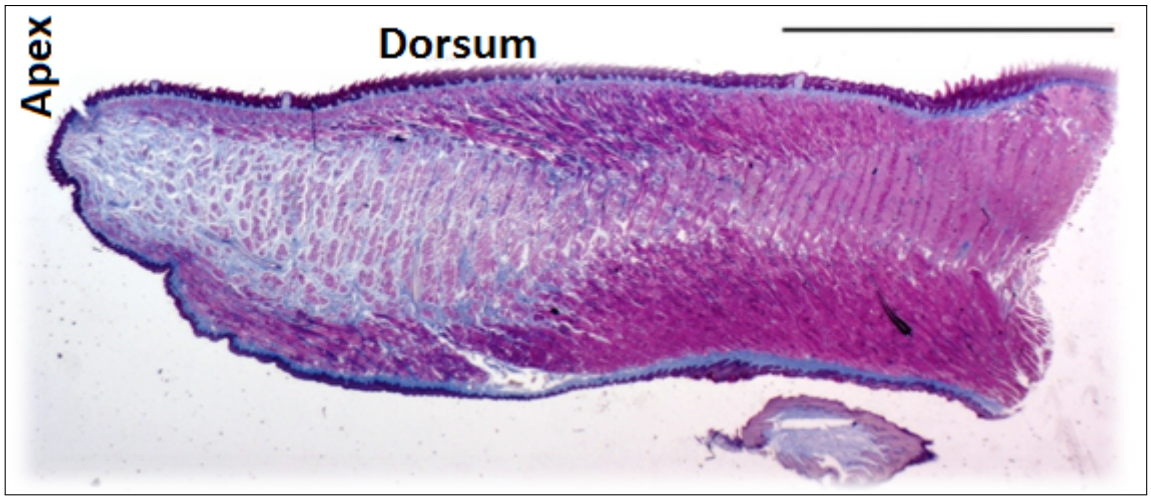
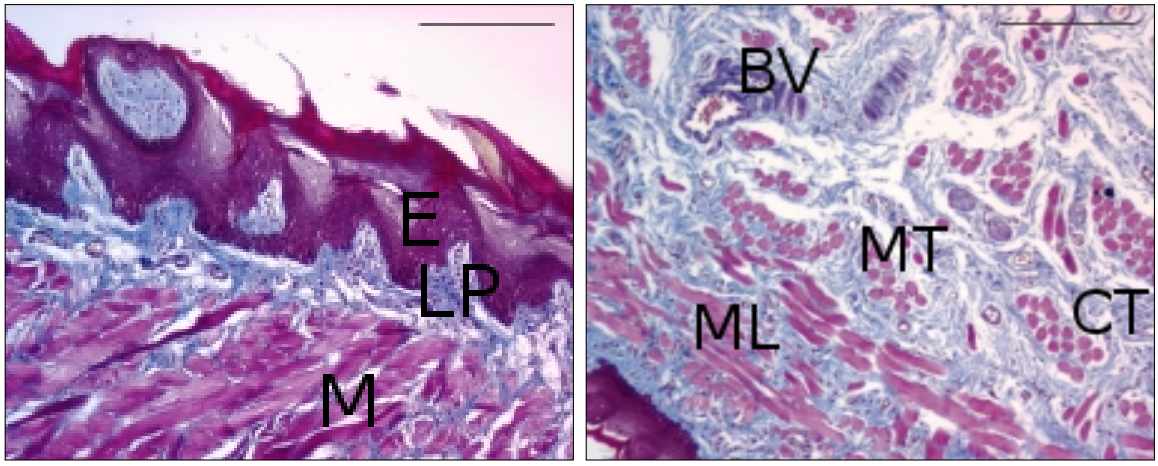


Figure 1:
RAT TONGUE
Masson's trichrome staining, bar 5000 µm (Photo by author)



(a) **(b)**

Figure 2:
(a):**RAT TONGUE EPITHELIUM:** M-muscular fibres, LP-lamina propria, E-keratinized stratified squamous epithelium
Masson's trichrome staining, bar 200 µm (Photo by author)
(b):**RAT TONGUE CORE:** CT-connective tissue, MT-muscular fibres transversal, ML-muscular fibres longitudinal, BV-blood vessel
Masson's trichrome staining, bar 200 µm (Photo by author)

The smallest and most numerous are filiform papillae; they are the most numerous on the dorsal surface of the tongue with a decreased amount on the caudal region (Reginato et al., 2014). A filiform papilla appears in general as a thin cone with its tip pointed backward; however, its shape and diameter can differ according to the tongue's region (Reginato et al., 2014). Its surface is covered by highly keratinized squamous epithelium and the core of the papilla consists of connective tissue. Its role in digestion is entirely mechanical; it does not contain TB, and therefore cannot participate in chemical perception of taste (Ross and Pawlina, 2011; Gray, 2000; Ovalle et al., 2008).

FP are mushroom-shaped structures that are scattered among the filiform papillae and project above them, (Figure 3a). In contrast to filiform papilla, FP is bigger and contains one TB that is situated at the dorsal surface of the papilla in the stratified squamous epithelium. Because of the presence of the TB it can perceive taste (Ross and Pawlina, 2011; Gray, 2000; Ovalle et al., 2008; Mistretta et al., 1999; Fish et al., 1944). The epithelium of FP is much thinner than the one covering filiform papillae (Fish et al., 1944). On average, there are approximately 180 FP in a rat tongue (Fish et al., 1944).

Foliate papillae occur on the dorso-lateral part of the tongue. They contain many TB in the epithelium of the facing walls of neighbouring papillae (Ross and Pawlina, 2011). Among the papillae there, grooves or furrows are formed, that are generally uniform in length and spacing (Fish et al., 1944). Serous glands empty into the basis of the furrows (Ross and Pawlina, 2011; Fish et al., 1944). On average, 12 foliate papillae can be found on rat tongue (Fish et al., 1944).

There is only one circumvallate papilla on the rat tongue, and it differs noticeably from that of other mammals (Fish et al., 1944). Circumvallate papilla is located in the centre of the tongue near its base (Ross and Pawlina, 2011) and the papilla proper is separated from the surrounding structures by a trench on three sides only, lateral and posterior (Fish et al., 1944). The TB are situated on both sides of the deeper portion of the trench; they are similar to those of the foliate papillae, but are relatively more numerous. Mistretta and Baum (1984) claim that there are approximately 400 to 600 TB in a rat circumvallate papilla. As in the case of foliate papillae, the circumvallate papilla is surrounded by crypts, extending from the floor of the trench, into which open the ducts of serous glands (Fish et al., 1944).

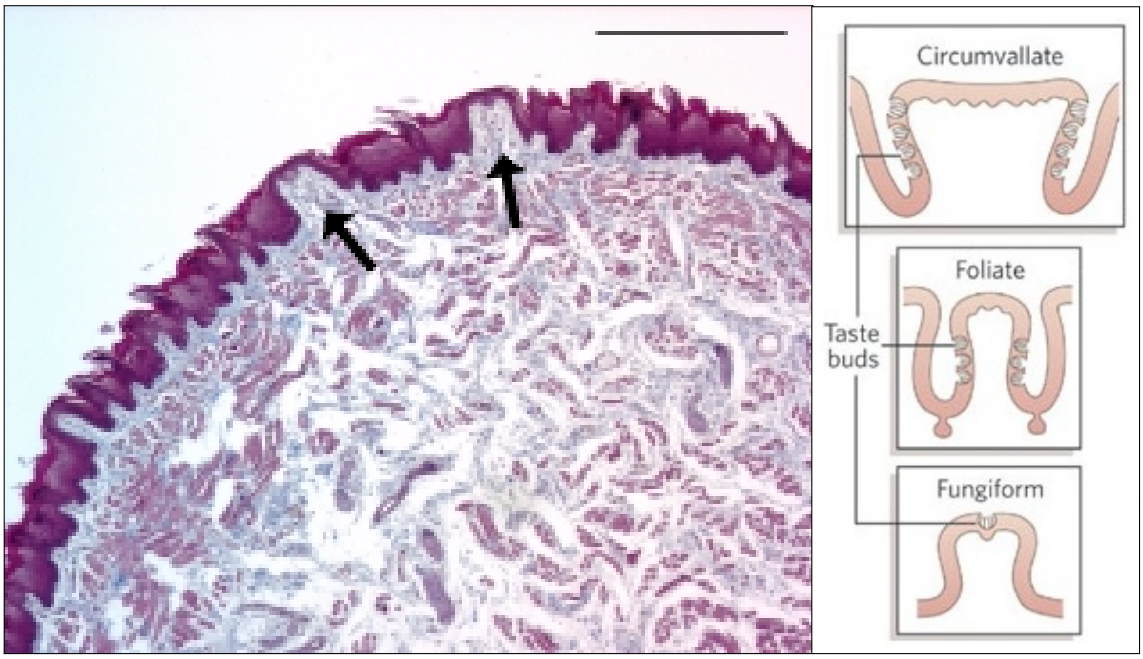
2.1.2 Taste buds

TB are onion-like peripheral gustatory organs that are localized in FP, circumvallate and foliate papillae, as well as over the fimbriae linguae, under the surface of the soft palate or on the posterior surface of the epiglottis (Gray, 2000). On the top of each TB is a small opening between the cells of the epithelium, called the taste pore, (Figure 4a), (Ross and Pawlina, 2011).

Each mature TB contains 50 to 75 taste cells (in rats) (Hosley and Oakley, 1987), classified into four different types, as shown in Figure 4b (Trivedi 2012). Some of the cells may detect nutrients and other compounds, while others provide support. The apical tip of taste cell forms microvilli and directly contacts the external environment in the oral cavity; its body spread out in the central portion of the bud and terminates in a basal lamina. The apical tips of taste cells are connected with tight junctions (Ross and Pawlina, 2011).

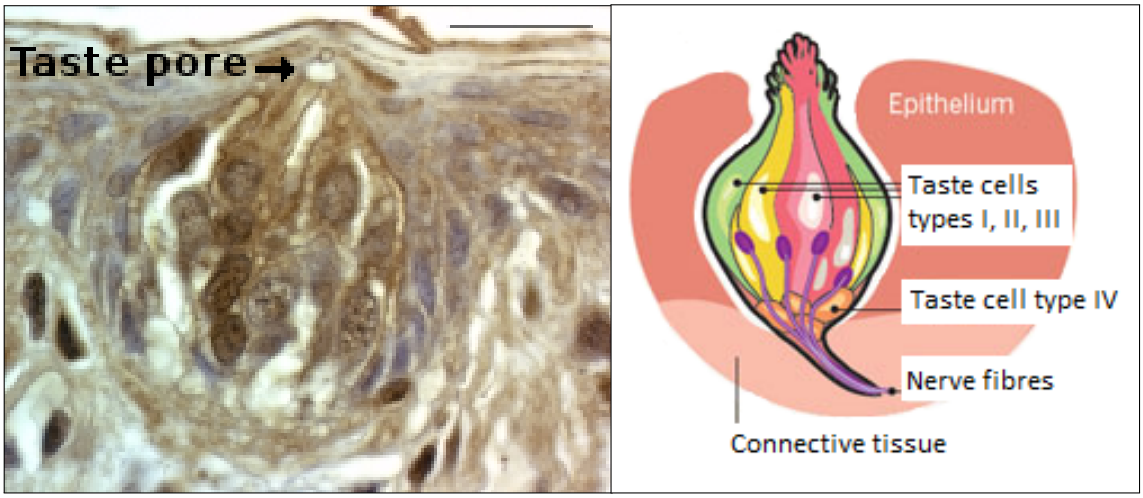
2.2 Ghrelin

Ghrelin is an orexigenic hormone that is produced in stomach (Kojima et al., 1999). Ghrelin is a peptide of 28 amino acids. Rat ghrelin is similar to the human hormone, apart from two amino acids. For its activation, the serine3 residue is n-octanoylated by ghrelin-O-acyltransferase (GOAT) (Kojima et al., 1999).



(a) **(b)**

Figure 3:
(a):**FUNGIFORM PAPILLAE AMONGS FILIFORM PAPILLAE:**
Masson's trichrome staining, bar 200 µm (Photo by author)
(b):**DIFFERENT TYPES OF PAPILLAE:**
Adapted from (Chandrashekar et al., 2006)



(a) **(b)**

Figure 4:
(a):**TASTE BUD WITH TASTE PORE:**
Anti-ghrelin counterstained with haematoxylin, bar 20 µm (Photo by author)
(b):**TASTE BUD WITH DIFFERENT TYPES OF TASTE CELLS:**
Adapted from (Trivedi, 2012)

Ghrelin is primary secreted in gastric fundus, yet it is also present within TB of the tongue. Recently, it was shown in mice that ghrelin is expressed in type I, II, III and IV taste cells of taste buds and can be found together with preproghrelin, prohormone convertase 1/3, its cognate receptor (GHSR) and ghrelin-O-acyltransferase (Shin et al., 2010). Additionally ghrelin and its receptor co-localize in the same taste cell suggesting its autocrine signaling (Shin et al., 2010).

The concentration of ghrelin in circulating blood changes during a day. There is a clear pre-prandial increase and postprandial decrease of ghrelin levels, and also an increase during prolonged fasting in rats (Cummings et al., 2001). Ghrelin signals hunger and increases food intake and adiposity via its effect on hypothalamus, moreover, it interacts with the brain reward pathways to alter food preferences and to intensify food reward (Menzies et al., 2013).

It is not fully understood how ghrelin differs in its plasma levels, receptors reactions and effective pathways and food reward in individuals with diet-induced obesity from lean individuals (Skibicka and Dickson, 2011).

2.3 Obesity

As the World Health Organization states: "Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk of health" (WHO, 2015).

The fundamental cause of obesity is the imbalance in energy intake and consumption. It is a combination of the increased intake of food, that is, food with high-fat and high-calories, on one hand, and decreased physical activity due to suburbanization and usage of transportations, on the other hand, that is responsible for the increase in body weight.

Body mass index (BMI) is a quantity to describe the estimated population measure of obesity: a person's weight (in kilograms) divided by the square of his or her height (in meters). The normal range of BMI extends between 18.5 and 24.9. A person with BMI of 25 or greater is considered overweight, although a person with a BMI equal to 30 or greater is generally considered obese. Severe obesity is defined as BMI greater than or equal to 40 and coincides with an extensively higher health risk than a BMI of 30 (WHO, 2015).

Being overweight or obese not only has esthetical and social impacts, but also is major risk factor for a number of chronic diseases, including cancer, diabetes, musculoskeletal disorders and cardiovascular diseases. Obese children have a higher risk of bone fractures, breathing difficulties, hypertension and early symptoms of cardiovascular diseases, insulin resistance and psychological effects. Additionally, childhood obesity correlates with a higher chance of obesity in adulthood, premature death and disability (WHO, 2015).

Nowadays obesity is not only a problem of western countries, but also arises in low and middle-income countries all over the world (WHO, 2015).

2.4 Surgical treatment of obesity

Surgical treatment of obesity is indicated for patients with a BMI exceeding 40 or for patients with a lower BMI (higher than 35) who are simultaneously suffering from other comorbidities, which were resistant to conservative therapy, including pharmacotherapy, and which failed to lose bodyweight (Češka et al., 2010).

There are three main types of bariatric surgeries: restrictive, malabsorptive and a combination of the two. Restriction physically limits the amount of food the stomach can hold, which leads to limited number of calories one can eat. Malabsorption shortens or bypasses part of the small intestine, which reduces the amount of calories and nutrition the body absorbs (Fried, 2009). Together, they present four main types of bariatric surgeries: vertical SG, adjustable gastric binding, Roux-en-Y gastric bypass and biliopancreatic diversion with a duodenal switch (Češka et al., 2010).

Vertical sleeve gastrectomy Vertical SG belongs to the restrictive surgeries. The surgeon removes about 75% of the stomach, it means resecting the part of great curvature and fundus and leaving only a narrow tube (sleeve), which connects the intestine. The results are faster sense of satiety, changes in hormone levels, acceleration of stomach emptying and speeding up of the passage through the intestine (Fried, 2009; Griffin and Derrer, 2014).

Adjustable gastric binding Another very common restrictive surgery is adjustable gastric binding. An inflatable band is placed around the upper part of the stomach and fixed, so the stomach starts to look like an asymmetric sandglass. A smaller upper pouch and a larger lower section create two parts that are still connected by a narrow channel. The binding slows down the emptying of the upper pouch and ensures that the patient feels satiated after consuming very small amount of food (Fried, 2009; Griffin and Derrer, 2014).

Roux-en-Y gastric bypass Roux-en-Y gastric bypass surgery combines the restrictive and malabsorptive approaches. The stomach is divided into two parts. The upper part is connected directly to the lower section of the small intestine while bypassing the lower part of stomach and 150-200cm of small intestine. The shortcut skipping these two parts of digestive system causes a lower absorption of calories and an earlier sense of being satiated (Češka, 2010; Fried, 2009; Griffin and Derrer, 2014).

Biliopancreatic diversion with a duodenal switch Biliopancreatic diversion with a duodenal switch is a surgery that restricts both food intake and the amount of nutrients the body absorbs. Approximately 70% of the stomach is removed. The rest of the stomach is connected with a digestive loop to the second part of the ileum, where it meets with pancreatic enzymes and bile coming through biliopancreatic loop (Češka et al., 2010).

2.5 Rat stomach

The rat stomach is divided into two parts: non-glandular (forestomach), where the oesophagus enters into and bacteria digest, and the glandular part, into where the acid and the enzymes are secreted.

The analogue for vertical SG in humans is horizontal SG at rats; as is shown in Figure 5.

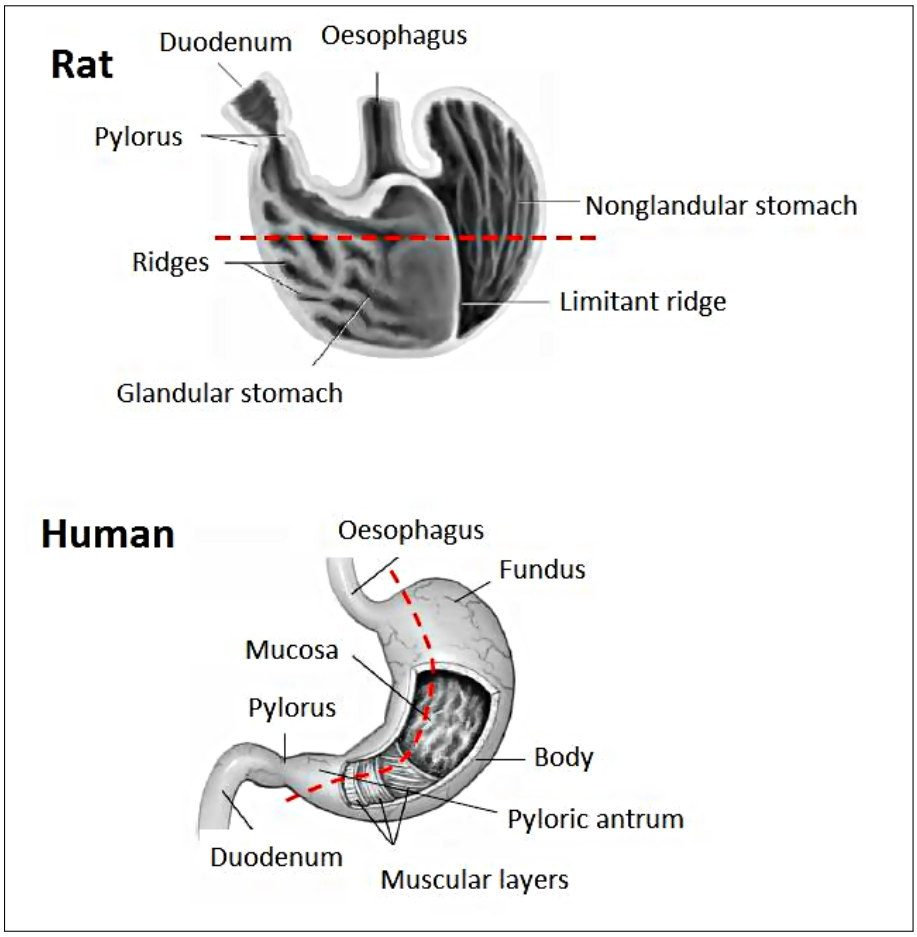


Figure 5: SLEEVE GASTRECTOMY IN RAT AND HUMAN
The red dashed line indicates the place of resection.
Adapted from (DeSesso and Jacobson, 2001)

2.6 Apoptosis

Apoptosis is a programmed cell death. It is a highly regulated cell suicide process activated when the cell is no longer needed or it poses a threat to the organism. Its activation can be triggered by the cell itself (intrinsic pathway) or by extracellular stimuli (extrinsic pathway). The advantage of apoptosis is its neat and smooth course without damaging the cell's neighbours. On the other hand, cell dying by necrosis (of acute stress) undergoes acute swelling and lysis and causes extensive surrounding tissue damage and intense inflammatory response (Alberts et al., 2002; Ross and Pawlina, 2011).

Apoptosis features itself with morphological and biochemical characteristics that resemble controlled autodigestion.

The whole process of cell degradation is irreversible and begins in nucleus by DNA fragmentation; nuclear endonucleases selectively cleave DNA into small oligonucleosomal fragments. At the same time chromatin aggregates and the nucleus disintegrates into several fragments enclosed in the nuclear envelope (Ross and Pawlina, 2011).

The cell volume decreases. Cytoplasm is shrinking, endocytotic vesicles fuse with the plasma membrane and cytoskeletal elements reorganize (Ross and Pawlina, 2011).

The mitochondria loose their function and integrity. Changes instantly appear in the permeability of mitochondrial membrane channels, the transmembrane potential drops and electron transport chain disrupts. Proteins, such as cytochrome c, are released from intermembrane into the cytoplasm and activate caspases. Caspases are the main instrument for dismantling the cell (Ross and Pawlina, 2011).

Certain molecules translocate from the cytoplasmic surface to outer surface of the plasma membrane; consequently, the physical and chemical characteristics of the cell membrane change and lead to blebbing without loss of membrane integrity (Ross and Pawlina, 2011).

The final stage of apoptosis is formation of apoptotic bodies. These membrane-bounded vesicles originating from cytoplasmic bleb contain organelles and nuclear material. They display properties on their surface to be rapidly recognized and phagocytized, either by a macrophage or by a neighbouring cell. The removal of apoptotic bodies is so fast and efficient that no inflammatory response occurs (Alberts et al., 2002; Ross and Pawlina, 2011).

3 Aim of the study

The aim of this work is to clarify whether there is a significant difference in the number of fungiform papillae in the tongues of Wistar rats in different experimental conditions, in diet-induced obesity and after sleeve gastrectomy.

4 Experimental part

4.1 Animals

4.1.1 Breeding conditions

Sixteen 21-day-old male Wistar rats were used. Body weight was 148 ± 66g at the beginning of the experiment. Breeding characteristics included room temperature (RT) 22 ± 2°C, relative humidity $50 \pm 10\%$, individual caging, pathogen-free conditions and 12:12 h controlled light-dark cycle with lights on from 8:00 AM.

The rats were divided into six groups (A, B, C, E, N and H) as shown in Table 1. For the first 12 weeks of the experiment, all of the 16 rats were fed ad libitum; 13 of them were fed HFD to induce obesity, whereas 3 rats, belonging to the control group (group N), were fed ND. The weight was monitored once a week and food intake twice.

After this period, 6 rats underwent SG (3 from the group B and 3 belonging to the group E), 3 rats underwent sham intervention (rats from the group A); meanwhile, the rest of the rats maintained intact. A sham surgery comprised the same laparotomy incision, as well as handling of the stomach, except for the gastrectomy.

For the next four weeks 5 rats (2 belonging to the H control group and 3 from the experimental group E), stayed on HFD; the rest of the rats were fed ND. All the rats were fed ad libitum, except of the 2 rats of the experimental group C, which were pair-fed (PF) to the average amount of the food eaten by the animals of the experimental group B. During this period, the food intake and weight gain were measured every day.

All the experimental procedures were approved by the Ethical Committee for Animal Experimentation of the University of Navarra (Protocol 052-10).

4.1.2 Diets

As ND was used Tekland Global 14% Protein Rodent Maintenance Diet obtained from HARLAN Laboratories, Spain, described in Table 2. As a HFD was used Mouse Diet, High fat, Fat Calories 60% (F3282), Bio-Serv, bought from Frenchtown, NJ, USA, (Table 2).

Table 1:
EXPERIMENTAL GROUPS OF RATS

Group	Control		Experimental			
Diet before intervention 12 weeks	ND	HFD	HFD			
Intervention	-	-	SG		Sham	-
Diet after intervention 4 weeks	ND	HFD	ND	HFD	ND	ND (PF)
Number of rats	3	2	3	3	3	2
Label of the group	**N**	**H**	**B**	**E**	**A**	**C**

ND-normal diet, HFD-high-fat diet, SG-sleeve gastrectomy, PF-pair-fed
A, B, C, E, N, H – designation of the group

Table 2:
TYPES OF DIETS

	Proteins		Carbohydrates		Lipids		Energy
	%weight	%kcal	%weight	%kcal	%weight	%kcal	%weight
ND	14.5	20.0	63.9	67.0	4.0	13.0	**3.1**
HFD	20.0	14.7	36.3	26.7	35.5	58.0	**5.5**

HFD: high-fat diet, ND: normal diet

4.1.3 Surgical procedures

Anaesthesia Nine rats from the groups A, B and E were subjected to general isoflurane (Laboratorios Esteve S.A., Barcelona, Spain) anaesthesia 14 hours after their last feeding. The inhalatory anaesthesia was performed in a special anaesthetizing chamber that was connected to the vaporizer (Medical Supplies & Services International Ltd., Keihley, UK). Through a vaporizer a mixture of isoflurane and oxygen was administered at a constant flow rate of 0.5l/min to 0.7l/min. 4.0% mixture of isoflurane-oxygen was used to induce the anaesthesia. At the moment the rat stopped responding to reflexes, it was positioned on its back at the surgery area with the constant flow of 2% isoflurane-oxygen mixture. The 2% concentration ensured that the animal undergoing surgery did not suffer pain or a change in a cardiac rhythm.

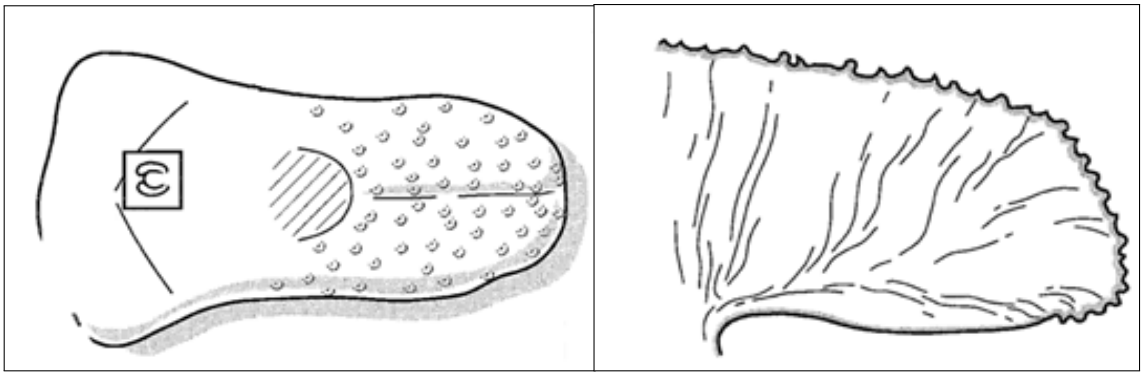
Sleeve gastrectomy To imitate human SG in a rat, approximately 60% of the rat stomach was resected (both glandular and non-glandular parts), being careful not to cut off the passage to duodenum and to preserve both parts for the correct function of the stomach, see Figure 5 in Theoretical part.

Sham surgery Three rats from the group a helped to distinguish the effect of SG from the effects of a surgery. The rats underwent a surgery called sham that was similar to SG but without the actual stomach resection. It also consisted of a laparoscopic incision, extraction of the stomach out of the abdominal cavity and hydration of the cavity with saline.

Food intake For the first 48 hours after the surgery, all the animals had free access to a liquid diet consisting of 5% glucose and 0.9% saline. After these two days, they started receiving solid diet according to their experimental groups.

Sacrifice The animals were sacrificed after 16 weeks of the experiment. After 14 hours of fasting, the rats were decapitated without anaesthesia due to its possible modification of metabolic variables (Pérez-Llamas et al., 1992; Frühbeck, 1995). Immediately afterwards, the tongues were dissected and processed for the optical microscopy.

4.2 Tissue processing



(a) **(b)**

Figure 6:
RAT TONGUE:
(a): dorsal view on the rat tongue
(b): saggital section
Adapted from (Mistretta et al., 1999)

4.2.1 Fixation

The tongues were dissected from the rat heads and then cut longitudinally in the middle, as is shown in Figure 6a. The sagittal orientation of the anterior tongue with FP provides an orientation that optimizes potential for complete cross sections through most papillae and TB (Mistretta et al., 1999) (Figure 6b). The half tongue pieces were fixed in 10% neutral formalin (pH 7.4) for 12 to 24 hours. Then they were rinsed with water and immersed in 70% ethanol until the inclusion.

4.2.2 Paraffin embedding

The embedding was done in an automatic processor (Vacuum Infiltration Processor: SAKURA 4893-Floor E150, BAZER, Barcelona, Spain). The whole process involves two phases. Firstly, the tissue undergoes a series of washes at 40 °C: one in 70% ethanol for 50 min, two in 96% ethanol for 60 min and 90 min, three in absolute alcohol each one for 60 min and finally, three 60-min-washes in xylene. Secondly, the tissue is immersed in a four different washes of liquid paraffin for 90, 60, 120 and 120 min at 60 °C. At the end, the paraffin blocks were made with correct orientation of both halves of the tongue.

4.2.3 Sectionning

The paraffin blocks were cut up into 3 µm slices using a microtome Microm HM 340E (Bio Optica, Milan, Italy). All slices were placed on slides. Those slices intended to be further stained with immunohistochemical methods were placed on special slides (Menzel-Glaser, J1800AMNZ, Braunschweig, Germany) for a better adherence and thus preventing loss or damage of the tissue while being worked with.

4.2.4 Staining techniques

Conventional staining: Masson's trichrome
The protocol that has been followed:

1. Heat the slides in an oven at 60 °C for 30 min; deparaffinize them in two xylene washes: the first wash for 30 min, and the second one for 15 min at RT.

2. Hydrate sections through a series of washes in decreasing concentration of ethanol in aqueous solutions (100%, 96%, 80% and 70% ethanol) for 5 min in each solution. Rinse with running tap water for 5 min.

3. Stain in Weigert's iron haematoxylin working solution for 7 min. Make a fresh solution of haematoxylin by adding equal volumes of Solution 1 (1% haematoxylin in 95% ethanol) and Solution 2 (1.2% ferric chloride, 1% hydrochloric acid in distilled water).

4. Differentiate in picric acid solution (13 g of picric acid in 2 l of 96% ethanol) for 5 min.

5. Rinse properly in running tap water for 10 min.

6. Stain in Solution a for 15 min. Add one part of solution a (1 g of acid fuchsin, 0.5 cc of acetic acid in 100 cc of distilled water) and two parts of Solution b (1 g of Ponceau S, 0.5 cc of acetic acid in 100 cc of distilled water).

7. Rinse in running tap water.

8. Differentiate in a 1% aqueous solution of phosphomolybdic acid for 5 min.

9. Wash in running tap water.

10. Stain in Solution B (2 g of aniline blue, 0.5 cc of acetic acid in 100 cc of distilled water) for 2 min.

11. Wash in running tap water.

12. Dehydrate through 96% ethanol (two washes, each 5 min), absolute ethanol (two washes, each 5 min) and clear in xylene (two washes, each 5 min).

13. Mount with DPX (a synthetic resin used as a mounting medium in histology).

Immunohistochemistry The specific rabbit polyclonal antibody anti-ghrelin was used to identify ghrelin producing taste cells. To reveal positivity we used horseradish peroxidase with 3,3'-diaminobenzidine (DAB) as chromogen.

The protocol that has been followed:

1. Heat the slides in an oven at 60 °C for 30 min, deparaffinize them at RT in two xylene washes: the first wash for 30 min, the second wash for 15 min.

2. Hydrate sections through a series of washes in decreasing concentration of ethanol in aqueous solutions (100%, 96%, 80% and 70% ethanol) for 5 min in each solution. Rinse with running tap water for 5 min.

3. Carefully wipe around the specimen to remove any remaining liquid. To block endogenous peroxidase cover the tissue with enough peroxidase block from Bottle (kit – Envision + system-HRP, see table below). Incubate for 10 min at RT.

4. Wash the samples in running tap water.

5. Immerse the slides in a fresh TB (tris-buffered saline – pH 7.36) bath for 5 min.

6. Tap off excess buffer and wipe the samples as before. Block background by incubating them in a normal serum diluted 1:20 at RT in a humidity chamber for 30 min.

7. Eliminate the excess of normal serum. Apply enough optimally diluted primary antibody to cover the tissue. Incubate it overnight at 4 °C in a humidity chamber.

8. Place the samples in a fresh buffer bath for 5 min.

9. Tap off excess buffer and wipe slides as before. Apply enough labelled polymer from Bottle 2 (kit – Envision + system-HRP, see table below) to cover the specimen. Incubate for 30 min in a humidity chamber at RT.

10. Wash samples in a buffer bath for 5 min.

11. Perform this step in a fume hood. After wiping the slides apply enough of the 1:200 diluted liquid 3,3'-Diaminobenzidine (DAB) + substrate-chromogen(bottle 3 and 4 of the kit, see table below) solution to cover specimen and incubate for 2 min. Monitor the revealing process in the microscope. Stop the reaction by rinsing the slides with water. Collect substrate-chromogen waste in a hazardous materials container for proper disposal.

12. Contrast the tissue by immersing the specimen in an aqueous solution of Harris's haematoxylin for 4 s. Differentiate haematoxylin in running water.

13. Dehydrate the slides through 96% ethanol (two washes, each 5 min), absolute ethanol (two washes, each 5 min) and clear in xylene (two washes, each 5 min).

14. Mount with DPX.

Positive control Rat stomach was used as a positive control in each experiment to ensure that all the reagents and equipment were functioning properly and that no human mistake occurred.

Primary Antibody Specific antibody Rabbit Anti-Ghrelin (Human) Antiserum (Phoenix Pharmaceuticals INC. Catalog No: H-031-30) was used at the optimal dilution 1:400.

Sequence: Gly - Ser - Ser(Octanoyl) - Phe - Leu - Ser - Pro - Glu - His - Gln - Arg - Val - Gln - Gln - Arg - Lys - Glu - Ser - Lys - Lys - Pro - Pro - Ala - Lys - Leu - Gln - Pro – Arg

Kit – EnVision+ System-HRP (DAB) The kit K 4011 (Dako, Glostrup, Denmark) was used in processing the protocol.

Bottle 1 - Peroxidase block (0.03% hydrogen peroxide containing sodium azide)

Bottle 2 – Labelled polymer (Peroxidase labelled polymer conjugated to goat anti-rabbit immunoglobulins in Tris-HCl buffer containing stabilizing protein and an anti-microbial agent; optimal dilution 1:400)

Bottle 3 - Substrate Buffer (Substrate Buffer solution, pH 7.5, containing hydrogen peroxide and a preservative)

Bottle 4 - Liquid DAB+Chromogen (3,3'-diaminobenzidine chromogen solution)

Normal serum Normal Goat Serum diluted 1/20 in TB (X 0907, Dako, Glostrup, Denmark).

Apoptosis detection: Tunel assay We optimised a method for apoptosis detection in taste cells of rat tongues, to be able to determine in subsequent studies whether in some groups of rats the taste cells die more frequently and in higher numbers than in others.

TUNEL assay i.e. terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling label broken DNA in a place of nick.

The protocol that has been followed:

1. Deparaffinize and hydrate the slides according to the immunohistochemical protocol described above.

2. To block endogenous peroxidase cover the tissue with 1 drop of commercial H_2O_2 (3% concentration) for 5 min to 10 min, stop the reaction by washing in deionized water.

3. Immerse the slides in phosphate buffered saline pH 7.4 for 5 min.

4. As a pretreatment of the tissue, incubate it with proteinase K in a humidity chamber for 30 min at 37 °C. Optimize this step by using different times and different concentrations of the enzyme.

5. Wash the slides in a phosphate buffer saline for 5 min.

6. Incubate the tissue for 5 min in terminal deoxynucleotidyl transferase (TdT) buffer (10 ml of buffer TdT 5x, 3 ml of 25 mM Cobalt dichloride, 37 ml of sterile distilled water) at RT.

7. Prepare the solution for detecting reaction by mixing the following: 20 µl of TdT buffer 5x, 6 µl of 25 mM Cobalt dichloride, 2 µl of 1 mM Deoxythymidine triphosphate (dTTP), 1 µl of 1 mM dUTP-16-biotin (Deoxyuridine Triphosphate (dUTP)), 69 µl of sterile distilled water and 2 µl of TdT.

8. Apply enough of the detecting solution (described in a step 7) on a tissue and incubate in a humidity chamber at 37 °C. The length of the incubation depends on type of the tissue and needs to be optimised.

9. Shake the slides with a stop wash buffer 2x (10x concentration: sodium chloride 87.65 g, sodium citrate 44.10 g, water to 1.0 l; dilute 5 times) for 15 min at RT. Rinse the buffer twice with distilled water.

10. Incubate the tissue with 2% normal serum at RT for 10 min, and then wash it with distilled water.

11. Immerse the samples in TB for 5 min.

12. Incubate the tissue with the avidin-biotin complex (prepared 30 min in advance) for 30 min at RT.

13. Wash the slides in TB for 5 min.

14. Reveal using the DAB technique described above in the protocol of Immunohistochemistry.

15. Contrast the tissue by immersing the specimen in an eosin solution for few seconds, and then rinse it with water.

16. Dehydrate the slides following the immunohistochemical protocol described above.

17. Mount them with DPX.

4.3 Statistical Analysis

4.3.1 Image capture

All slides were observed with the optical microscope and some of them were selected for further capture of structures and tissues of interest. The images were taken using the digital camera DXM 1200 (Nikon, NY, USA) joined to the optical microscope Eclipse E800 (Nikon) and the program NIS-Elements (NIS-Elements D, version 3.2).

4.3.2 Counting procedure

One of the goals of the experiment was to disclose whether there is a significant difference in the number of FP in the tongues belonging to rats from different experimental groups.

Only those structures that were clearly identified as FP were counted, the numbers were recorded for each half of the tongue separately. We considered a TB only when at least one taste cell was observed. In case of any doubts, two investigators had to agree on the identification.

4.3.3 Statistical analysis

Normality was assessed with the Shapiro-Wilk test. The results obtained are summarized as mean and standard deviation in Table 3 and means with 95% confidence interval (CI) in Figure 16 (in Results).

The differences between all experimental groups were studied using nested one way ANOVAS with type I (sequential) sum of squares as shown in Table 4 (in Results).

The Stata/IC 12.1 for Windows (32-bit) (Stata Corp LP, Texas, USA) was used to carry out the analyses. The statistical significance level was set at $P = 0.05$.

5 Results

5.1 General histological Traits

Masson's trichrome staining was used to better distinguish various tissues and structures in the rat tongue. Connective tissue appeared light blue; nuclei were stained dark violet; whereas muscles, keratin, cytoplasm and erythrocytes formed background and displayed red coloration (Bancroft and Gamble, 2003) (Figure 7).

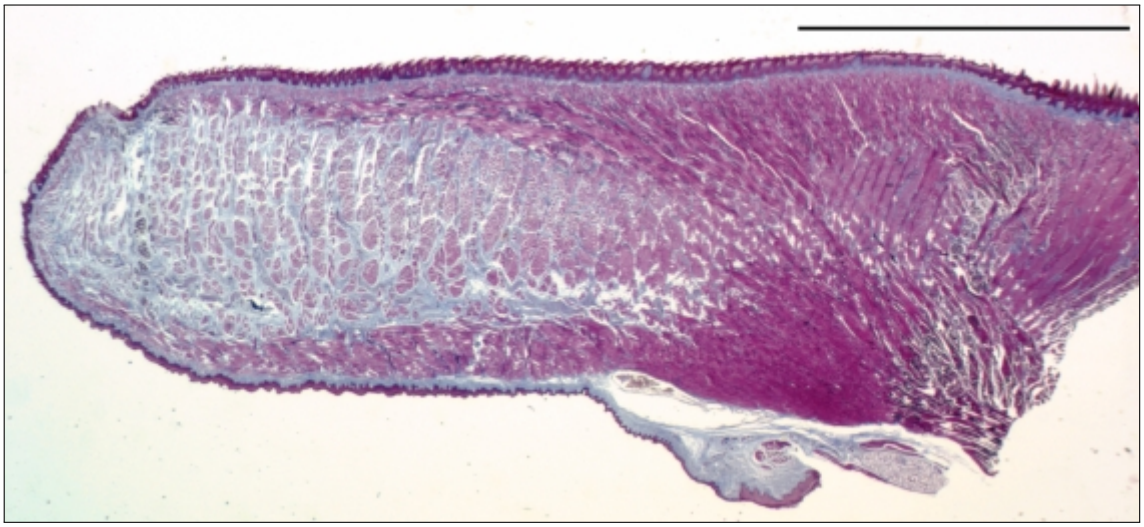
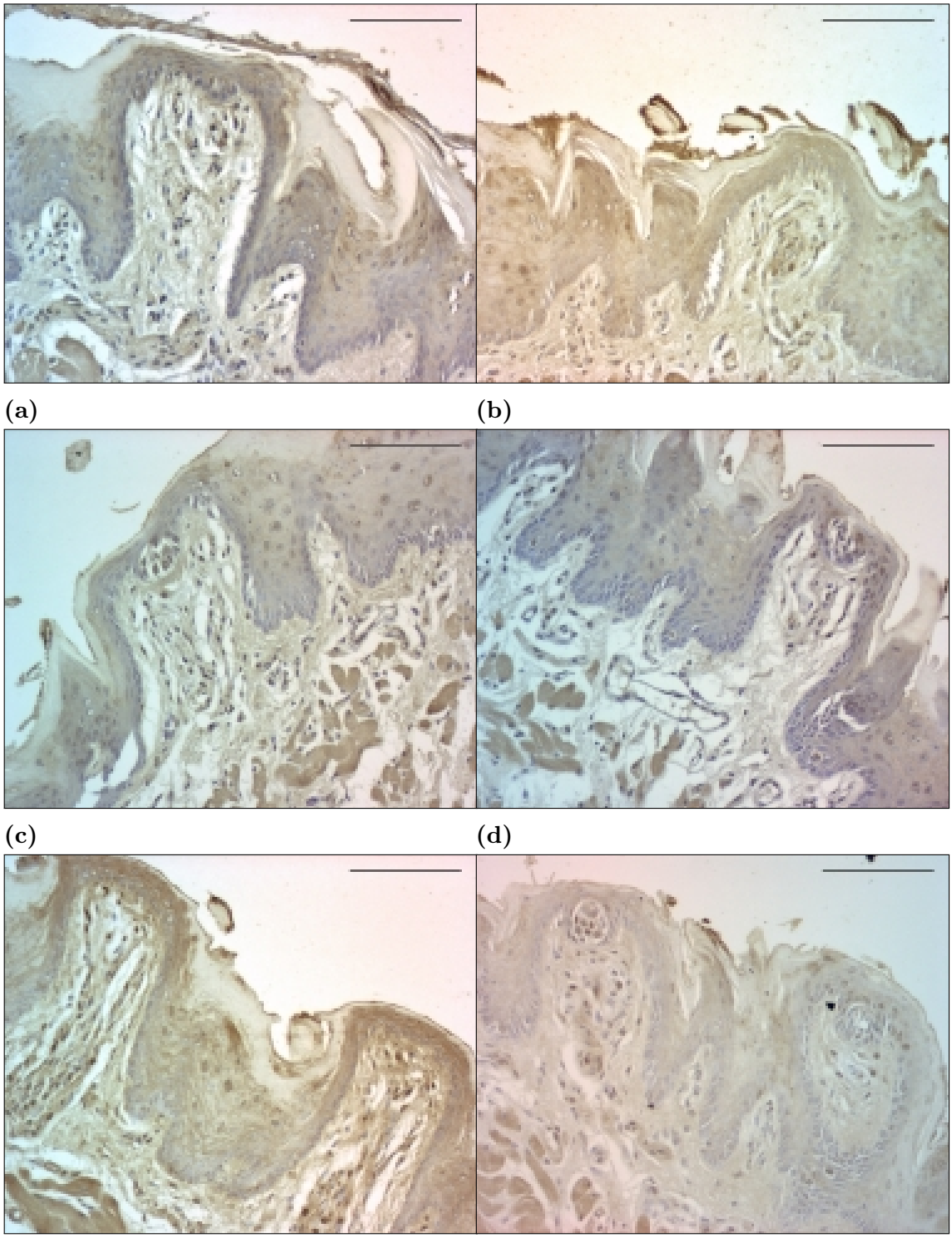


Figure 7: RAT TONGUE
Group N, Masson's trichrome staining, bar 5000 µm

5.2 Morphological characteristics of fungiform papillae

We observed no two papillae are exactly the same; there are great differences in size and shape. Some FP are high and narrow, some are low and wide, some extend others by far at both height and width; and some are very hard to distinguish, because their size is similar to the size of filiform papillae.

The technique of fixation and paraffin embedding is very important and can change the view we have on papillae. Our goal was to observe all papillae in their sagittal section (Figure 8). Nevertheless, due to laboratory limits and work with biological material (that is not perfect), some sections through papillae were made transversally (Figure 9) or obliquely (Figures 10, 11). Thus, we can observe not only different sizes and shapes of papillae, but also their different sectioning.



(a) **(b)**

(c) **(d)**

(e) **(f)**

Figure 8:
SAGGITAL SECTIONS OF FUNGIFORM PAPILLAE
Anti-ghrelin counterstained with haematoxylin, bar 100 µm

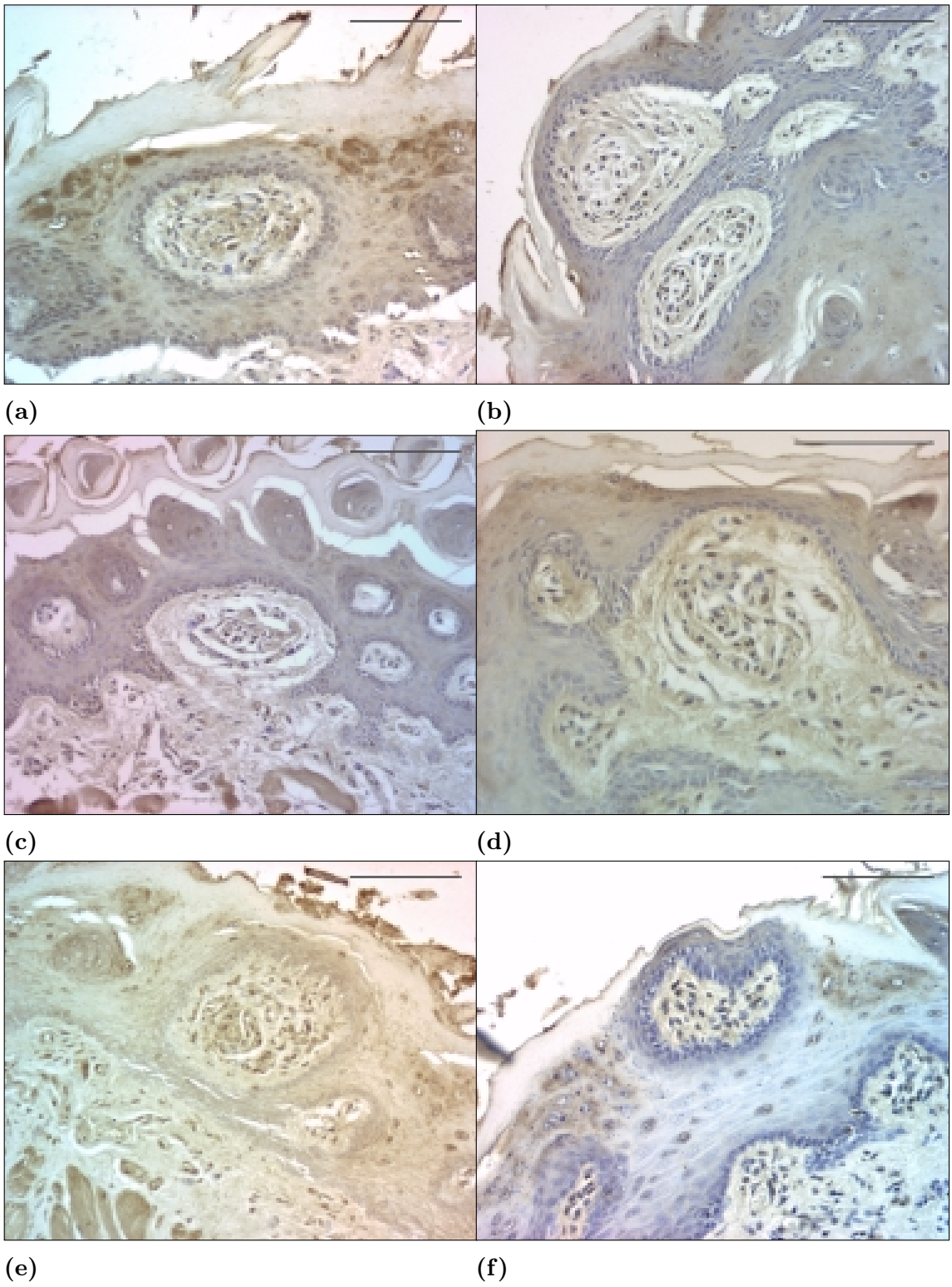
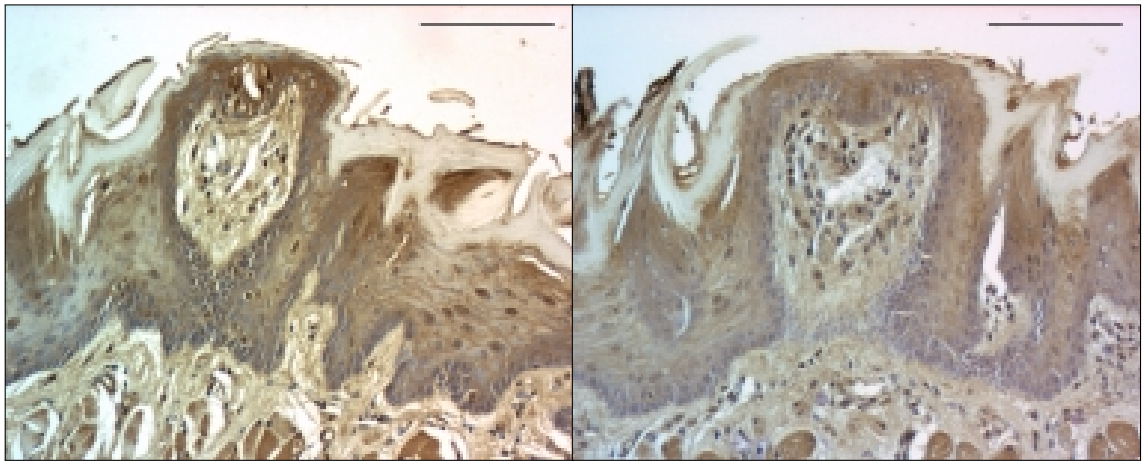
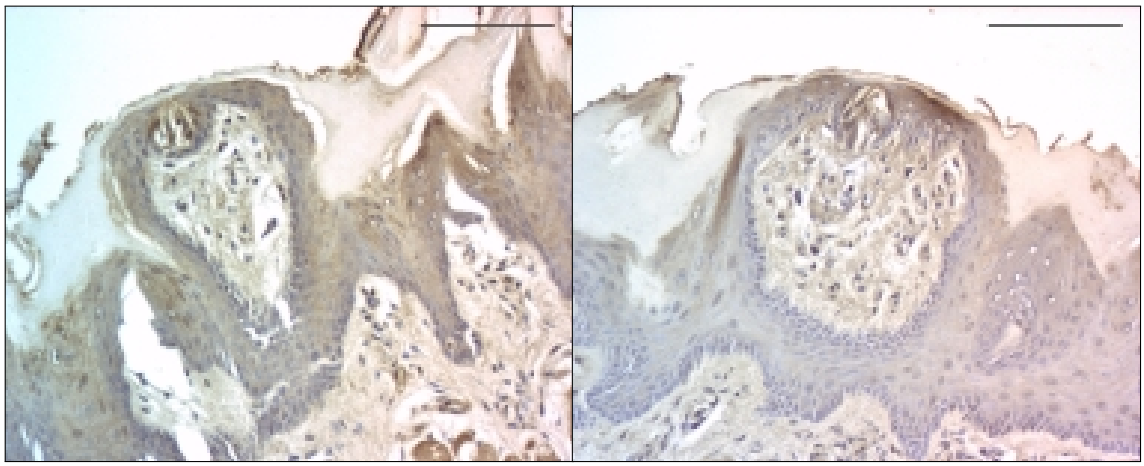


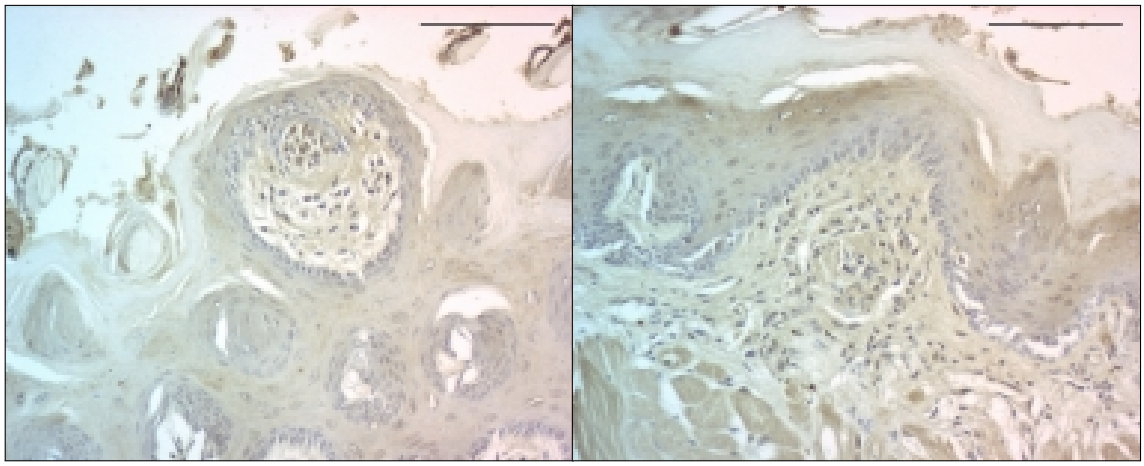
Figure 9:
TRANSVERSE SECTIONS OF FUNGIFORM PAPILLAE
Anti-ghrelin counterstained with haematoxylin, bar 100 µm



(a) (b)

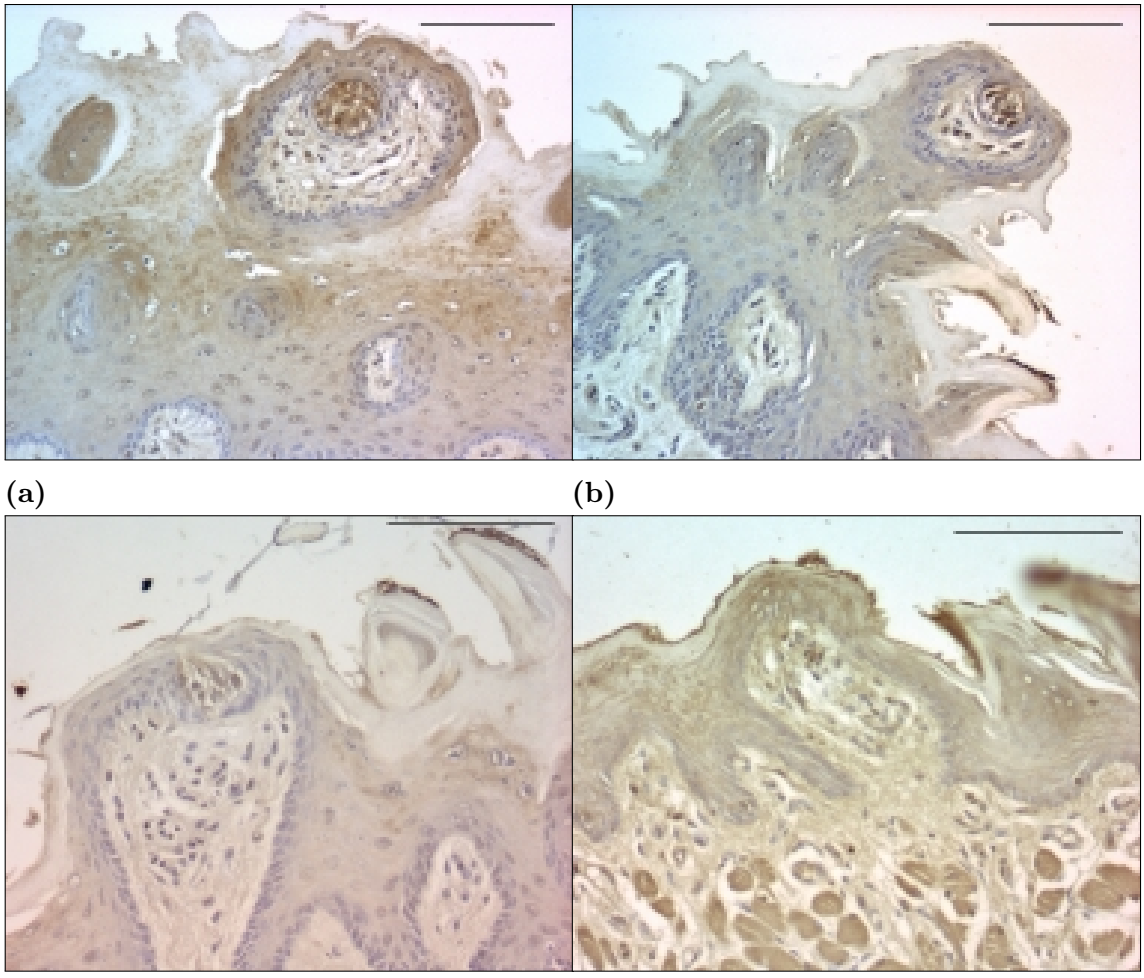


(c) (d)



(e) (f)

Figure 10:
OBLIQUE SECTIONS OF FUNGIFORM PAPILLAE
Anti-ghrelin counterstained with haematoxylin, bar 100 µm



(a) **(b)**

(c) **(d)**

Figure 11:
OBLIQUE SECTIONS OF FUNGIFORM PAPILLAE
Anti-ghrelin counterstained with haematoxylin, bar 100 µm

5.2.1 Curiosities of fungiform papillae

Despite the fact that different types of papillae are precisely described and it is clear and easy to distinguish and classify them, sometimes, it may be bit more complicated. The biggest papilla on Figure 12a seems like a hybrid between filiform papilla and FP. On one hand, it contains nerve fibres and it is bigger than filiform papillae in the surrounding, as typical FP. On the other hand, it is conical and it has a keratinized layer on its top, of the same shape and thickness as filiform papilla.

Another curious sight that surprised us during examination under the microscope; there were two FP appearing inversed, positioned right next to each other (Figure 12b).

Although the papillae are in proximity, they can have opposite orientation, as is apparent on (Figures 12c and 12d).

In a few cases, we observed 3 FP standing together (Figures 12e and 12f).

Not at all expected TB were located close to the radix of the tongue belonging to the rat from the control group N (Figure 14), view on the whole tongue is in Figure 7.

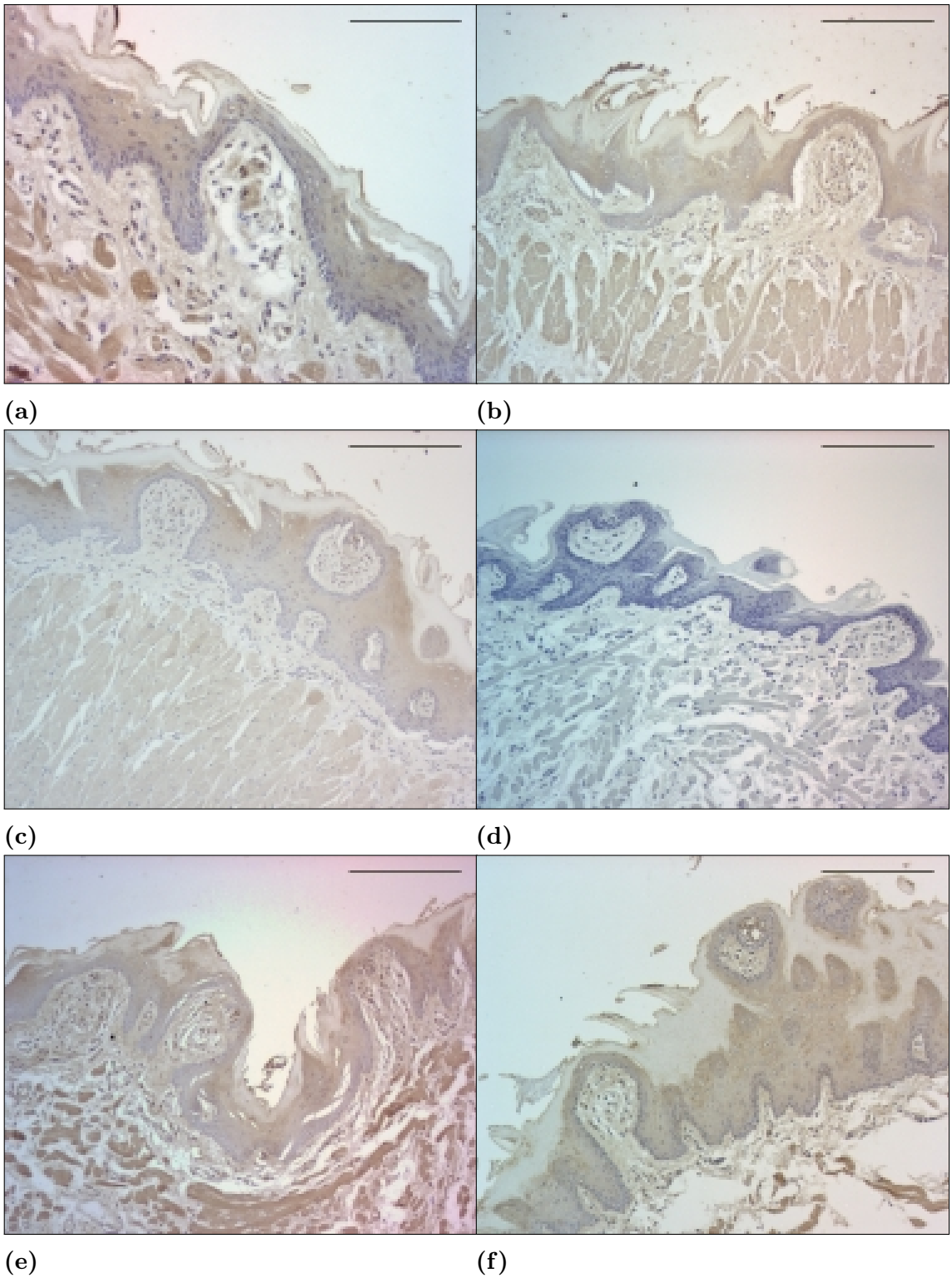
5.3 Taste buds and ghrelin marked taste cells

Ghrelin and ghrelin containing taste cells were marked and stained dark brown (Figure 13). Taste cells with ghrelin were therefore easy to distinguish from the others that were stained very light brown, light grey or appeared bluish.

Each batch undergoing the immunohistochemical staining process contained one slide with rat stomach as a positive control (Figure 15).

Statistical analysis showed that there was no significant difference in a number of TB in the different groups of rats (Table 3).

At least one ghrelin marked taste cell appeared in basically every TB we observed (with only one exception that we consider staining imperfection) (Table 3).

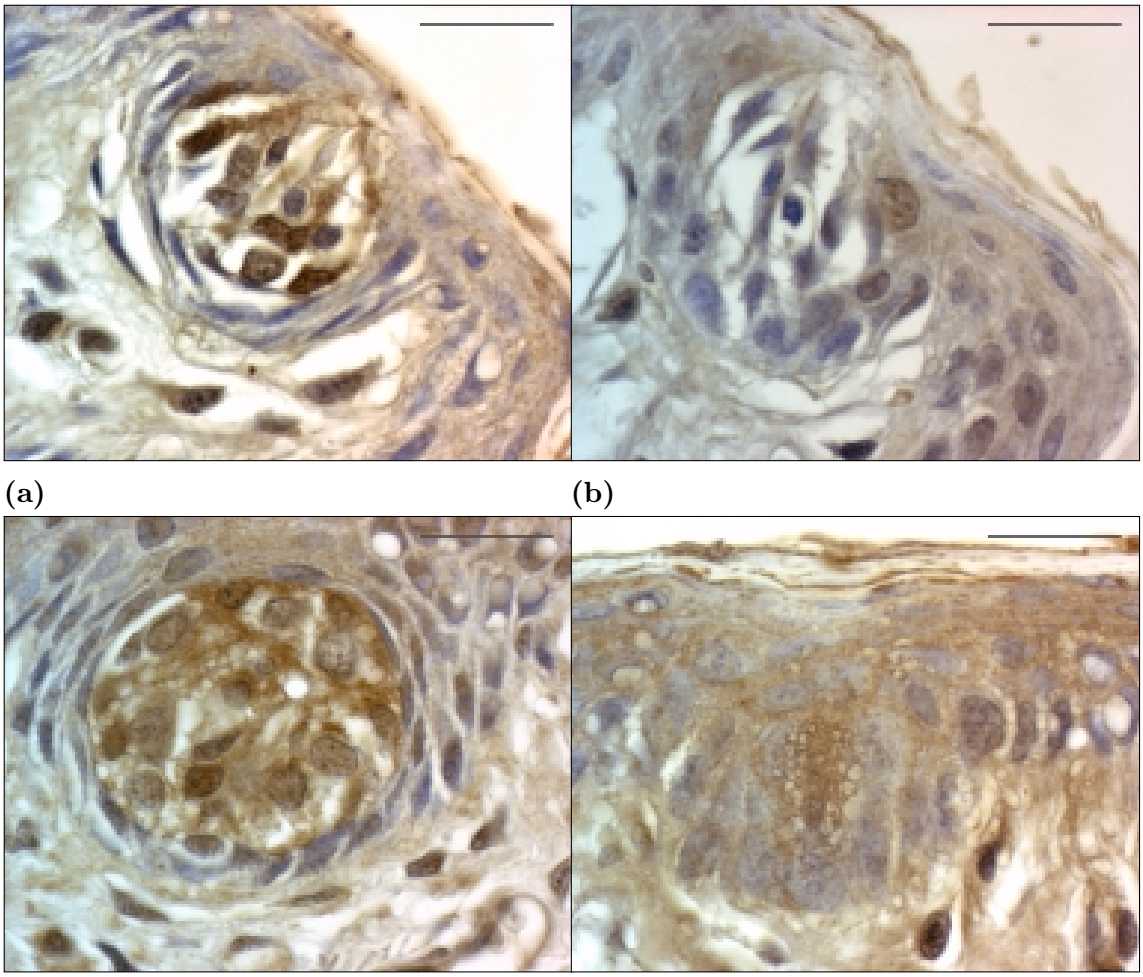


(a) **(b)**

(c) **(d)**

(e) **(f)**

Figure 12:
CURIOSITIES
(a): group B, bar 100 µm
(c): group E, bar 200 µm
(b), (e): group A, bar 200 µm
(d), (f): group B, bar 200 µm
Anti-ghrelin counterstained with haematoxylin



(a) **(b)**

(c) **(d)**

Figure 13:
TASTE BUDS WITH GHRELIN MARKED TASTE CELLS
Brown marked taste cells with detected ghrelin
Anti-ghrelin counterstained with haematoxylin, bar 20 µm

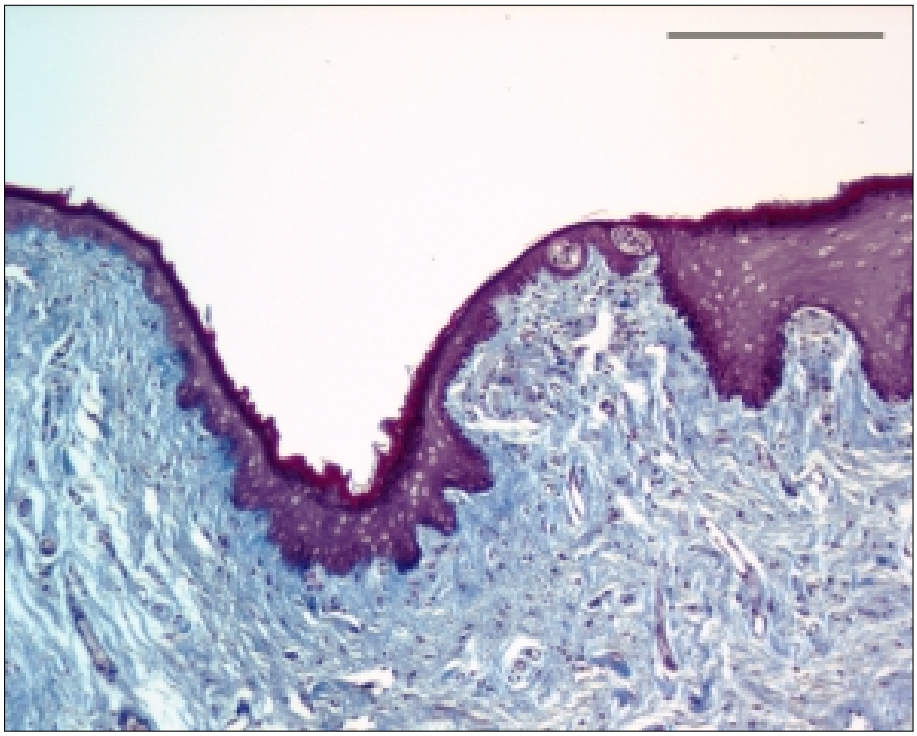
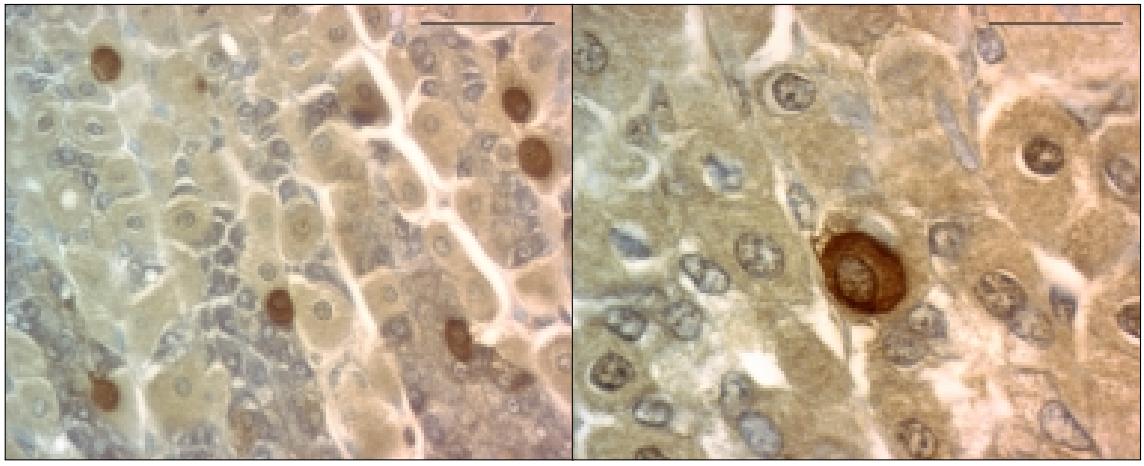


Figure 14:
TASTE BUDS CLOSE TO THE TONGUE RADIX
Group N, Masson's trichrome staining, bar 200 µm



(a) **(b)**

Figure 15:
POSITIVE CONTROL – RAT STOMACH
(a) bar 50 µm (b) bar 20 µm
Anti-ghrelin counterstained with haematoxylin

5.4 Results of statistical analysis

Normality was assessed with the Shapiro-Wilk test. The results obtained are summarized as mean and standard deviation in Table 3 and means with a 95% confidence interval in Figure 16.

The highest mean number of FP (5.11 ± 1.45) was counted in rats belonging to the experimental group C (first part of the experiment were fed ad libitum with a HFD, second part were PF with a ND, no intervention). The lowest number of FP (3.38 ± 1.45) was found in the experimental group E (rats undergoing SG and fed a HFD for the entire duration of the experiment).

When comparing the number of FP in the different groups of rats, no significant differences were observed ($P = 0.0710$), although the value was very near the statistical significance level set at $P = 0.05$ (Table 4).

Our null hypothesis stated that there is no difference in the mean number of FP between rats from the different experimental groups.

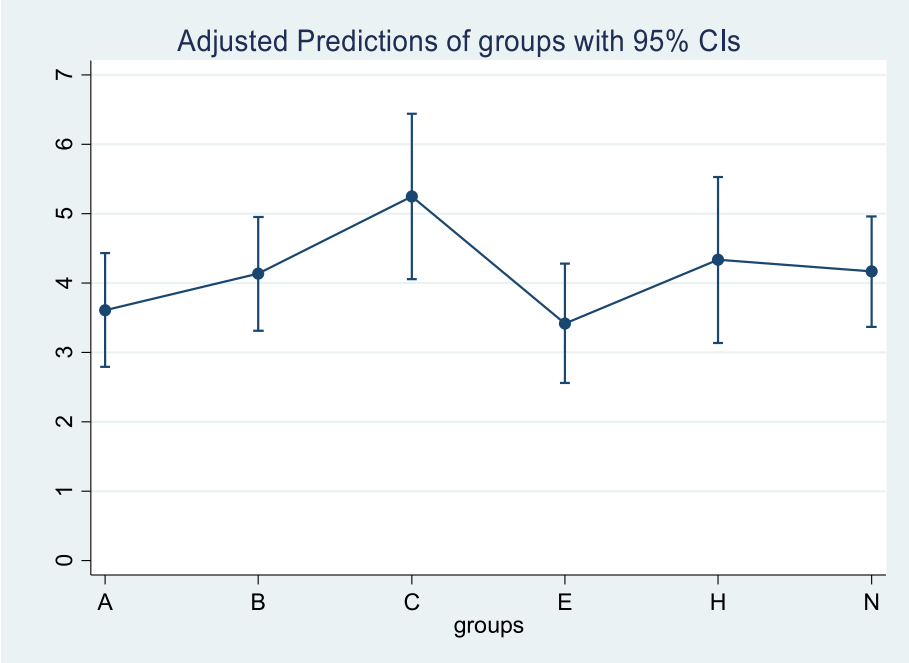


Figure 16:
MEANS OF COUNTS OF FUNGIFORM PAPILLAE WITH 95% CONFIDENCE INTERVALS (CIS)

Table 3:
NORMALITY ASSESSED WITH SHAPIRO-WILK TEST

group	mean	sd	min	max	n
Fungiform papillae					
A	**3.65**	1.69	1	6	17
B	**4.12**	1.76	1	9	17
C	**5.11**	1.45	3	7	9
E	**3.38**	1.45	1	7	16
H	**4.44**	1.33	3	7	9
N	**4.17**	1.95	2	10	18
total	**4.03**	1.7	1	10	86
Taste buds					
A	0.94	0.97	0	3	17
B	1.35	0.86	0	3	17
C	1.56	1.23	0	4	9
E	0.69	0.60	0	2	16
H	1.56	1.13	0	3	9
N	1.17	1.10	0	4	18
total	1.15	0.99	0	4	86
Ghrelin positivity					
A	0.88	0.86	0	2	17
B	1.29	0.85	0	3	17
C	1.56	1.23	0	4	9
E	0.69	0.60	0	2	16
H	1.56	1.13	0	3	9
N	1.18	1.13	0	4	17
Total	1.13	0.97	0	4	85

Mean, standard deviation (sd), minimum (min), maximum (max), number (n).

Table 4:
ANOVA FP GROUPS/TONGUES; GROUPS, SEQUENTIAL

Number of obs = 86; R-squared = 0.1509;
Root MSE = 1.72358; Adj R-squared = -0.0311

Source	Seg. SS	df	MS	F	Prob > F
Model	36.9453488	15	2.46302326	0.83	0.6427
Groups	21.8871789	5	4.37743578	2.91	**0.0710**
Tongues/groups	15.0581699	10	1.50581699		
Residual	207.95	70	2.97071429		
Total	244.895349	85	2.88112175		

P value = 0.0710

5.5 Preliminary results of apoptosis

We optimised the technique TUNEL assay for apoptosis detection. The optimal results of detecting apoptosis in taste cells of rat tongues seemed to be while using proteinase K in a concentration of 10 µg/ml and its time of incubation set for 45 min (Table 5); the array in Figure 17.

Table 5:
OPTIMIZING OF CONCENTRATION OF PROTEINASE K AND TIME OF INCUBATION

Concentration of proteinase K (µg/ml) / Time of incubation		
PK 10 / 45 min	PK 20 / 45 min	PK 40 / 45 min
PK 10 /2 h	PK 20 / 2 h	PK 40 / 2 h

TUNEL assay technique, bar 20 µm
PK - the concentration of proteinase K in (µg/ml), Time of incubation - 45 min or 2 h;
The optimal results were by using PK 10 for 45 min.

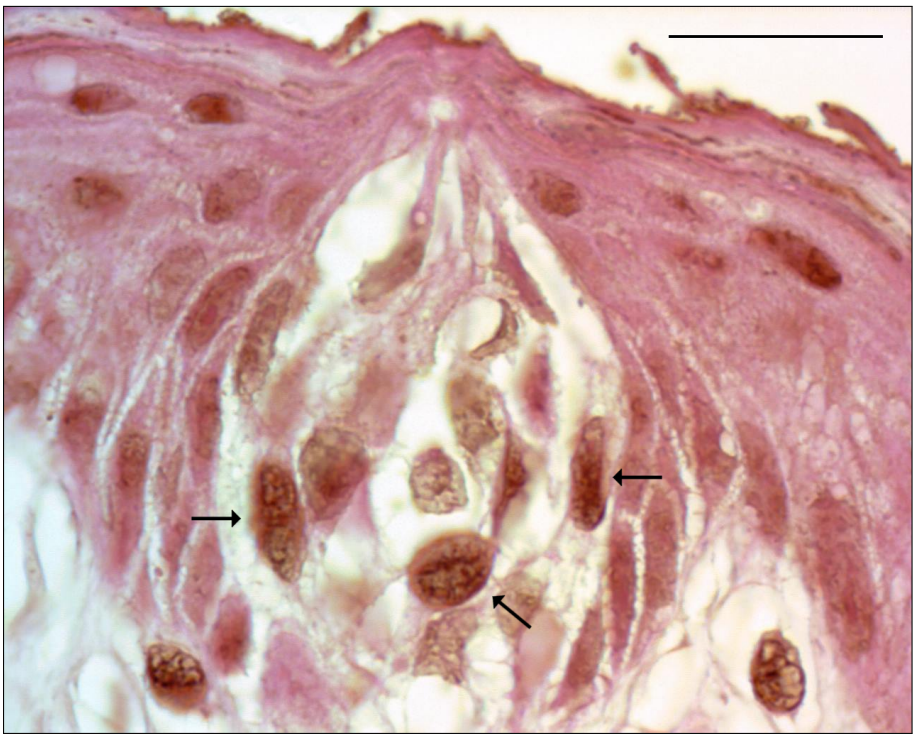


Figure 17:
DETECTION OF APOPTOSIS
TUNEL assay, counterstained with eosin, bar 20 µm

6 Discussion

The role of SG together with other types of bariatric surgeries in weight loss and the mechanism of maintaining a lower body weight for many years are not very clear. The first idea for the explanation of weight loss after SG is the actual gastric restriction. Decreased stomach volume may activate stretch mechanoreceptors very early, restrict food intake and induce satiation. This theory is supported by the fact that a significant weight loss follows up other restrictive procedures like adjustable gastric binding, which change less the levels of gut hormones (Scott and Batterham, 2011). An animal study carried out by Nausheen et al. (2013) concludes that rats after SG reduce food intake.

A change of food preference and a different caloric intake may be another possible explanation for long-lasting weight loss after SG or any other bariatric surgery (Münzberg et al., 2015; Graham et al., 2014). Human studies are almost uniform stating the change in food preference from high energy-dense to less energy dense food with lower content of fat and sugar (Miras and Le Roux, 2010; Tichansky et al., 2006; Laurenius et al., 2013; Kenler et al., 1990; Scruggs et al., 1994).

Unfortunately, the studies and experiments on rats result in ambivalent conclusions. Wilson-Pérez et al. (2012) found that rats undergoing vertical SG decreased intake of dietary fat and started to prefer low-caloric food after the surgery. These rats also developed an aversion to oil administered intra-gastrically. Shin et al. (2012) discovered the shift towards low-fat food occurs after Roux-en-Y gastric bypass. On the other hand, there is also the outcome of two studies suggesting that SG does not change preoperative food preferences or any other eating behaviour parameter in rats (Saeidi et al., 2010; Kodama et al., 2010).

Another answer for the appetite reduction may be decreased levels of ghrelin after SG, as a consequence of the removal of ghrelin secreting tissue – gastric fundus – and the degree of its dysfunctionality (Frühbeck et al., 2004; Scott and Batterham, 2011; Langer et al., 2005). The study carried out by Karamanakos et al. (2008) concludes that patients undergoing SG not only show great appetite suppression, but also reduced ghrelin levels. The same conclusion was also stated by Scott and Batterham (2001), Depaula et al. (2009) and Langer et al. (2009). Moreover, the level of ghrelin remains low for at least five years after surgery (Bohdjalian et al., 2010). Wang and Liu (2008) reported lower levels of ghrelin after SG in rat model.

Plasma ghrelin levels are lower after SG (Bohdjalian et al., 2010), but the presence of ghrelin in taste cells remains (even though the levels are not yet measured). Our study shows that ghrelin is present in some levels in all TB, either belonging to lean rats, obese rats, rats undergoing SG or sham surgery, rats on a HFD or rats fed with a ND.

There is surprisingly not much written and known about a change in taste sensitivity and acuity after any type of bariatric surgery (none after SG), and the results of such studies differ. Burge et al. (1995) disclosed that mean recognition thresholds for sucrose fall significantly after Roux-en-Y gastric bypass, although there was no difference in urea (bitter) acuity. In contrast, a completely opposite conclusion was made by Pepino et al. (2014), stating that neither Roux-en-Y gastric bypass nor laparoscopic adjustable gastric binding affect taste detection thresholds, and that changed eating behaviour is not associated with any change in taste sensitivity. Scruggs et al. (1994) described a significant up-regulation in taste acuity for bitter and sour with a tendency for a reduction in salt and sweet detection after gastric bypass surgery, and connected this fact with the weight loss after surgery.

There are no studies describing the effect of HFD or of bariatric surgery, on alteration of the tongue histology; therefore, we performed this preliminary study. Our results are only close to the statistical significance level (P value is 0.0710), but they are noteworthy. Rats fed a HFD ad libitum for 12 weeks and then fed a ND with food restriction for 4 weeks had the highest number of FP. On the other hand, the rats fed a HFD ad libitum for the entire duration of the experiment and that underwent SG had the lowest amount of FP. As this is a preliminary study it would be interesting to increase the number of animals in each group in order to count FP in a higher number of slides.

7 Conclusion

The difference in the number of FP in rats from the different experimental groups was close to the statistical significance level $P = 0.0710$. The highest mean number of FP (5.11 ± 1.45) was counted in rats belonging to the experimental group C (first part of the experiment were fed ad libitum with a HFD, second part were PF with a ND, no intervention). The lowest number of FP (3.38 ± 1.45) was found in the experimental group E (rats undergoing SG and fed the entire duration of the experiment a HFD).

There was no difference in the number of TB in the tongue of rats belonging to the different experimental groups.

At least one taste cell, of all the TB we observed, was immunoreactive to ghrelin. There was no difference in staining among the experimental groups.

We optimised the method for apoptosis detection in taste cells of TB in rats. Optimal detection was achieved while using proteinase K in a concentration of 10 µg/ml with its time of incubation set for 45 minutes.

Abbreviations

ATP Adenosinetriphosphate

BMI Body mass index

DAB 3,3'-Diaminobenzidine

dTTP Deoxythymidine triphosphate

dUTP Deoxyuridine triphosphate

FP Fungiform papilla / papillae

HFD High-fat diet

DPX mounting medium

ND Normal diet

PF Pair-fed

RT Room temperature

SG Sleeve gastrectomy

TB Taste bud / buds

TdT Terminal deoxynucleotidy transferase

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Bibliography

1. ALBERTS B, JOHNSON A, LEWIS J, RAFF M, ROBERTS K, WALTER P. Molecular biology of the cell. 4th ed. New York: Garland Science, 2002. ISBN 0815340729. Programmed cell death (Apoptosis).

2. BANCROFT JD, GAMBLE M. Theory and practice of histological techniques. 5th ed. Edinburgh: Churchill Livingstone, 2003,125-138. ISBN 0443064350.

3. BOHDJALIAN A, LANGER FB, SHAKERI-LEIDENMÜHLER S, GFERER L, LUDVIK B, ZACHERL J, PRAGER G. Sleeve gastrectomy as sole and definitive bariatric procedure: 5-year results for weight loss and ghrelin. Obesity Surgery. 2010;20(5):535-540.

4. BURGE JC, SCHAUMBURG JZ, CHOBAN PS, DISILVESTRO RA, FLANCBAUM L. Changes in patients' taste acuity after Roux-en-Y gastric bypass for clinically severe obesity. Journal of the American Dietetic Association. 1995;95(6):666-670.

5. ČEŠKA R, DÍTĚ P, ŠTULC T, TESAŘ V. Interna. 1st ed. Praha: Triton, 2010, 855, 274-275. ISBN 978-80-7387-423-0.

6. CHANDRASHEKAR J, HOON MA, RYBA NJP, ZUKER CS. The receptors and cells for mammalian taste. Nature. 2006;444(7117):288-294.

7. CUMMINGS DE, PURNELL JQ, FRAYO RS, SCHMIDOVA K, WISSE BE, WEIGLE DS. a preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes. 2001;50(8):1714-1719.

8. DEPAULA AL, MACEDO ALV, SCHRAIBMAN V, MOTA BR, VENCIO S. Hormonal evaluation following laparoscopic treatment of type 2 diabetes mellitus patients with BMI 20–34. Surgical Endoscopy. 2009;23(8):1724-1732.

9. DESESSO JM, JACOBSON CF. Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. Food and Chemical Toxicology. 2001;39(3):209-228.

10. FISH HS, MALONE PD, RICHTER CP, RICHTER GMA. The anatomy of the tongue of the domestic Norway rat. I. The skin of the tongue; the various papillae; their number and distribution. The Anatomical Record. 1944;89(4):429-440.

11. FRIED M. Laparoskopické bariatrické operace. Endoskopie. 2009;18(1):19-21

12. FRÜHBECK G, DIEZ-CABALLERO A, GIL MJ, MONTERO I, GÓMEZ-AMBROSI J, SALVADOR J, CIENFUEGOS JA. The decrease in plasma ghrelin concentrations following bariatric surgery depends on the functional integrity of the fundus. Obesity Surgery. 2004;14(5):606-612.

13. FRÜHBECK G. Inexpensive continuous-infusion swivel: towards more physiological measurements. Physiological research 1995;44:121-126.

14. GRAHAM L, MURTY G, BOWREY DJ. Taste, smell and appetite change after Roux-en-Y gastric bypass surgery. Obesity Surgery. 2014;24(9):1463-1468.

15. GRAY H. Anatomy of the human body [online]. 20th ed. New York: Bartleby.com, 2000. ISBN 1587341026. Available from: http://www.bartleby.com/107/222.html[cit. 2015-03-06]

16. GRIFFIN, MR, DERRER DT. WebMD. Choosing a Type of Weight Loss Surgery [online]. 2014. Available from: http://www.webmd.com/diet/weight-loss-surgery/weight-loss-surgery-making-the-choice?page=1[cit. 2015-04-10]

17. HOSLEY MA, OAKLEY B. Postnatal development of the vallate papilla and taste buds in rats. The Anatomical Record. 1987;218(2):216-222.

18. KARAMANAKOS SN, VAGENAS K, KALFARENTZOS F, ALEXANDRIDES TK. Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy. Annals of Surgery. 2008;247(3):401-407.

19. KENLER HA, BROLIN RE, CODY RP. Changes in eating behaviour after horizontal gastroplasty and Roux-en-Y gastric bypass. The American Journal of Clinical Nutrition. 1990;52(1)87-92.

20. KODAMA Y, ZHAO C, KULSENG B, CHEN D. Eating behaviour in rats subjected to vagotomy, sleeve gastrectomy, and duodenal switch. Journal of Gastrointestinal Surgery. 2010;14(10):1502-1510.

21. KOJIMA M, HOSODA H, DATE Y, NAKAZATO M, MATSUO H, KANGAWA K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999;402(6762):656-660.

22. LANGER FB, REZA HODA MA, BOHDJALIAN A, FELBERBAUER FX, ZACHERL J, WENZL E, SCHINDLER K, LUGER A, LUDVIK B, PRAGER G. Sleeve Gastrectomy and gastric banding: effects on plasma ghrelin levels. Obesity Surgery. 2005;15(7):1024-1029.

23. LAURENIUS A, LARSSON I, MELANSON KJ, LINDROOS AK, LÖRONTH H, BOSAEUS I, OLBERTS T. Decreased energy density and changes in food selection following Roux-en-Y gastric bypass. European Journal of Clinical Nutrition. 2013;67(2):168–173.

24. MENZIES JRW, SKIBICKA KP, LENG G, DICKSON SL. Ghrelin, reward and motivation. Endocrine development. 2013;25:101-111.

25. MIRAS AD, LE ROUX CW. Bariatric surgery and taste: novel mechanisms of weight loss. Current Opinion in Gastroenterology. 2010;26(2):140-145.

26. MISTRETTA CM, BAUM BJ. Quantitative study of taste buds in fungiform and circumvallate papillae of young and aged rats. Journal of Anatomy. 1984;138(2):232-332.

27. MISTRETTA CM, GOOSENS KA, FARINAS I, REICHARDT LF. Alterations in size, number, and morphology of gustatory papillae and taste buds in BDNF null mutant mice demonstrate neural dependence of developing taste organs. The Journal of Comparative Neurology. 1999;409(1):13-24.

28. MÜNZBERG H, LAQUE A, YU S, REZAI-ZADEH, BERTHOUD HR. Appetite and body weight regulation after bariatric surgery. Obesity Reviews. 2015;16:77-90.

29. NAUSHEEN S, SHAH IH, PEZESHKI A, SIGALET DL,CHELIKANI PK. Effects of sleeve gastrectomy and ileal transposition, alone and in combination, on food intake, body weight, gut hormones, and glucose metabolism in rats. AJP: Endocrinology and Metabolism. 2013;305(4):E507-E518.

30. OVALLE WK, NAHIRNEY PC, NETTER FH. Netter's essential histology. 1st ed. Philadelphia: Saunders/Elsevier, 2008, 30-45. ISBN 9781929007868.

31. PEPINO MY, BRADLEY D, EAGON JC, SULLIVAN S, ABUMRAD NA, KLEIN S. Changes in taste perception and eating behaviour after bariatric surgery-induced weight loss in women. Obesity. 2014;22(5):e13-e20. DOI: 10.1002/oby.20649.

32. PÉREZ-LLAMAS F., ZAMORA S, ROSIQUE MJ, SASTRE JF. Effects of inhalation of ethyl-ether on glycemia and on some variables of intermediate metabolism in rats. Archives of Physiology and Biochemistry. 1992;100(5):335-337.

33. RODRÍGUEZ MM. Factores reguladores del balance energético en la obesidad. Estudio de los mecanismos de adaptación histológicos y funcionales tras restricción calórica impuesta o inducida mediante cirugía bariátrica (gastrectomía tubular). Pamplona, 2011. Doctoral thesis. Facultad de Ciencias, Universidad de Navarra.

34. ROSS MH, PAWLINA W. Histology: a text and atlas: with correlated cell and molecular biology. 6th ed. Philadelphia: Wolters Kluwer/Lippincott Williams, c2011, xviii, 526-534, 556-562, 574-586. ISBN 978-0-7817-7200-6.

35. SAEIDI N, NESTORIDI E, KUCHARCZYK J, UYGUN MK, YARMUSH ML, STYLOPOULOS N. Sleeve gastrectomy and Roux-en-Y gastric bypass exhibit differential effects on food preferences, nutrient absorption and energy expenditure in obese rats. International Journal of Obesity. 2012;36(11):1396-1402.

36. SCOTT WR, BATTERHAM RL. Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: understanding weight loss and improvements in type 2 diabetes after bariatric surgery. AJP: Regulatory, Integrative and Comparative Physiology. 2011;301(1):R15-R27

37. SCRUGGS DM, BUFFINGTON C, COWAN JR GSM. Taste Acuity of the morbidly obese before and after gastric bypass surgery. Obesity Surgery. 1994;4(1):24-28.

38. SHIN AC, ZHENG H, TOWNSEND RL, PATTERSON LM, HOLMES GM, BERTHOUD HR. Longitudinal assessment of food intake, faecal energy loss, and energy expenditure after Roux-en-Y gastric bypass surgery in high-fat-fed obese rats. Obesity Surgery. 2012;23(4):531-540.

39. SHIN YK, MARTIN B, KIM W, WHITE CM, JI S, SUN Y, SMITH RG, SÉVIGNY J, TSCHÖP MH, MAUDSLEY S, EGAN JM, BARTELL PA. Ghrelin is produced in taste cells and ghrelin receptor null mice show reduced taste responsivity to salty (NaCl) and sour (Citric Acid) tastants. PLoS ONE. 2010;5(9):e12729.

40. SJÖSTRÖM L. Bariatric surgery and reduction in morbidity and mortality: experiences from the SOS study. International Journal of Obesity. 2008;32:93-97.

41. SKIBICKA KP, DICKSON SL. Ghrelin and food reward: The story of potential underlying substrates. Peptides. 2011;32(11):2265-2273.

42. SOUZA GR, SOUSA BOLINA C, WATANABE I, CIENA AP. Three-dimensional aspects of the lingual papillae and their connective tissue cores in the tongue of rats: a scanning electron microscope study. The Scientific World Journal. 2014;2014:1-6.

43. TICHANSKY DS, BOUGHTER JD, MADAN AK. Taste change after laparoscopic Roux-en-Y gastric bypass and laparoscopic adjustable gastric banding. Surgery for Obesity and Related Diseases. 2006;2(4):440-444.

44. TRIVEDI BP, GRAY J. Gustatory system: The finer points of taste. Nature. 2012;486(7403):351-394.

45. WANG Y, LIU J. Plasma ghrelin modulation in gastric band operation and sleeve gastrectomy. Obesity Surgery. 2008;19(3):357-362.

46. WHO. [online]. Available from: http://www.who.int/topics/obesity/en/ [cit. 2015-03-18]

47. WILSON-PÉREZ HE, CHAMBERS AP, SANDOVAL DA, STEFATER MA, WOODS SC, BENOIT SC, SEELEY RJ. The effect of vertical sleeve gastrectomy on food choice in rats. International Journal of Obesity. 2012;37(2):288-295.