
UNIVERSITÀ POLITECNICA DELLE MARCHE

FACOLTÀ DI MEDICINA E CHIRURGIA

Scuola di Dottorato in Medicina e Chirurgia

*Corso di Dottorato in Scienze Biomediche*

Coordinatore: Prof. Andrea Giacometti

# Taste sensitivity in cancer patient

Dottorando:

***Dott.ssa Sofia Pugnaloni***

Tutor: ***Chiar.ma***

***Prof.ssa Arianna Vignini***

*XXXII Ciclo*

*Triennio Accademico 2016/2019*

# 1. PROLOGUE

Taste alteration is one of the common side effects of chemotherapy. However, little is known about early diagnosis of taste modifications and the influence on patients' food behavior.
The aim of this thesis is to analyze taste alterations in physiological and pathological conditions, and in particular in a cancer patient population.
The focus of this Doctoral project is on studying how dysgeusia could be analyzed with a simple and inexpensive method in order to immediately intervene in taste alterations with nutritional counseling or personalized diet to prevent the patients' changes in food intake behavior. This method could allow to make a diagnosis of dysgeusia, but also to assess its degree and characteristics.
I analyzed taste alterations also in relation to body mass index (BMI), age and gender. Taking into consideration those variabilities, this procedure could open up a new approach for a personalized diet, considering those variabilities, to prevent and/or reduce taste alterations among populations at risk (drug consumption, age, smoking, disease…) especially among cancer patients who have received or are receiving surgical or oncological treatment.
Eating in those patients had become something that was necessary for survival rather than a pleasure. It was clear that this loss severely affected both family and social life and it would be important if clinicians could give advice on how to alleviate taste alterations.

My project started with the evaluation of the relationship between taste identification ability and BMI by studying the response to the administration of different taste stimuli on the surface of the tongue in three BMI-different groups of subjects (normal weight, overweight and obese) and this work resulted in a scientific article published in Disease Markers Journal (Vignini A. et al., Disease Markers, 2019). We showed a general lowering of taste sensitivity with the increase of BMI. Other variables affecting taste sensitivity are age, with a negative association, gender (women generally show higher sensitivity) and taste stimuli concentration with a positive association.
My interest in taste alterations has grown during the doctoral school years where I have become increasingly conscious of the importance of the evaluations of taste loss especially in cancer patients. So I continued in the taste-project in the Medical Oncology Clinic at Umberto I University Hospital of Ancona. During interviews with patients with different types of cancer, I have become

more aware of the variation in taste alterations and the effect they can have on daily food intake and food preference.

The aim of Study I was to analyze taste alterations in a patient population compared to heathy people as controls, also in relation to gender.
In this way, last year I started Study II that concentrated on two of the most common types of cancer, breast cancer and gastrointestinal cancer, focusing on the differences in taste sensitivity and in food preferences and food intake between pre-and post-chemotherapy.

The common purpose of this thesis is to contribute to preventing and/or reducing taste alterations and malnutrition by opening future perspectives that consider early evaluation of alterations in taste for better management of future food behavior alterations of the patient. This may lead to the development of evidence-based advice and interventions available to healthcare staff.

It has been an interesting and fascinating experience interviewing the patients in this study who have so generously shared their difficulties and feelings and their experience of taste alterations.
My ambition is that this thesis will do justice to their participation and contribute to the development of knowledge in this area.

# 2. BACKGROUND

## 2.1 The sense of taste

### 2.1.1 The chemical senses

Among the sensory modalities, gustatory and olfactory systems are phylogenetically older than the visual, auditory and tactile systems. They are the "chemical senses", as the receptors are activated by a chemical stimulus. Through taste and smell, the environmental molecules provide humans with important information of which they make continuous use in everyday life, as is the case with other sensory stimuli. This information concerns the external world, but it is related to the internal environment of the organism, with its needs, its defense and its state of satisfaction. In particular, the olfactory and gustatory sensitivities in humans intervene in a complex mechanism such as the selection of food and drinks, together with the trigeminal transmission and visual characteristics, determining what is perceived as taste, "taste", palatability of food. The aroma is the complex interaction between somatic sensation, taste and smell. Furthermore, the olfactory and gustatory sensitivities mediate the transmission to the Central Nervous System (CNS) of information regarding the presence of unwanted and potentially dangerous substances in food and air, including natural gas, smoke and pollutants, toxic or poisonous substances, acting as monitors for inhaled and ingested chemicals, and this is an important function in many animal species (Kandel ER et al., Principles of Neuroscience 2005).

Olfactory signals originate in the nasal epithelium, while through the tongue and other gustatory tissues the perception of the six primary modes (sweet, salty, acid, bitter, umami and fat) occurs, each evoked by compounds of a different chemical nature and mediated by different mechanisms of signal capture and transduction. These six fundamental flavors can be schematically attributed to different functions: ensuring the energy reserves for the sweet, maintaining the electrolyte balance for the salty, controlling the acid-base balance for the acid, avoiding toxic substances for bitter, ensuring the protein reserves for umami and guaranteeing fat reserves for fat (Chaudhari N. and Roper SD, The Journal of Cell Biology 2010). The trigeminal fibers are responsible for pain, tactile and thermal components of the olfactory and gustatory sensation and also play an important role in the identification of irritating or poisonous substances. These fibers reach the trigeminal nucleus

in the medulla and are carried contralaterally where they contract synapses in the thalamus and then in the anterior parietal cortex. The gustatory function is closely related to the olfactory one: during the meal the first perceived sensation is smell, starting from volatile molecules released from the foods that bind the receptors of the olfactory mucosa. Only later, when food is introduced into the oral cavity and comes into contact with specific receptor cells, does gustatory perception originate.

The volatile particles can stimulate the olfactory epithelium in two ways: the orthonasal one, associated with the identification of a food, with the preparation and the desire of its introduction, and the retronasal one, correlated to the perception of the aroma during the meal and then to the induction of the sense of satiety. When the chewed bolus is swallowed, this second olfactory and gustatory perception occurs through the nasopharynx and the larynx and only finally does trigeminal stimulation complete the perception of pleasant food intake (Kandel ER et al., Principles of Neuroscience 2005; Rolls ET , Biological Science 2006; DeVere R., Continuum 2017; Yin W. Et al., Appetite 2017).

### 2.1.2 Taste sensitivity

There are several aspects that influence taste sensitivity and consequently the food choice of each subject and the feelings that derive from it. A fundamental aspect regarding the intake of food in humans is the sensation of pleasantness that it is able to cause, and which is often particularly implemented by sweet substances, which are also an important source of energy. The pleasantness of the food is perceived starting from the triggering of salivation reflexes, swallowing and preparation of the gastrointestinal tube to digestion and absorption, just as happens with the bitter taste, contained in many organic poisons, which triggers the rejection of the substance and, if very intense, also stimulates the vomiting reflex (Kandel ER et al., Principles of Neuroscience 2005; Yin W. Et al., Appetite 2017). The perception of pleasantness of food, however, depends on several factors, which include purely subjective aspects and are influenced by individual experience and also others related to the nutritional need of the moment and the sense of satiety. For this reason, for example, the pleasantness of a food can be different in different moments of the day or even between the beginning and the end of a meal and based on the gustatory information associated

with the olfactory ones you have the ability to choose between different foods preferring those that provide the nutrient supply best suited to the body's needs (Kershaw JC and Mattes RD, World Journal of Otorhinolaryngology Head and Neck Surgery 2018).

Furthermore, perception can be modified by the concentration of the substance taken, as happens for example with kitchen salt which at low concentrations is perceived as sweet and at higher concentrations as bitter. A further determining factor in taste sensitivity is sex, as in men the perception of sweet and salty is inferior compared to women, while that of acid stimulation is greater; finally age, with the increase of which there is a reduction and modification of taste (Curtis K.S. et al., Physiology & Behavior 2005; Kandel E.R. et al., Principles of Neuroscience 2005). The desire to introduce more food and the sense of satiety are modulated by many factors including also the stimulation time of the receptors, at least as regards the sweet taste it has been seen that after the ingestion of 135g of sucrose contained in a drink, the feeling of being satiated is greater if consumed within 10 minutes compared to 2 minutes (Yin W. Et al., Appetite 2017).

In addition to the nutritional and social function, the role played on the perception of quality of life, the gustatory function has a biologically important role in the control of digestive and absorptive processes. The connections between the gustatory centers and the nuclei involved in the control of vegetative efferences, especially vagal ones, activate and modulate the secretions of numerous glands of the gastrointestinal tract, including salivary, gastric and pancreatic glands, with reflex mechanisms, influencing both quantity and quality. For example, the sweet taste seems to be responsible for the secretion of hormones and enzymes involved in glucose metabolism, bitterness stimulates the secretion of enzymes in the gastric glands, acid stimuli determine an increase in the aqueous and bicarbonate component of von Ebner's salivary glands and fats, when present in the oral cavity stimulate the secretion of salivary, pancreatic and gastric lipases. With regard to the recognition of potentially harmful substances, related to bitter taste, humans recognize low concentrations of often highly toxic compounds, including amino acids, peptides, polyphenols, isoflavonoids and glucosinolates, alkaloids such as quinine, isothiocyanates, fatty acids, produced for example by plants as a protection mechanism against predators, limiting their consumption thanks to rejection responses. At the same time, the possibility of recognizing some classes of bitter compounds that can act as antibacterials and / or antioxidants, such as those found in tea, coffee, some fruits, citrus fruits and chocolate, can bring health benefits (Huang AL et al., Nature 2006;

Rolls ET, Biological Science 2006; Frings S., PNAS 2010; Gilbertson TA et al., CRC Press / Taylor & Francis 2010).

### 2.1.3 Gustatory receptors

The chemical substances in food stimulate gustatory chemoreceptors, specialized cells of the oral cavity, which are found in the epithelium of the dorsal surface of the tongue, of the soft palate, of the pharynx, of the upper part of the esophagus, on the mucosa of the lips and cheeks. Taste receptors are found in gustatory buttons, which can be considered as the functional unit of taste: they are more numberous in the embryo and in the child but in the adult tend to total between 3000 and 10000, due to degeneration which usually occurs around 45 years of age and involves a reduction of physiological taste sensitivity. They are roundish-ovoid structures, of about 70 µm of diameter and with an opening of 3-5 µm, the gustatory pore, which puts them in communication with the oral cavity. In turn, the gustatory buttons are grouped in the tongue to form further specialized structures called taste buds, of which three types exist in humans. The papillae fungiform each contain 1 to 5 taste buds, they are located in the anterior two thirds of the tongue and altogether they make up 25% of the taste cells. Another 25% is found in the papillae, which are in the posterolateral part of the tongue; finally, the remaining share of receptor cells is contained in the circumvallate, large round papillae structures surrounded by a furrow and arranged to form an inverted V in the back of the tongue. There is also another type of papilla on the tongue, but without a gustatory function: these are the filiform papillae, whose function is probably that of keeping some substances on the lingual surface and then favor their amalgamation and bolus formation (Figure 1) (Kandel E.R. et al., Principles of Neuroscience 2005; Lalwani TO THE. et al., Harrison 2009; Chaudhari N. and Roper S.D., The Journal of Cell Biology 2010; Frings S., PNAS 2010; Kinnamon S.C., BMC Biology 2013).

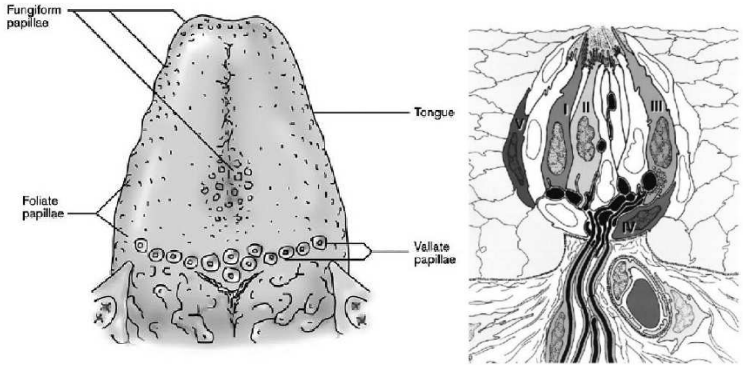


Figure 1. Taste papille (on the left); Longitudinal taste button section (on the right) (DeVere R., 2017).

Each button contains, in addition to 50-150 receptor cells, also basal cells (cells of type IV), which are undifferentiated cells that are precursors of the other cellular elements, and cells of support or type I, support and secretion elements provided with apical granules, perhaps involved in the perception of salt. Type II receptor cells are those with heterotrimeric G protein-coupled receptors (GPCRs, guanine-binding family protein) of T1R type for sweet and umami and T2R for bitterness, which are immunoreactive to gustducine and which respond by secreting ATP towards afferent fibers, with a mechanism not mediated by synaptic vesicles: they have a fusiform form and because they do not have granules or synapses appear clear under the microscope. These cells have no axons and receive afferent nerve fibers at their basal ends. In the last ten years different channels have been called into question as being involved in the release of ATP molecules that would then act on the P2X2 and P2X3 purinergic receptors: among these, above all, the pannexin-1 channel. It has more recently been identified as CALHM1 (calcium homeostasis modulator 1), a subunit of a non-selective and voltage-dependent cation channel, which is selectively expressed in type II receptor cells; furthermore, in mice lacking the CALHM 1 gene, the release of ATP from these cells in response to gustatory stimuli is lacking, with a loss of perception of sweet, umami and bitter, but not acid and salty. CALHM1 is therefore proposed as a component of the ATP permeable channel, probably forming a heterodimer with CALHM3, which allows a faster release of the molecule. (Kinnamon S.C., BMC Biology 2013; Oka Y., Neuron 2018). Type II cells also express numerous hormones such as GLP-1 (glucagon-like peptide 1), CCK (cholecystokinin), NPY (neuropeptide Y), VIP (vasoactive intestinal peptide), ghrelin, of which is known the regulatory function on the digestive processes, in glucose homeostasis, in the appetite and in the sense of satiety and for which receptors are found

at the level of the gustative buttons and on the afferent fibers, suggesting a local endocrine regulation: for example the GLP- 1 would increase the perception of sweet, while ghrelin that of acid and salty and NPY in basal cells would modulate cell renewal. Furthermore, the taste cells would be sensitive to circulating hormones, as demonstrated for example by the expression of receptors for circulating leptin that would be involved in sensitivity to sweet taste (Yoshida R. et al., Diabetes 2015). Finally, type III cells present apical and basal processes that create synapses with axons, provided with vesicles, characterized by the release of serotonin, GABA, and norepinephrine: they seem to perceive the acid taste directly but they also intervene indirectly and are not strictly necessary for the perception of sweet, umami and bitter through the connections with type II cells. The average life of the receptor cells is about half of that of the surrounding epithelial cells and is around 10-11 days: they have a remarkable capacity for renewal.

Therefore in the gustatory button both young and mature cells coexist, the latter located towards the center of the structure, while the cell progenitor, or basal cells, are found in the epithelial layer at the base of the gustatory button, which corresponds to the germinative layer; this involves rapid cell regeneration following physical or chemical damage caused by stimuli that are too hot or cold, or by irritating or acidic substances on the oral cavity. As the gustatory cell grows, it migrates towards the gustatory pore with its apical extremity, from which numerous microvilli, called gustatory hairs, protrude right through the pore into the oral cavity and on whose surface the chemoreceptors start. The chemical substances only if dissolved in the saliva can bind the receptors, determining the stimulus that allows to perceive the typical taste of the food (Figure 2)

(Chaudhari N. and Roper S.D., The Journal of Cell Biology 2010; Besnard P. et al., Physiological Review 2016).

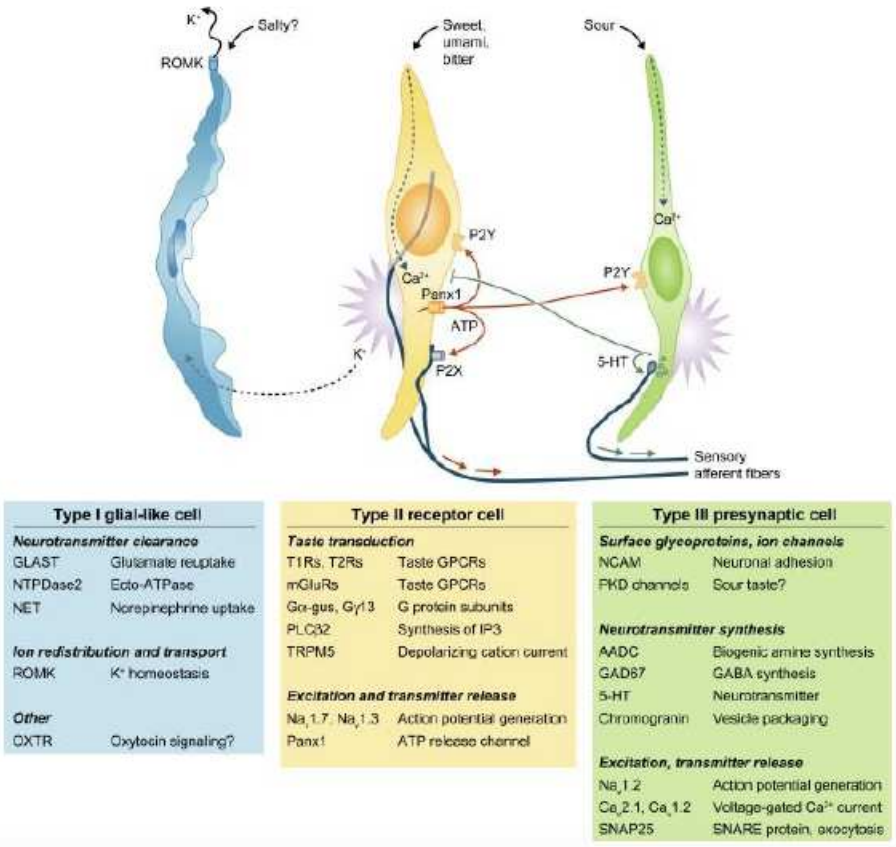


Figure 2. The three main types of cells in the taste button. (Chaudhari N. and Roper S.D, 2010)

### 2.1.4 The taste signal processing

Each receptor cell contracts a chemical-like synapse at the level of the basal portion with an afferent nerve fiber: the facial nerve (VII pair of cranial nerves) is involved through one of its branches, the chord of the tympanum, as regards the innervation of the two anterior thirds of the tongue, and through the large superficial petrous, another sensory branch, for the gustatory buttons of the mucous membrane of the palate. The afferent fibers reach the bodies of the pseudo-unipolar neurons, in the geniculate ganglion. The tympanic cord also carries the preganglionic efferent fibers parasympathetic to the submandibular ganglion, for the innervation of the sublingual and submandibular salivary glands, while the large superficial petrosal nerve contains the parasympathetic preganglionic fibers that reach the pterygopalatine ganglion where they contract

synapses with the postganglionic cells, which innervate the mucous glands of the hard and soft palate. The glossopharyngeal nerve (IX pair of cranial nerves) is instead involved through its lingual branch, which reaches the posterior third of the tongue, the palatoglossal arches and part of the oropharynx, while in the petrosal ganglion the gustatory neurons are found, which are particularly sensitive to toxic substances and able to mediate reflexes of avoidance of the same. Finally, the vagus nerve (X pair of cranial nerves), with its upper laryngeal branch, reaches the posterior end of the tongue but also the epiglottis, the larynx and the upper third of the esophagus. Its neurons, located in the nodose ganglion, are sensitive to acids and water and participate in the swallowing reflex and other reflexes that protect the airways from aspiration of food or drink (Kandel ER et al., Principles of Neuroscience 2005; Lalwani AL et al., Harrison 2009). Most gustatory buttons, as well as the non-specialized epithelium of the oropharynx, also receive innervation by trigeminal fibers (V pair of cranial nerves): they play a role in the perception of food temperature and consistency in addition to probably having other functions that are still not well known (Kandel ER et al., Principles of Neuroscience 2005; Lalwani AL et al., Harrison 2009; Fletcher ML et al., The Journal of Neuroscience 2017).

The centripetal branches of the primary gustatory fibers form the solitary fascicle and are carried to the level of the bulb in the rostral part of the nucleus of the solitary tract, a portion that is therefore also defined gustatory nucleus: here the second neuron of the gustatory pathway is located. The second-order gustatory fibers, which originate from here, ascend into the brainstem, included in the dorsomedial part of the medial lemniscus, to contract synapses with the more medial parvicellular neurons of the Ventroposterior medialis parvocellularis (VPMpc) of the thalamus, in a portion also called accessory arcuate core. From the VPM the following order fibers radiate into the internal capsule until they reach the anterior insula and the inner part of the frontal operculum, where most of the taste cells respond to more than one gustatory quality. Other ascending pathways lead to numerous nuclei of the hypothalamus, leading to gustatory information of the limbic system and favoring functional adaptations of the visceral nervous system (Kandel ER et al., Principles of Neuroscience 2005; Fletcher ML et al., The Journal of Neuroscience 2017). The gustatory stimulation reaches an area of granular cortex located in the lower portion of the precentral gyrus, on the lateral convexity of the hemispheres: it is this latter region that constitutes the primary gustatory area. The neurons that respond to gustatory stimuli are relatively few, representing 2-11% of the entire population, and are mixed with other neurons activated for

example by the movements of the tongue or by tactile stimulation. Neurons sensitive to gustatory stimuli are also present in primates in the latero-posterior portion of the orbito-frontal cortex, in areas 12 and 13 of Brodmann, which receives projections from the anterior insula and constitutes the secondary gustatory area. In this region neurons have different and more complex characteristics: they are generally activated by a single gustatory quality, but they can also respond to stimuli related to other sensory modalities such as olfactory, creating an integration between the different information and the consequent eating behavior. Moreover, animal studies on primates, which unlike other animals such as rodents have an anatomically organized taste system similar to humans, have shown that the secondary taste area neurons are activated by food intake, but only when the animal is hungry, thus being closely connected to the sense of satiety, which also send signals related to gastric distension, the presence of chemosensory elements in the gastrointestinal tract, the levels of plasma glucose and hormones such as leptin (Rolls ET, Philosophical Transaction of the Royal Society of London. Series B, Biological Science 2006); MRI investigations have confirmed the response to complex stimuli deriving from the co-presence of the gustatory component with the olfactory and also visual and tactile components (Fletcher M.L. et al., The Journal of Neuroscience 2017). Signals that reach the striatum, the cingulate cortex and the dorsolateral prefrontal cortex originate from the orbitofrontal cortex, on which behavioral and affective responses to food intake depend.

From the core of the solitary tract there are also fibers that reach the lower and upper salivating nuclei, to regulate the salivation reflex, and fibers directed to the Pontine parabrachial complex, in which there are portions that respond to gustatory stimuli. The latter then sends fibers to the central nucleus of the amygdala, to the interstitial nucleus of the terminal stria and to the hypothalamus, as well as to the reticular substance of the brainstem, giving way to circuits implicated in reflex responses to gustatory stimuli, such as the production of saliva, vasoconstriction and retraction in response to bitter taste. Moreover, through the fibers that reach the hypothalamus, the Pontine parabrachial complex is probably also involved in the transmission of gustatory information to the centers of hunger, thirst and satiety. There is also the possibility of the presence of a direct pathway from parabrachial nuclei to the cortex: smell and taste would be the only ones among the sensory systems in which at least some of the fibers do not pass through the thalamus. In this integrated system, the ventral tegmental area and the nucleus accumbens are involved in terms of motivation and reward, the amygdala, the cingulate cortex and the hippocampus for the affective component,

the frontal regions of the cortex, such as the prefrontal cortex , orbitofrontal and cingulate, for the decisional aspect: combined together they contribute to establishing the hedonic aspect of the introduction of food and eating behavior.

Added to these is the hypothalamus which constitutes the metabolic center and, with the information coming from the gastrointestinal tract, is involved in controlling the chemical composition of the "internal medium" and consequently in the general homeostasis of the organism (Figure 3 and 4) (Kandel ER et al., Principles of Neuroscience 2005; Besnard P. et al., Physiological Review 2016; Fletcher ML et al., The Journal of Neuroscience 2017; Peterschmitt Y. et al., Nutrients 2018).

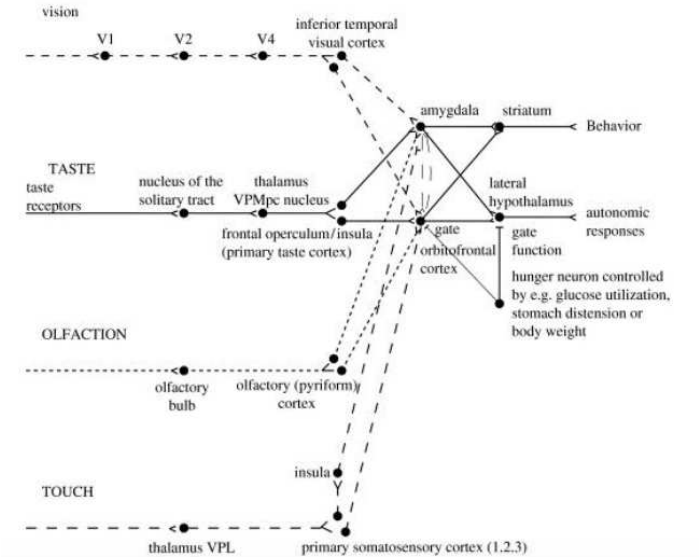


Figure 3. Diagram of the taste and olfactory pathways that shows how they converge with other sensory abilities (Rolls E.T., 2006).

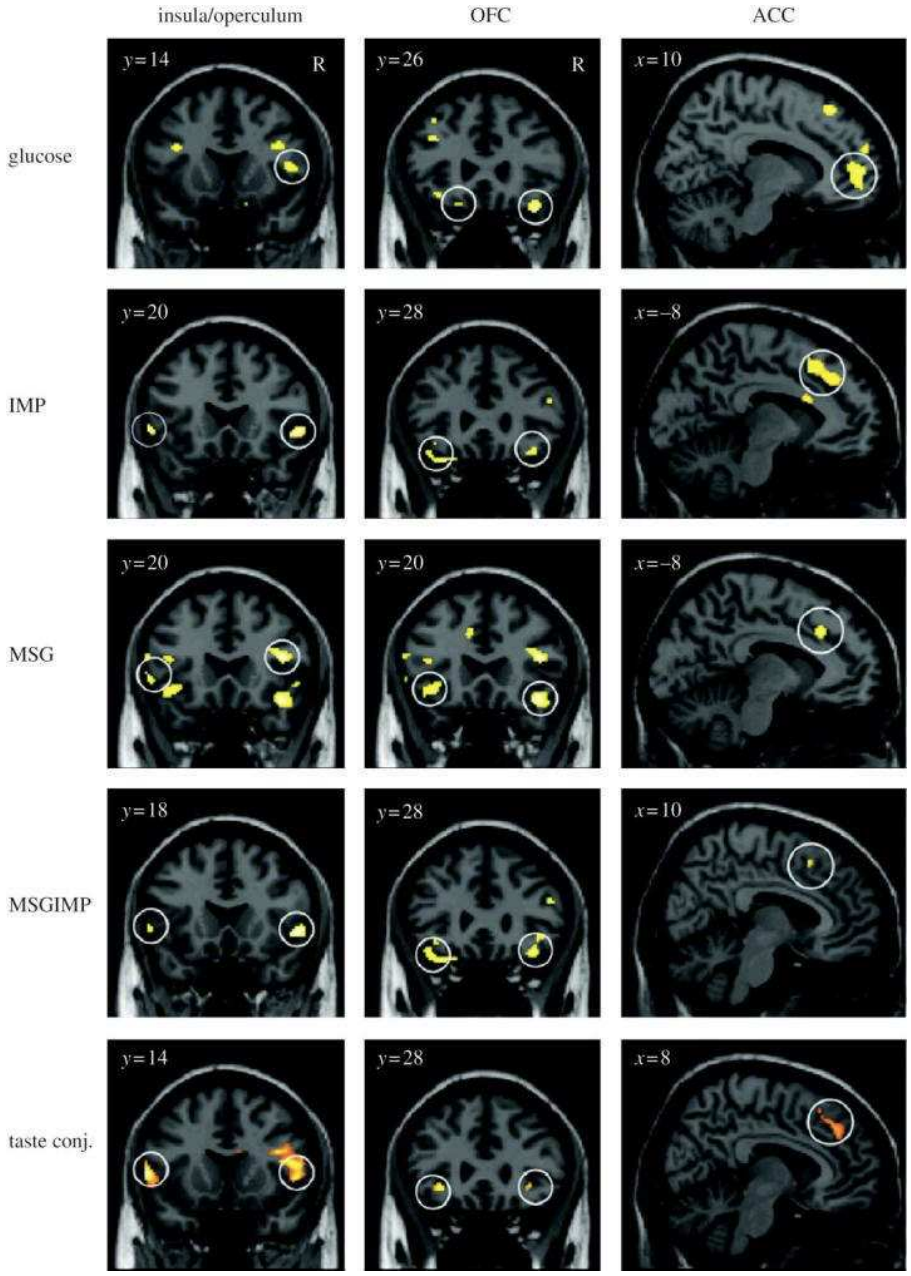


Figure 4. Activation of the insular and frontal regions, the orbitofrontal cortex (OFC) and the anterior cingulate cortex (ACC) in response to taste stimuli. The stimuli used include glucose, glutamate monosodium (MSG), monophosphate inosine (IMP) and the two stimuli for umami together. (Rolls E.T., 2006)

There are several theories on the codification modalities of gustatory perception, developed since the 1940s, starting from the registration experiments in cats of the activity of single fibers of the tympanic cord (Pfaffman C., Journal of Cellular Physiology 1941; Pfattman C., Journal of Neurophysiology 1955).

The hypothesis according to which each zone of the tongue is assigned to the recognition of a specific gustatory stimulus, and in particular the anterior one to the sweet, the lateral anterior region to the acid and posterior to the salty, and the area behind to the bitter, has now been overcome : more recent molecular and functional investigations have shown that each flavor is recognized throughout the language, but with different sensitivity thresholds in different areas (Honn MA et al., Cell 1999; Adler E. et al., Cell 2000; Nelson G. et al., Cell 2001; Huang AL et al., Nature 2006).

Two contrasting models have been proposed: on one hand, some studies show a discrimination at receptor cell level, so the different types would be able to selectively respond to different gustatory stimuli, on the other there are studies on animals that highlight how each fiber responds to different chemical stimuli and in particular how the fibers that respond to salt are also activated by acid, and those that respond to acid are also activated by bitter (Kimura K. and Beidler LM, Journal of Cellular and Comparative Physiology 1961).

The stimuli used include glucose, monosodium glutamate (MSG), inosine monophosphate (IMP) and the two stimuli for umami together (Rolls E.T., 2006) according to which each flavor is transmitted through a specific dedicated neural line, so that each receptor cell and every gustatory fiber are exclusively assigned to the perception of a single gustatory quality. According to this model, single receptor cells perceive more gustatory qualities and then each fiber transmits complex qualitative multiple information, or the single receptor cells are connected to a single gustatory stimulus but then each fiber carries multimodal information (Figure 5) (Frank M., Journal of General Physiology 1973; Smith DV et al., Journal of Neurophysiology 1983; Chandrashekar J. et al., Cell 2000; Chandrashekar J. et al., Nature 2006).

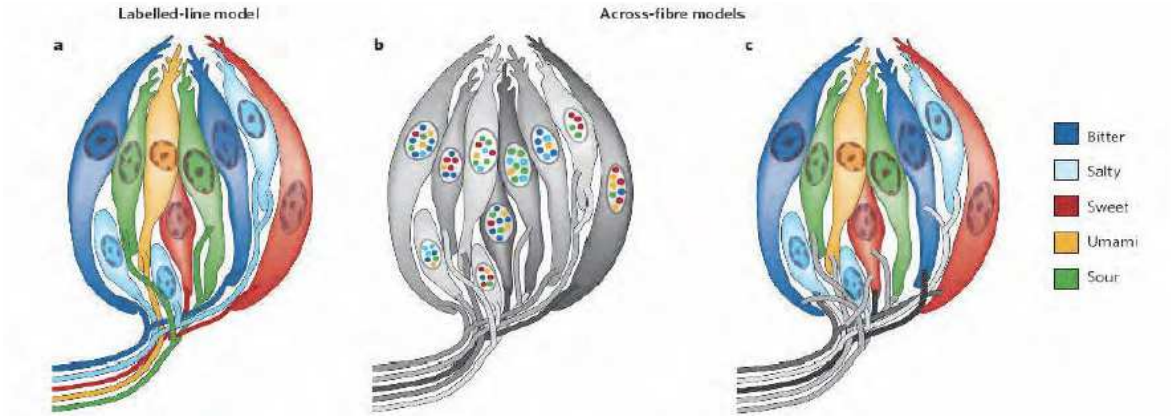


Figure 5. Encoding of taste stimuli. In a, taste receptor cells (TRCs) are specialized in responding to individual taste modes and are innervated by dedicated individual nerve fibers. In b and c, it seems that individual TRCs are tunable to multiple cross-taste modes and that the same afferent fibers carry information about more than one mode, or that TRCs perceive individual taste modes, but the same afferent fibers carry multimodal taste information. (Chandrashekar et al., 2006).

Thus the model was developed where it is the global type of discharge generated by a population of fibers that determines the gustatory decoding: a central group of neurons receives afferent fibers of a whole population of gustatory elements and decodes the different types of activities of different gustatory stimuli in the fibers themselves. Through intracellular recordings performed on single gustatory cells, it was also shown that each of them is able to respond to all the different gustatory qualities, although with different sensitivities (Kimura K. and Beidler L.M, Journal of Cellular and Comparative Physiology 1961). There are probably neurons excited by multiple taste stimuli and others for which a preferential stimulus would strongly exist. This happens both for the single receptor cells and for the relative afferent fibers connected to their basal portions, but in both cases a certain degree of response is possible even for all the other tastes, albeit with a different threshold. In favor of the “labeled line” model, the results of cellular receptor replacement for bitter or sweet have been reported with a modified opioid receptor on mice: in the case of sweet the synthetic ligands, which normally would not give any gustatory perception, were recognized and caused a strong preference for the solutions in which they were contained, because they were perceived as sweet. When the opioid receptor was instead expressed in the receptor cells for bitter the ligand was recognized as strongly repulsive; all this to confirm the existence of specific receptor

cells for the transmission of certain gustatory qualities, while the same has not been demonstrated at the level of nerve fibers. Probably the recognition of each fundamental flavor derives from the comparison between the degree of activation of the individual neural lines and the global pattern of activity of the system, while as regards the decoding of the intensity of the stimulus, it may depend on the frequency of the action potentials of gustatory neurons. For each gustatory stimulus it is possible to define a perception threshold (protopathic) and a recognition threshold (epicritic). The first has a lower value and is defined as the minimum concentration of a specific substance capable of stimulating the taste receptors and inducing a gustatory sensation. The recognition threshold is instead given by the minimum concentration of the substance necessary so that the subject can identify taste with certainty. The thresholds are different based on the different taste stimuli, being related to their need and danger: they will therefore be lower for bitter flavors, thus allowing the defense against potentially toxic substances (Table 1) (Mueller KL et al., Nature 2005; Frank ME et al., Progress in Neurobiology 2008; Yoshida R. et al., The Journal of Physiology 2009). The gustatory system is capable of linking a large number of chemical molecules at the receptor level, but these are now grouped into six fundamental and well-recognized taste sensations that are sweet, bitter, salty, sour, umami and fat.

| ***Taste sensation*** | ***Substance*** | ***Perception Threshold*** | ***Recognition Threshold*** |
|---|---|---|---|
| **Salty** | NaCl | 0,00238 mM | 0,00815 mM |
| **Sour** | HCl | 0,179 mM | 0,226 mM |
| **Sweet** | Fructose | 0,266 mM | 1,33 mM |
| **Bitter** | Chloride quinine | 3,99 µM | 4,75 µM |
| **Umami** | MSG | 0,0126 M | 0,00207 M |

Table 1. Perception and recognition of certain substances

The sweet taste is caused by the stereochemical configuration of sucrose, the sensation of bitter taste is a result of the bond with nitrogen-containing compounds, the salty one is evoked by NaCl, the sensation of sour is due to the presence of hydrogen ions. Particular attention should be given

to the last two fundamental tastes, as they are more recent than the other recognized states. In 1985, in fact, a fifth fundamental gustatory stimulus was added, defined as umami, associated with MSG and accentuated by the IMP (Kawamura Y. and Kare MR, Marcel Dekker 1987, Rolls ET, The journal of Nutrition 2000). It ensures the body's protein reserves by recognizing the content of the food in amino acids, precisely the basic components of proteins (Shigemura N. et al., PloS One 2009; Yasumatsu K. et al., The Journal of Physiology 2015). Finally, in the last decade fat has been recognized as a further fundamental taste, until then it was thought to be primarily of somatosensory origin as it depends on its structure. It has been seen that free fatty acids, produced in the oral cavity by the rapid hydrolysis of triglycerides ingested by the salivary lipase, secreted by the Von Ebner glands, and abundant in the feeding of different animal species and of man himself, constitute strong gustatory stimuli , recognized by specific receptors placed on the taste cell membrane. It can therefore be added to basic tastes (Gilbertson TA, Current opinion in neurobiology 1998; Kawai T. and Fushiki T., American Journal of Physiology. Regulatory Integrative and Comparative Physiology 2003; Laugerette F. et al., The journal of Clinical Investigation 2005; Mattes RD, Annual Review of nutrition 2009; Wellendorph P.et al., Molecular Pharmacology 2009; Gilbertson TA et al., CRC pres /Taylor & Francis 2010; Peterschmitt Y. et al., Nutrients 2018).

The receptor sites on the apical microvilli of taste cells, emerging from the pore of the gustatory button, bind the dissolved molecule in saliva, recognize its chemical configuration and initiate different transduction mechanisms that lead to the perception of taste. This process involves ion channels located on the apical membrane and on the basolateral membrane of the receptor cell. The latter are voltage type channels dependent, for sodium ($Na^+$), potassium ($K^+$) and calcium ($Ca2^+$) ions, which generate a receptor potential when the cells are stimulated by chemicals. The activation of the potential leads to an increase in intracellular $Ca2^+$ with consequent fusion of synaptic vesicles up to transmission, due to the initiation of the action potential of the afferent axonal fibers of the cranial nerves involved. The signal transduction mechanism for each individual gustatory quality was investigated. Regarding sweet, umami and bitter transduction, GPCRs receptors are involved, consisting of 7 transmembrane α-helix domains, a C-terminal intracellular domain and in the case of the TAS1R family (taste receptor, type 1), also called T1R, composed of three genes, also from a large extracellular N-terminal domain; the third long cytoplasmic loop (which elapses between the fifth and sixth α-helix) of the receptors corresponds to the region of the molecule that is coupled to the G protein. For example, one of these proteins is gustducine, whose α subunit is specifically

expressed in the taste cells of the circumvallate, leafy and fungiform papilla: in it the activation of the beta and γ subunits can provoke the activation of the phospholipase C β2 (PLC β2), which in turn hydrolyses the phosphatidylinositol-4,5-diphosphate (PIP2) producing the two intracellular messengers diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3). Then the IP3R3 ion channels located on the endoplasmic reticulum open, causing a release in the cytosol of the $Ca2^+$ receptor cells. When a $Ca2^+$ threshold level is reached in the cell, the TRPM5 receptor-channels (Transient Receptor Potential Channel M5) open and determine transient potential changes responsible for the receptor cell depolarization (Liu D. and Liman E.R., PNAS 2003). The genic inactivation of PLCβ2 induces a drastic, but not total, drop in taste sensitivity. Studies in mice have shown that in the absence of the TRPM5 receptor there is a reduced, but not abolished sensitivity to sweet, umami and bitter, suggesting the presence of an additional independent mechanism of stimulus transduction (Zhang Y. et al., Cell 2003). This consists of the TRPM4 channels, dependent and selective voltage for $Ca2^+$ ions, present on type II and III cells, and whose absence is associated with reduced sensitivity while the perception of the three gustatory qualities is completely abolished when both channels are missing (Roper SD, Pflugers Archiv 2007; Dutta Banik D. et al., PNAS 2018). As regards the transduction of the sweet taste, it is induced by sucrose and molecules with a similar structure, which by binding to the membrane receptors provoke the activation of gustducine, with consequent formation of cyclic AMP, which in turn activates the protein kinase. The latter catalyzes the phosphorylation and consequent closure of the channels for $K^+$ and thus the ion accumulates in the cell resulting in a cellular depolarization, which results in the opening of the voltage-dependent channels of calcium. This eventually leads to the release of the neurotransmitter by exocytosis and activation of the afferent fiber. There are two transduction mechanisms for the bitter taste. They are related to the introduction into the oral cavity of a large number of substances, including toxic compounds. Some of these substances, such as quinine, act through their ability to block the $K^+$ channels, with consequent increase of the ion inside the cell, depolarization and opening of the voltage-dependent calcium channels, $Ca2^+$ entry and release of the neurotransmitter. Other substances instead bind the membrane chemoreceptor and activate the transducin, which is a G protein, closely related to gustducine. This in turn, activating the PLCβ2, determines the production of IP3, which favors the release of the $Ca2^+$ contained in the intracellular deposits and consequently the release of the neurotransmitter. The mechanism of transduction of umami taste activates the bond to the gustatory receptor by glutamate: it causes, finally, the opening of $Na^+$ channels that

allow the entry of the ion into the cell, and of $K^+$ channels that allow the leakage. The net result is cellular depolarization and therefore the entry of $Ca2^+$ through the voltage-dependent channels and the release of the neurotransmitter (Boughter J.D. et al., The journal of Neuroscience 1997; Stone L.M. et al., Chemical Senses 2007).

Regarding the salty taste the stimulation transduction depends on the presence of sodium ions. Their increased concentration outside the cell following the introduction of salty foods causes an increase in the electrochemical strength and a consequent entry of these ions into the cell, with membrane depolarization. Therefore, the voltage dependent calcium channels are opened and the neurotransmitter release takes place as above (Chaudhari N. and Roper SD, The Journal of Cell Biology. 2010; Kikut-Ligaj D. and Trzcielińska-Lorych J., Cellular & Molecular Biologic Letters 2015).

The perception of the sour taste depends instead on the presence of hydrogen ions in food, as they bind the potassium channels blocking the escape of this ion from the cell. Intracellular $K^+$ accumulation causes cellular depolarization with the usual consequent opening of voltage-dependent calcium channels, entry into the $Ca2^+$ cell and release of the neurotransmitter via exocytosis.

Finally, as far as fat is concerned, the large lipid molecules undergo the action of the lingual lipase, secreted by the von Ebner glands near the leafy and circumvallate papillae, which splits them into molecules of lower molecular weight, capable of stimulating the receptor taste. In particular, it causes triglycerides to release fatty acids, for which there are transporters at the level of the apical membrane of gustatory buttons, allowing their entry into the cells. Lipase plays a fundamental role in the pleasant perception of fat and in the propensity to eat fat rich foods (Figure 6) (Laugerette F. et al., The Journal of Clinical Investigation 2005; Kulkarni BV, and Mattes RD, American Journal of Physiology Regulatory Integrative and Comparative Physiology 2014).

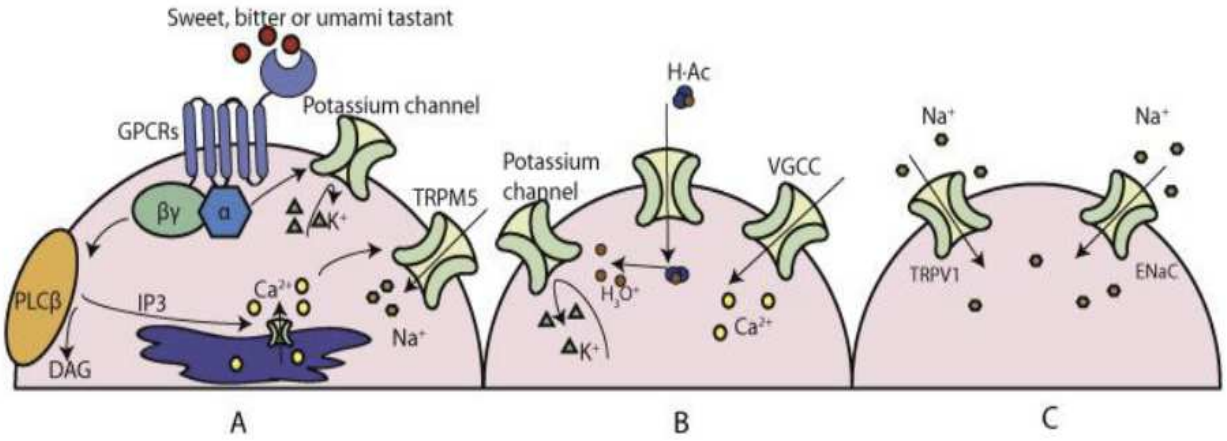


Figure 6. Proposed mechanisms of signalling of five taste qualities (Liu D. et al. 2016).

Following the improved knowledge, through RT-PCR and western blot techniques, of the numerous mechanisms of signal transduction at the level of the taste cells, proteins and channels involved, the gene expression of the receptors involved in the perception of taste was also investigated. Previous observations, based on ultrastructural and then immunohistological tests, demonstrate that there would be a diffused system consisting of solitary chemosensory cells (CCS), single or in groups, present in the oral cavity but also in digestive tracts. These cells would be entirely similar to the taste cells that we find in gustatory buttons, but they would play a role in secretions, in the immune response, in absorption and probably in many other functions of the organism. In these studies, sweet and bitter taste receptors were detected at the level of the upper airways up to the trachea, and as far as bitterness is concerned, even to the lungs, with a role in secretion and ciliary movement; in addition, bitter receptors are also found in the gastrointestinal tract, where they modulate secretion and absorption, but also the bacterial population and detection of irritants. We then tried to identify possible gene modification strategies, able to modulate the functions mediated by the cells themselves, having seen that prolonged diets are able to modify the expression of proteins in these cells. Hyperlipidic diets, for example, drastically reduce the expression on the enterocytes of the CD36 protein, with a high affinity for fatty acids, and modulate fat-induced satiety signals, mainly dependent on the production of the lipid hormone Oleoyl ethanolamide (OEA). The role played by taste cells and their interaction with the chemical molecules

contained in food in the regulation of food intake and processing would therefore go well beyond their presence on the lingual papillae (Finger TE et al., Proceedings of the National Academy of Science of the US A. 2003; Sbarbati A. and Osculati F., Progress in Neurobiology 2005; Rozengurt E., American Journal of Physiology Gastrointestinal and Liver Physiology 2006; Mace OJ et al., The Journal of Physiology 2007; Gulbransen et al., Journal of Neurophysiology 2008; Sbarbati A. et al., Progress in Neurobiology 2010; Lee RJ et al., Science Signaling 2017; Maina IW et al., World Journal of Otorhinolaryngologyl Head and Neck Surgery. 2018; Patel NN: et al., Journal of Pathogens 2018).

### 2.1.5 The fundamental tastes

#### *Sweet taste*

Sweet, as well as umami, is an attractive taste: both involve a family of three GPCRs receptors, named T1R1, T1R2 and T1R3, partly related to the metabotropic receptors of glutamate, pheromones, extracellular $Ca2^{+}$ sensor and receptors type B for GABA. These three receptors are assembled in homodimeric or heterodimeric complexes (Pin JP and Acher F., Current Drug Targets CNS and Neurological Disorders 2002) and present long amino-terminal extracellular domains, responsible for the recognition of the ligand (Kunishima N.et al., Nature 2000). There are several subpopulations of cells, some of which express T1R1 and T1R3, others T1R2 and T1R3, and others only T1R3 (Nelson G. et al., Cell 2001; Nelson G. et al., Nature 2002; Yoshida R. and Ninomiya Y., The Biochemical Journal 2016).

Genetic studies performed on mice have shown the presence on these cells of a single main locus with an important role in the response to numerous sweet substances (Fuller J.L., the Journal of Heredity 1974; Lush I.E., genetical Research 1989). This locus, called "Sac" determines different levels of ability to discriminate solutions containing sucrose and / or saccharin compared to pure water. Later it was shown that the Sac locus encodes the T1R3 receptor and that a member of the T1Rs family of receptors is involved in the perception of sweet taste (Kitagawa M.et al., Biochemical and Biophysical Research Communication 2001).

Subsequent functional expression studies in heterologous cells, then confirmed that T1R3 combined with T1R2 (T1R2+3) determines the formation of the sweet taste receptor, which is capable of

responding to all classes of sweet gustatory stimuli such as natural sugars, sweeteners artificial, D-amino acids, glycine, metabolites of plants such as sativoside and intensely sweet proteins, such as monellin, miraculin, pentadiene, curculin and thaumatin; the range of detected substances varies according to the species (Ravi K., Nutrition Journal 2005). Studies of heterologous co-expression of T1R2 and T1R3 in cell lines have allowed us to understand that their heterodimer recognizes the sweet taste of numerous and varied sweet substances (Nelson G. et al., Cell 2001; Nakajima K.et al., FASEB Journal 2008; Sanematsu K. et al., Scientific reports 2016).

Comparing the studies carried out in humans with those on the mouse, numerous differences emerged in their perceptive capacity towards certain artificial sweeteners and intensely sweet proteins; it has been seen, for example, that the mouse cannot perceive aspartame and monellin. The introduction of the human T1R2 receptor in the mouse on the other hand significantly changes its perception of the sweet taste, which assumes a human-like response profile (Zhao GQ et al., Cell 2003; Assadi-Porter FM et al., Molecules 2018). The various genetic and functional studies definitively confirm the role of T1R2 and T1R3 receptors in the recognition of sweet gustatory stimuli and demonstrate the enormous importance of hetero dimerization in the functioning of the receptor complex. The definitive proof of the role played by the T1R2+3 receptor in the perception of sweet taste in mammals came from studies on knockout mice for T1R2 and T1R3 obtained by homozygous mutations that led to the absence of the two receptor subunits. These animals presented a clear loss of perception of sweet taste and a dramatic abolition of all behavioral and electrophysiological responses to artificial sweeteners, D-amino acids and natural sugars at low or moderately high concentrations (up to 300 mM). They retained only mild responses to very high concentrations of sugars (Damak S. et al., Science 2003). A further confirmation of the role of T1Rs in sweet taste perception derives from the observation in cats and in general in the family of felids, including tigers, of a natural deletion in the gene for T1R2, which would constitute the molecular basis of the absent response to sweet taste in these animals (Figure 7) (Li X. et al., PLoS Genetics 2005; Antinucci M. and Risso D., Frontiers in Molecular Neurosciences 2017).

### *Umami taste*

Umami enhances the sweet taste. The term umami in Japanese means "delicious taste"; in the western cuisine it corresponds to a sensation of "flavor" that is induced by natural foods such as

ripe tomatoes, peas, human milk, poultry meat and poor fish, such as cod or mackerel and which is well represented by beef broth. This perception in humans is evoked by two amino acids, aspartate and MSG, unlike what happens in many other mammals that are attracted to a greater number of L-amino acids (Pritchard TC and Scott TR, Brain Research 1982; Iwasaki K. et al. Physiology & Behavior 1985; Ikeda K., Chemical Senses 2002). Regarding the involvement of the T1Rs receptor family, studies on cellular expression have shown that in rodents T1R1 and T1R3 combine to form the broad-spectrum receptor of L-amino acids (Nelson G. et al., Nature 2002). Umami stimulates the T1R1+3 receptors thus activating the G protein and consequently the PLC β 2, with production of IP3. This promotes the release of $Ca2^{+}$ and activation of TRPM5 channels with cellular depolarization, formation of the action and release potential of the ATP neurotransmitter and probably also GLP-1. There are also other amino acid receptors, such as the ionotropic and metabotropic glutamate receptors, and in particular the taste-related mGluR4, which is a truncated form of the metabotropic glutamate receptor of the brain and constitutes part of the receptors coupled to the G-proteins (Chaudhari N. et al., Nature Neuroscience 2000; Ruiz CJ et al., Chemical Senses 2003; He W. et al., The Journal of Neuroscience 2004).

While the T1R1 and T1R3 subunits are coexpressed in the gustatory caloric receptor cells in the anterior portion of the tongue, the taste-related mGluR4 receptor is found in the cellular groups of gustatory buttons of circumvallate and leaf papillae (Yang H. et al., The Journal of Histochemistry and Cytochemistry 1999).

Other studies conducted on heterodimers T1R1+3 in both humans and rodents showed a strong potentiation in response to purine nucleotides, such as IMP and disodium guanosine monophosphate (GMP). Some authors have also seen that several gene variants of the T1R1 and T1R3 receptors are associated with different sensitivity to umami (Yamaguchi S., Journal of Food Science 1967; Shigemura N. et al., PLoS One 2009). Finally, similarly to what happened for the sweet taste, confirmation that the T1R1+3 receptor complex acts as a receptor of the amino acid taste (umami) was obtained from the study on knockout mice for T1R1 and T1R3. These homozygous mutant animals, missing the two subunits, showed a total loss of umami taste, also of IMP empowerment and included behavioral attraction responses to MSG and L-amino acids. More recent studies show that in mice lacking the receptor complex there is still a sensitivity to umami stimulation at high concentrations, probably due to its action on the T1R1 receptor only (Figure 7) (Yasuo T. et al., Biological & Pharmaceutical bulletin 2008; Kurihara K., Biomed Research

International 2015; Yasumatsu K. et al., The Journal of Physiology 2015; Blonde GD and Spector AC, Chemical Senses 2017).

### *Bitter taste*

The qualitative distinction of a bitter substance from others is probably of minor importance in the defense of the organism compared to the generic recognition of a substance as potentially dangerous. Since this is the fundamental task of the perception of bitterness, evolution has led to the development of a system that allows the recognition of a wide spectrum of chemical compounds, without making a fine distinction of each. The perception of the bitter stimulus occurs by receptors encoded by a large gene family, which includes about thirty very divergent GPCR receptors, as emerged from studies on gene sequencing (Matsunami H. et al., Nature 2000).
However, a fundamental role is played by T2R receptors, whose genes are expressed selectively in cellular subpopulations that differ from those expressing the T1Rs involved in the reception of sweet and umami tastes (Adler e. Et al., Cell 2000). Given the wide chemical diversity of the compounds detected it is clear that these receptors recognize a wide variety of different chemical groups. It is still not well understood how so few receptors are sufficient to monitor the presence of the many naturally occurring bitter compounds but it is known that the same compound can be recognized by different receptors and that some receptors can recognize a wide range of molecules while others have a more selective target, also with notable variation of the perception thresholds (Meyerhof W. et al., Chemical Senses 2010). The T2R genes form clusters in genomic regions that have been shown to be genetically linked to the transmission of bitter taste in both mice and humans and there are several studies that confirm this role (Lush IE and Holland G., Genetics Research 1988; Reed DR et al., American Journal of Human Genetics 1999; Chandrashekar J. et al., Cell 2000; Behrens M. et al., Biochemical and Biophysical Research Communication 2004; Behrens M. and Meyerhof W., Physiology & Behaviour 2011). It has also been shown that the presence of characteristic polymorphisms in these genes is associated with significant changes in the sensitivity of perception of bitter stimuli, both in the mouse, in chimpanzees and also in humans (Chandrashekar J. et al., Cell 2000; Kim UK et al., Clinical Genetics 2005; Wooding S et al., Nature 2006).

Regarding bitter taste, studies have also been carried out on knockout mice for receptors, which once again confirmed that they are fundamental for the perception of taste, bitter specifically, as in this case they are also necessary for the survival of the species. Animals lacking a specific T2R, namely T2R5, which is the candidate cycloheximide receptor, presented a selective and very marked loss of the perceptive ability of the corresponding bitter compound. As further confirmation, the mouse does not normally respond to phenylthiocarbamide (PTC) and salicylic, but genetically modified animals, which express the human receptor for these two bitter substances, assume an avoidance attitude towards them (Mueller KL et al, Nature 2005). Therefore the T2R receptors are necessary, sufficient and essential for the response to bitter taste stimulating substances, and the cells that express them on their apical membrane are those that mediate the perception of this taste in vivo, functioning as a very wide sensor for bitter substances in general, with little or no discrimination (Adler e. et al., Cell 2000). Several studies on murine species confirm this role of T2R-expressing cells as universal bitter sensors, capable of making a quantitative distinction regarding different stimulus intensities, but not qualitative discrimination of different bitter substances, and being able to respond to each of them (Figure 7) (Spector AC and Kopka SL, The Journal of Neuroscience 2002; Zhang Y. et al. Cell 2003; Ji M. et al., Chemical Biology & Drug Design 2014).

The genetically determined ability to perceive the bitter taste of thioureas, such as PTC and PROP (6-n-propylthiouracil), varies greatly among individuals, affecting the choice of food and body composition. Non-sensitive and sensitive individuals have been defined as "no tasters" and "tasters" respectively, distinguishing the latter further in "supertasters" and "medium tasters". Regarding PTC, the ability to perceive it in humans is a Mendelian trait, resulting from a balanced selection of both alleles. If the "PTC tasters" are less likely to eat foods that could be toxic, non-tasters, which are about 30% of subjects in the Caucasian population, have a diet with more variety of choice. Sensitivity to PROP is associated with PAV haplotypes, a dominant variant associated with high sensitivity, AVI, associated with low or no sensitivity, and other rare haplotypes associated with intermediate sensitivity, of the TAS2R38 receptor gene and could be associated with polymorphisms of the gustin gene (CA6), a zinc dependent enzyme present in human saliva implicated in the development of taste buds (Calò C. et al., Physiology & Behavior 2010). Further gene variations in members of the TAS2R receptor family are called into question in the individual response to the bitter taste of quinine, saccharin, acesulfame potassium, goitrin, alcohol, grapefruit juice and coffee. With regard to PROP, many works have highlighted how supertasters have a greater density of

fungiform papillae on the front surface of the tongue, when compared with the other groups: these anatomical differences could partially explain the greater taste sensitivity of the supertasters towards a wide range of taste stimuli, including other types of bitter-tasting compounds, sweet substances, irritating substances (ethanol or chili), fats and creamy compounds, so the taste sensitivity to PROP is often used as a general index of chemosensory perception (Tepper BJ et al., Obesity 2008). For example, several studies have reported that PROP "no tasters" compared to "tasters" have a lower fat taste perception, showing a higher acceptability towards the latter and a higher BMI. The condition of "super tasters" for the PROP has also been correlated to a greater emotionality, above all regarding the feelings of anger, tension and fear and also they could contribute to increased food intake (Macht M. and Mueller J., Physiology & Behavior 2007).

Decreases in the salivary secretion of gustin have been associated with reduced or distorted gustatory and olfactory function. It has been hypothesized that this protein may act as a trophic factor on stem cells; the enzymatic function of gustin depends on the presence of zinc in its active site and it is known that treatment with zinc can improve gustatory function in the elderly. Growing up, the genetic component in determining food preferences is strongly modified by experience and this characteristic has made human beings able to adapt to the most diverse environments (Fox AF, PNAS 1932; Mueller KL et al., Nature 2005; Pronin AN et al., Current Biology 2007; Roudnitzky N. et al., Human Molecular Genetics 2011 ; Melis M. et al., Laryngoscope 2019).

### *Salty and sour tastes*

Both of these types of sensory stimuli act on taste receptor cells through the direct entry of ions, Na+ for salt and H+ for acid, which use specialized membrane channels of the apical cell end.

As far as salty taste is concerned, the amiloride-sensitive Na+ channels are involved which allow ion entry and the activation of the receptor cells: they are located in the anterior portion of the tongue and mediate the perception with low salt concentrations, which are those that give a feeling of pleasantness (Heck GL et al., Science 1984; Avenet P. and Lindemann B., The Journal of Membrane Biology 1988). This could reflect the usefulness of moderate amounts of salt to maintain muscle contraction, action potentials and many other functions in the body. As for high concentrations of salt, related to diseases such as hypertension, they could instead induce a reaction of aversion to food; in this case the channels involved are not well known, but it is assumed that they are located

both on the anterior and posterior portion of the tongue and that they also respond to bitterness and sour. Recently the epithelial sodium channel (ENaC) has been shown to be involved in different $Na^+$ transport reactions through the epithelium. It would seem that the cells that perceive salt are those of type I (Vandenbeuch A. et al., BMC Neuroscience 2008; Chandrashekar J.et al., Nature 2010; Liman E.R. et al., Neuron 2014).

Regarding the sour taste many different receptors have been called into question in the perception of the stimulus, including the channels permeable to cyclic nucleotides (HCN, cyclic nucleotide-gated) which are activated by hyperpolarization, acid-sensitive ion channels (ASICs), potassium channels (K2P) and calcium channels permeable to $H^+$ (Waldmann R et al., Nature 1997; Ugawa S. et al., Nature 1998; Stevens DR et al., Nature 2001; Lin W. et al., Journal of Neurophysiology 2004; Richter TA et al., Journal of Neurophysiology 2004).

Also the proposed mechanisms are different: among them for example the involvement of the $Na^+$ / $H^+$ exchangers and the acid inactivation of the $K^+$ channels (Cummings TA and Kinnamon SC, The Journal of General Physiology 1992; Lyall V. et al., The Journal of Physiology 2004). More clarity regarding sour taste receptors has been made thanks to recent genetic and functional studies, which have shown in the acid-sensitive taste cells the presence of a member of the TRP ion channel family (channel receptors that cause transient variations in potential, Transient Receptor Potential Channels).

This is PKD2L1 (Polycystic kidney disease 2-like 1 protein, so named because the mutation causes polycystic kidney disease) and it is expressed in a population of TRCs cells completely distinct from those sensitive to sweet, bitter and umami tastes, in further confirmation of the thesis according to which there is a cellular segregation for the different gustatory modalities in the periphery. In particular, the heterodimer formed by the non-selective ion channels for PKD2L1 and PKD1L3 (Polycystic kidney disease 1-like 3 protein) cations has gained more credit as an sour taste receptor, that within the gustatory buttons are selectively expressed by type III cells, which are presynaptic cells (Huang AL et al., Nature 2006; Lopezjimenez ND et al., Journal of Neurochemistry 2006).

More recent mouse studies show that the gene deletion of the PKD1L3 subunit forming the heterodimer does not prevent the perception of sour taste; however, these channels retain their importance in the marking of cells used for sour sensing and remain the most promising markers. In fact a recent work reports that the current supplied by an apical acid stimulus, consisting of $H^+$ ions, exclusively of PKD2L1 expressing cells, causes a depolarization with a temporary increase in

the intracellular concentration of $Ca2^{+}$, which in the presynaptic cells induces the release of the transmitter (Chang RB et al., PNAS 2010; Nelson T. et al., Chemical Senses 2010). Also in this case, definitive proof of the correlation between PKD2L1-expressing cells and reception of the sour gustatory stimulus derive from experiments in gene ablation performed on mice. The transgenic animals in the study expressed the attenuated diphtheria toxin, which was directed in a targeted manner against tongue cells expressing the PKD2L1: this caused a specific and total loss of the acid taste, leaving the sensitivity to other stimuli, including salty, normal for the effective role of PKD2L1-TRCs cells as the only acid-sensitive and of PKD2L1 ion channels as acid-taste receptors (Huang AL et al., Nature 2006; Ishimaru Y. et al., Proceedings of the National Academy of Sciences The United States of America 2006; Ishimaru Y., Bioscience Biotechnology and Biochemistry 2015).

The sensitivity to sour taste could play an important role not only for the gustatory system, but could also have other functions in maintaining homeostasis, intervening in the processes of monitoring the levels of carbon dioxide in the blood, of the internal state of the cerebral spinal fluid and of the brain, thus deepening the distribution and participation of PKD2L1 channels also in other physiological mechanisms (Figure 7) (Lahiri S. and Forster R.E., The International Journal of Biochemistry & Cell Biology. 2003; Vigh B. et al., Histology and Histopathology 2004; Huang A.L. et al., Nature 2006).

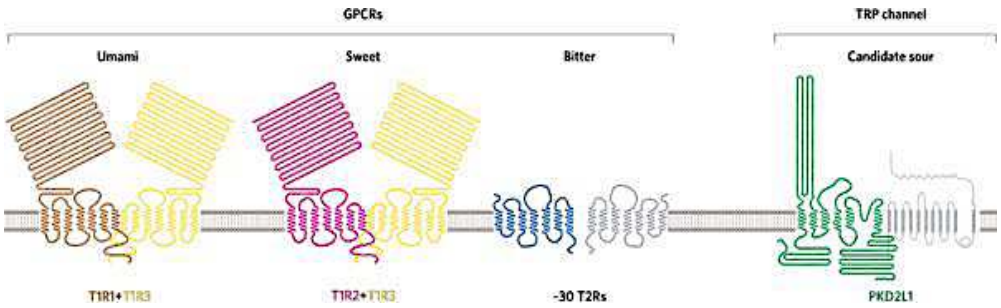


Figure 7. A schematic representation of taste receptors that mediate umami, sweet, bitter and sour. (Chandrashekar J. et al., 2006).

### *Fat taste*

For several years the recognition of dietary fats was considered primarily as a function of their consistency, and therefore a tactile stimulus; today it seems to derive from the union between a tactile sensation and a gustatory perception and has been proposed as a determinant in the selection of foods introduced and in the consequent predisposition to overweight and obesity (Kindleysides S. et al., Nutrients 2017).

Fatty taste has only been added to others in the last decade: a group of researchers at the Washington University School of Medicine highlighted the presence of papillae specialized in identifying fat in food. Human behavior tending to desire or avoiding fat intake would seem to be dependent on the activity of the CD36 gene, which regulates sensitivity to fat-containing foods by specific receptor cells. If the gene is active, the synthesis of large amounts of fat-localizing proteins takes place, with an easier regulation of the intake of high-calorie foods that contain them. Subjects with a hypoactive gene variant produce less proteins that are receptive to fat and do not reach a sense of satiety towards them. This situation would affect 20% of obese subjects, who would thus have difficulty meeting their desire for fat introduction. An increased desire for fat mediated by the CD36 gene's lack of activity on the other hand could also be partially induced by the introduction of high-calorie foods, with a high lipid content, which could thus start a vicious circuit with a tendency to overweight and obesity with its multiple complications. In addition to the preference for fat, its gene deletion also abolishes the cephalic digestion induced by the administration of free fatty acids in the oral cavity (Pepino MY, Journal of Lipid Research, 2011; Laugerette F. et al., The Journal of Clinical Investigation 2005). CD36 glycoprotein is a membrane protein with receptor function that binds saturated and unsaturated free fatty acids with a nanomolar range of affinity, thanks to its hairpin structure with a large extracellular domain and a hydrophobic pocket located between the two short cytoplasmic tails. The C-terminal tail can interact with the Src protein tyrosine kinase (PTK) forming a functional complex that allows the transfer of the signal into the receptor cell, causing an increase in intracellular $Ca2^{+}$ and release of neurotransmitters.

It is expressed in many cell types, including macrophages, platelets, enterocytes, hepatocytes, adipocytes, where it performs multiple functions, such as that of "scavenger receptor", control of homeostasis, perception of lipids, but at the level of the oral cavity its presence is limited to the

gustatory epithelium of the tongue, mainly in the circumvallate papillae (Rać MEet al., Molecular Medicine 2007).

Studies on rats have shown three possible signal transduction mechanisms with regards to food fats: they include not only that of the CD36 glycoprotein transporter, but also the involvement of G proteins and in particular the G protein-coupled receptor GPR120, or the family GPR40 which includes GPR40 for medium or long chain fatty acids and GPR41 and 43 for short chain ones. Finally, in studies starting from electrophysiological tests on rats, the involvement of delayed rectifying potassium channels (DRK Delayed Rettifying $K^+$ channels) has been highlighted: free fatty acids would inhibit these channels, thus blocking $K^+$ efflux with consequent depolarization and cell activation. Among these channels those mainly involved are KCNC2, KCNC1 and above all KCNA5 (Kv 1.5), with high sensitivity for cis-PUFAs (polyunsaturated fatty acids in cis conformation), which would also modulate the response to sweet and salty (Figure 8) (Khan NA and Besnard P., Biochemistry and Biophysica Acta. 2009; Mattes RD, Annual Review of Nutrition 2009; Gilbertson TA et al., CRC Press / Taylor & Francis 2010; Abdoul-Azize S. et al., Biochimie 2014; Ozdener MH et al., Gastroenterology 2014; Besnard P. et al., Physiological Review 2016; Liu D. et al., Progress in Lipid Research 2016; Peterschmitt Y. et al., Nutrients 2018).

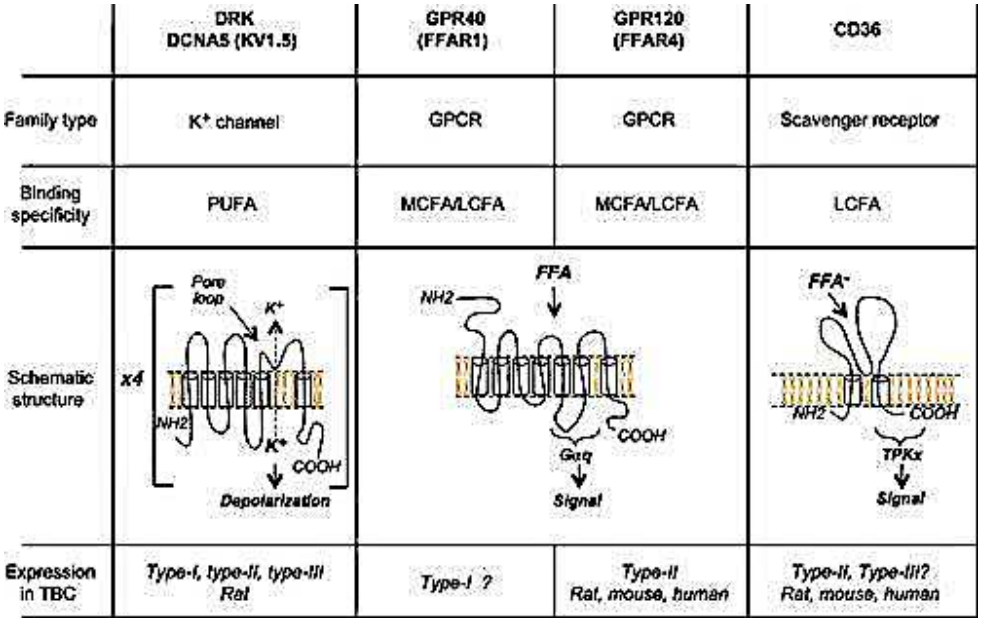


Figure 8. Main proteins candidate for the role of fat stimulus transducers. (Besnard P. et al., 2015).

### 2.1.6 Taste disorders

The alterations of taste sensitivity are responsible for severe consequences in humans, first of all because they are associated with a modification of eating behavior which results in a tendency to the onset or aggravation of nutritional deficits, but also because they prevent the avoidance of ingestion of substances potentially toxic to the body. While not enjoying great clinical attention compared to other sensory capacities the gustatory, as well as olfactory one, performs important functions and its disorders affect an estimated two million subjects in the United States (Schiffmann SS, Critical Reviews in Food Science and Nutrition 1993). Many patients suffering from loss of olfactory sensitivity also complain of a decrease in the sense of taste, but if tested, most of these subjects have normal taste detection thresholds. Moreover, in most cases the taste deficit coexists with that of smell, so that the most frequent condition is precisely that of anosmia, which is a total loss of olfactory sensitivity, with associated partial taste deficit or secondary hypogeusia. Only about 20% of subjects with altered taste have a primitive disorder of gustatory function. The alterations include (Table 2): true ageusia (complete loss of taste), hypogeusia (inability to identify only some of the fundamental gustatory qualities), specific ageusia refers to the inability to taste a specific chemical, dysgeusia ("distorted" taste or wrong gustatory perception), perception of a certain taste when no substance was ingested, hypergeusia (gustatory hypersensitivity), cacogeusia (unpleasant taste sensation) and lack of taste recognition.

| **Taste dysfunction** | |
|---|---|
| Ageusia | Complete loss of ability to taste |
| Hypogeusia | Decreased sensitivity to taste perception |
| Hypergeusia | Increased sensitivity to taste perception |
| Dysgeusia | Distortion of taste perception |
| Phantogeusia | Perception of taste without an external stimulus |
| **Smell dysfunction** | |
| Anosmia | Complete loss of ability to smell |
| Hyposmia | Decreased sensitivity to odour perception |
| Hyperosmia | Increased sensitivity to odour perception |
| Dysosmia | Distortion of odour perception |
| Phantosmia | Perception of odour without an external stimulus |

Table 2. Categorization of taste and smell changes (Schiffman SS, Annu Rev Nutr1993; Hummel T, Curr Top Otorhinolaryngol Head Neck Surg 2011).

The confusion between sour and bitter and sometimes between salty and bitter is common; sometimes there may be semantic confusions but mostly there is a physiopathological basis (Lalwani A.L. et al., Harrison 2009). Taste disturbances can be caused by factors that interfere with the access to the receptor cells located in the gustatory calyx causing a transport deficit, as in the case of drugs, intoxication by heavy metals, radiotherapy, Sjogren's syndrome, xerostomia.

In other cases there is a lesion of the receptor cells with consequent sensory deficiency: this happens for example in the case of candidiasis, endocrine disorders, drugs like antithyroid and antineoplastic ones, viral infections especially herpes, aging, oral neoplasms, pemphigus, radiotherapy, infections of the oral cavity, poor oral hygiene, gum or dental pathologies. A recent study carried out on 81 subjects with a reduction in taste compared to 40 healthy subjects highlighted the role played by a reduced density of fungiform papillae and alterations in the composition of saliva, in terms of protein concentration, proteolytic substances and antioxidants (Walliczek-Dworschak U. et al., Chemical Senses 2017). Another mechanism is damage to the afferent nerves and gustatory central pathways: for example, nerve deficit occurs in diabetes mellitus, stroke and CNS diseases, upper respiratory tract infections, oral surgery, hypothyroidism, kidney diseases, oral neoplasms, radiotherapy, trauma. The gustatory buds undergo degeneration when their gustatory afferents are dissected but remain intact when the somatosensitive afferents are interrupted. In particular, drugs and toxins frequently contribute to gustatory disorders with different mechanisms: they modify the qualitative and quantitative characteristics of saliva, and interfere with the function of taste buds and receptor cells, through inflammation of the epithelial layer of tongue, oral cavity and pharynx , with the alteration of the cranial nerves V, VII, IX, X and their central connections. These include some neurological drugs such as levodopa, phenytoin and carbamazepine. Some drugs inhibit the action of zinc in the salivary glands and taste cells, which is necessary for salivary function, the digestion of food and for normal cellular function. In some cases the drugs can trigger dysgeusia: for example therapeutic compounds containing sulfhydryl groups, including propylthiouracil, methimazole and penicillamine, chelating agents on the excretion of copper and zinc, associated with the perception of a bitter or metallic taste (Lalwani A.L. et al., Harrison 2009; DeVere R., Continuum 2017; Schiffmann S.S., World Journal of Otorhinolaryngology Head and Neck Surgery 2018).

Being able to identify a precise cause at the base of sensory alteration is important when trying to implement an etiological therapy (Table 3): patients who experience a taste reduction should be

subjected to tests to evaluate both the gustatory and olfactory function. As far as taste is concerned, anamnesis plays a decisive role in establishing the precise characteristics of the disorder, the extent, the circumstances in which it occurred, the coexistence of local or systemic symptoms and signs. Local causes include nose, paranasal or oral cavity pathologies, neurological disorders involving the olfactory nerve (I pair of cranial nerves) or V or the olfactory bulb, cranial tumors, cranial traumas, lesions of elongated marrow or bark, meningitis or encephalitis, temporal lobe epilepsy, xerostomia. Systemic pathologies in which there may be an alteration of taste are diabetes mellitus, hypertension, gastroesophageal reflux disease, vitamin B or zinc deficiency, Herpes Zoster, hypothyroidism, adrenal insufficiency, collagen diseases, polyneuritis, Ulrich-Turner syndrome. The alterations of taste sensitivity can also be genetically determined, as happens for the different perception of PROP and PTC, or for the existence of genetic variants for the CD 36 protein, as regards the fat taste, or of the umami receptor (Lalwani AL et al., Harrison 2009; DeVere R., Continuum 2017).

| |
|---|
| *SMOKING (cigar, pipe)* |
| *INFECTIOUS DISEASES (ORAL CAVITY)*<br>- Viral (adeno, rhino and virus flu, Herpes simplex)<br>- Bacterial (bothloadeniti)<br>- Fungina (oral candidiasis) |
| *SALIVARY PATHOLOGY*<br>- Sjogren Syndrome<br>- Scleroderma<br>- Cystic Fibrosis<br>- - Radiotherapy |

| |
|---|
| *TOXIC-CARENTIAL PATHOLOGY*<br>- Malnutrition and celiac disease<br>- Kidney and liver failure<br>- Hypovitamins A and B<br>- Zinc and iron deficit<br>- Cancer Cachexia |
| *ENDOCRINE PATHOLOGY*<br>- Hyper or hypo corticosurrenalism<br>- Panipopituitarism<br>- Hypothyroidism<br>- Pseudoipoparathyroidism<br>- Diabetes mellitus<br>- |
| *PSYCHIATRIC DISORDERS (depression, chizophrenia)* |
| *DRUGS*<br>- Antihypertensive: ACE-inhibitors, Ca-antagonists, Spironolactone, Beta-blockers<br>- DM: Oral hypoglicemic<br>- Hypocholesterolemizers<br>- Antihistamines, Fluticasone<br>- Anidrasicarbon inhibitors<br>- Imidazole<br>- Psychopharmaceuticals<br>- Antibiotics: chinololithic, macrolid, amoxicillin<br>- Diclofenac, Ibuprofen, Tramadol<br>- Antiepileptics: CBZ, phenytoin, lamotrigine<br>- Unpleasant: Baclofen<br>- L-dopa<br>- Antimicranial: triptans |

Table 3. Main causes of non-neurological taste alterations

A thorough medical history must be followed by a thorough physical examination starting from an otolaryngology one (ORL), to verify the possible presence of local causes that can justify the sensory alteration. Of fundamental importance is also the systematic semiological examination of the cranial nerves, in particular those involved in the gustatory and olfactory sensory afferents, that is, I, V, VII, IX, and X. It then proceeds with functional tests, such as electrogustometry, through which the gustatory function of the cranial nerves VII, IX and X is evaluated by sending an electrical stimulus to an electrode positioned on the free edge of the tongue, on the lingual V and on the roof of the palate. This examination allows to determine the presence and the entity of the gustatory alteration, with the limit consisting in the prevalent activation of specific receptors for the salty and the sour. It is possible to perform tests on the gustatory capacity of the entire oral cavity beyond the threshold, gathering information regarding the quality, intensity and pleasure of the four flavors: sweet, salty, sour and bitter. The most commonly used reagents for this purpose are sucrose, citric or hydrochloric acid, caffeine or quinine (sulfate or hydrochloride) and sodium chloride. Taste stimuli must be freshly prepared and have a similar viscosity. For quantification, the detection thresholds are obtained by applying graduated dilutions to the lingual quadrants or to the entire oral cavity. Regional gustatory tests are useful for evaluating a possible gustatory reduction localized to one or more receptor fields, as a consequence of a central or peripheral lesion. A widely used test, of simple execution, was developed in Germany: it consists of "taste strips" and constitutes a psychophysical test that allows a good evaluation of taste sensitivity (Landis B.N. et al., J Neurol 2009). To elaborate normal values, a multicenter study was carried out on 537 subjects with a normal sense of smell and taste (318 females and 219 males), aged between 18 and 87 years with an average age of 44 for a total of 32 surveys. The results indicate that the gustatory function decreases significantly with age. In each age group, women show better results than men with significantly higher scores. The 10th percentile of taste score for recognition was used as the cut-off value for distinguishing normogeusia from hypogeusia. Results on a small group of patients with ageusia confirm the clinical utility of the proposed normal values (higher or equal to 19 for women and higher or equal to 17 for men).Regarding the difference between the two sides of the tongue, the ninetieth percentile in the 18-40 age group was 3.3, so a pathological difference can be suspected with a higher score difference between the right and left side of the tongue (Landis BN et al., J Neurol 2009).

Further investigations are then performed on the basis of suspicion in the individual subjects, in order to clarify or confirm a diagnosis: they include blood chemistry tests, gustatory evoked potentials (PEG), magnetoencephalography (MEG), computed tomography (CT); magnetic resonance imaging (MRI); positron emission tomography (PET) and single-photon emission computed tomography (SPECT), or specialist consultations of a neurological, internal or psychiatric nature or whatever else is deemed necessary.

The therapeutic possibilities are in most cases limited and there are often no valid strategies for neurosensitive disorders. Many potential treatments for dysgeusia have been reported based on individual experiences. In idiopathic forms the disorder remains stable or progressively worsens: a therapy based on zinc and vitamins has been proposed, but its efficacy has not been proven. In 2005 Heckmann et al studied 116 cases of dysgeusia: 50 were idiopathic and the rest due to poor oral hygiene, allergies to dental material, poorly controlled diabetes mellitus, reduced salivation following drugs, reduced function of salivary glands, low levels of zinc, side effects of medicines.

Zinc gluconate 120-140 mg / day was used and in 50% of cases there was an improvement in taste and quality of life (Heckmann S.M. et al., Journal of Dental Research 2005). Taste dysfunctions resulting from traumas tend to resolve spontaneously, those due to injury by surgical stretching of the nerve of the tympanic cord improve in 3-4 months, while in the case of nerve section they are permanent. In some cases the etiological therapy can improve the gustatory function: artificial saliva for xerostomia or pilocarpine, modification or substitution of therapy where drugs are concerned. Useful recommendations and practical advice for patients are to label and date every perishable food and keep it in the fridge or freezer to avoid food poisoning, ensure that every chemical and cleaning product is properly labeled, make changes in the food and its preparation. For example, making a strong coffee, using artificial sweeteners instead of sugar, replacing the salt with monosodium glutamate to enhance smell and taste properties, drinking a cold glass of orange juice or grapefruit juice with lots of pulp (which stimulate sour and sweet receptors but also the trigeminal system for temperature and consistency), using artificial flavors such as chocolate, vanilla or strawberry at different concentrations according to preference, flavoring with pepper, balsamic vinegar or hot sauce that stimulate taste and trigeminal receptors; it is important to pay attention because patients often increase their use of salt and sugar, thus increasing their risk of developing hypertension and diabetes mellitus (Lalwani AL et al., Harrison 2009; DeVere R., Continuum 2017; Cecchini MP et al. , Journal of Neurology 2018; Doty RL, World Journal of Otorhinolaryngology Head

and Neck Surgery 2018; Schiffman SS, World Journal of Otorhinolaryngology Head and Neck Surgery 2018; Melis M. et al., Laryngoscope 2019).

## 2.2 Cancer disease

### 2.2.1 The disease

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues (National Cancer Institute).

Two essential characteristics of cancer cells are uncontrolled growth and the ability to metastatize. The malignant phenotype of a cell is the end result of a series of genetic changes that remove mechanisms for controlling cell growth and induce new characteristics that make the cell capable of metastasis, including the expression of surface receptors for binding to basal membranes, enzymes that can alter anatomical barriers by creating continuous solutions, cytokines that facilitate mobility and angiogenetic factors that determine the development of new vessels that ensure nutrient and oxygen factors.

As a rule, such genetic changes imply the increased or abnormal expression or activity of certain genes, known as proto-oncogenes (often growth factors or their receptors; enzymes involved in growth mechanisms or factors of deletion or inactivation of tumor suppression genes and defects of DNA repair enzymes). These genetic changes can occur due to point mutations, gene amplification, gene rearrangement or epigenetic modification such as the gene alteration.

Once the cells have assumed the characteristics of malignancy, their growth kinetics are similar to that of normal cells but lack regulation. For unclear reasons, tumor growth kinetics follow a *Gompertzian curve* (Figure 9): as the tumor mass grows, the portion of cells that multiply is reduced; at the time when a tumor is large enough to be clinically identifiable, its growth rate is often small. Unfortunately, the growth of a tumor usually does not stop before the tumor itself reaches lethal proportions (Lalwani A.K., Harrison's Principles of Internal Medicine, 2018).

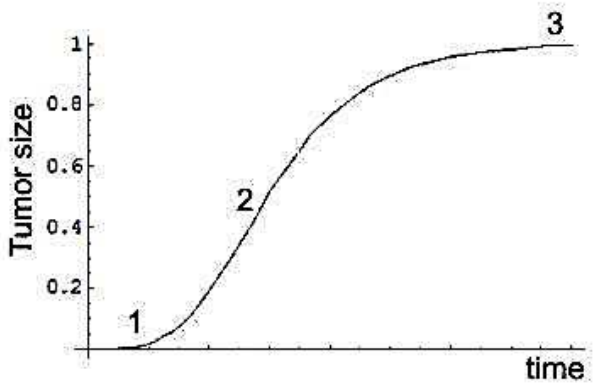


Figure 9. Tumor grows exponentially (point 1) until crossing the inflection point (point 2) whereupon its growth decelerates. As the tumor grows its core lacks oxygen and becomes necrotic. When the rate of growth equals the rate of death (necrosis) tumor size remains constant (point 3) (Laird, 1964; Laird, A.K. et al., 1965).

Cancer cells proceed through the same stages of the cell cycle as normal replication cells. Some non-division cells can remain in the G0 or quiescent phase for long periods. Some chemotherapy agents are specific to cells at certain stages of the cell cycle, which is important in developing effective chemotherapy regimens (Lalwani A.K., Harrison's Principles of Internal Medicine, 2018).

### 2.2.2. Epidemiology

In 2018, more than 18 million cases of cancer have been diagnosed worldwide, a figure expected to increase by about 60% by 2040 due to aging and increase in the world population.

These are some of the data published in the third edition of the Cancer Atlas - the result of a collaboration between the American Cancer Society, the International Union for The Control of Cancer and the International Agency for Research on Cancer - reported on the Epicenter website Higher Institute of Health (Iss).

The Atlas provides an overview of the incidence of cancer around the world through graphs, statistics and maps on the geographical distribution of the disease and risk factors. The printed version is associated with the website where the data is available in interactive format. The theme

of this edition is in fact "Access Creates Progress", to emphasize the importance of the accessibility of the data. One focus is on lung cancer, which is the most diagnosed and responsible for the highest number of deaths: in 2018, 2.1 million cases were diagnosed worldwide and 1.8 million deaths were recorded. Eastern European countries have the highest incidence rates. Tobacco consumption (two-thirds of cases) is the main culprit. It is estimated that there are 1.3 billion smokers, the majority residing in low-income or developing countries. In pointing out viable solutions, the Atlas shows that tax policies are an effective weapon to combat tobacco consumption. In particular, rising prices discourage the purchase of tobacco products in the less affluent population. For example, in South Africa, rising consumer costs have led to a decline in smokers.

Overall, there were 3.9 million new cancer cases and 1.9 million deaths in Europe in 2018. Half of the cases were represented by 4 types of cancer: breast (523 thousand new cases, 13% of the total), colorectal (500 thousand, 13%), prostate (470 thousand, 12%) lungs (450,000, 12%). As for mortality, in 2018 20% of cancer deaths were due to lung cancer (388,000 deaths), 13% to colorectal (242,000 deaths) and 7% to breast cancer (138,000 deaths).

In future years, expenditures for cancer care are likely to increase as the population ages and cancer prevalence increases. Costs are also likely to increase as new, and often more expensive, treatments are adopted as standards of care.

Cancer is among the leading causes of death worldwide. In 2012, there were 14.1 million new cases and 8.2 million cancer-related deaths worldwide.

Fifty-seven percent of new cancer cases in 2012 occurred in less developed regions of the world that include Central America and parts of Africa and Asia; 65% of cancer deaths also occurred in these regions.

The number of new cancer cases per year is expected to rise to 23.6 million by 2030.

The best indicator of progress against cancer is a change in age-adjusted mortality (death) rates, although other measures, such as quality of life, are also important. Incidence is also important, but it is not always straightforward to interpret changes in incidence. For example, if a new screening test detects many cancer cases that would never have caused a problem during someone’s life (called overdiagnosis), the incidence of that cancer would appear to increase even though the death rates do not change. However, a rise in incidence can also reflect a real increase in disease, as is the case when an increase in exposure to a risk factor causes more cancer cases. In this scenario the

increased incidence would likely lead to a rise in mortality from the cancer. Although death rates for many individual cancer types have also declined, rates for a few cancers have stabilized or even increased.

As the overall cancer death rate has declined, the number of cancer survivors has increased. These trends show that progress is being made against the disease, but much work still remains. Although rates of smoking, a major cause of cancer, have declined, the U.S. population is aging, and cancer rates increase with age. Obesity, another risk factor for cancer, is also increasing.

New cases of cancer in Italy are tending to decrease. In 2019 there are an estimated 371,000 diagnoses (196,000 men and 175,000 women).

The five most common cancers are breast cancer (53,500 cases in 2019), colorectal (49000), lung (42,500), prostate (37,000) and bladder (29,700). In particular, there is a drop in the incidence of cancers of the rectum, stomach, liver and prostate in men, although lung cancer continues to increase among women (2.2% per annum), due to the worrying spread of smoking among the Italians.

Breast cancer and, in both genders, pancreatic, thyroid and melanomas (especially in the South) are also on the rise.

These are some of the data that emerge from the volume Cancer Numbers in Italy 2019, the official census of the Italian Association of Medical Oncology-AIOM, the Italian Association of Tumor Registers-AIRTUM, the AIOM Foundation and PASSI (Progress of Health Health Companies in Italy) and the Italian Society of Pathological Anatomy and Diagnostic Cytology (SIAPEC-IAP) now in its ninth edition and presented on September 24$^{th}$ in Rome in the Auditorium of the Ministry of Health at a national conference.

The highest incidence is recorded in Friuli Venezia Giulia (716 cases per 100,000 inhabitants), the lowest in Calabria (559 cases per 100,000 inhabitants).

Nearly 3 and a half million Italians (3,460.025, 5.3% of the entire population) live after cancer diagnosis, a number that is constantly growing (there were 2.24 million in 2006, 2 million and 587 thousand in 2010, about 3 million in 2015), thanks to increasingly effective weapons and adherence to screening programmes.

Survival is also on the rise: 63% of women and 54% of men are alive 5 years after diagnosis. At least one in four patients, or nearly a million people, has returned to having the same life expectancy as the general population and can consider themselves cured.

### 2.2.3 Cancer treatment

Tumors occur in distinct stages: the initial, or localized, disease in which there is only one tumor in a single location; the phase of recurrences, possible and post-surgery ones, in which the disease recurs, but always and only in the place where it first appeared; the scattered form, in which the malignant cells leave the organ of origin to colonize other organs even at a distance (metastasis).
Blood cancers are by their nature widespread from the beginning, but they are also distinguished in the early stages in minimal residual disease and recurrence. Each tumor requires a different approach and often even different treatment times. In general the earlier a diagnosis the more timely and effective the treatment can be. But there are exceptions. Some cancers, such as that of the testicle, respond well to chemotherapy and can heal completely even when they have already given rise to metastases. Other cancers, however, such as some brain tumors, are difficult to treat even when they are initial and localized (Lalwani A.K., Harrison's Principles of Internal Medicine, 2018). A person who no longer shows signs or symptoms of illness after 5 years after the end of treatment is generally considered to be healed. In some cases, such as some forms of lung or prostate cancer, it is preferable to wait 10 years before dissolving the prognosis. This does not mean that the person is constantly undergoing treatment, on the contrary: often these are concentrated in the first months after diagnosis and, later, only periodic checks are carried out to check for the presence of residual cancer cells or recovery disease.
There are many types of cancer treatment. The types of treatment depend on the type of cancer and how advanced it is. The so-called *active surveillance* is reserved for tumor forms with very slow growth, such as some prostate tumors: it keeps the disease under close observation with repeated examinations, without intervening. Only if the doctor notices a sudden acceleration of the disease does it pass to actual treatment (Lalwani A.K., Harrison's Principles of Internal Medicine, 2018).

- Surgery is the main option in most solid tumors. Sometimes, to facilitate the work of the scalpel, an attempt is made to reduce the size of the tumor with a pre-operative chemotherapy or radiotherapy.
- Radiotherapy uses X-rays to destroy cancerous cells. It is typically concentrated as much as possible in the area affected by the disease to avoid damaging healthy cells. It can be used before surgery to reduce the size of a solid tumor or sometimes as a unique therapy if the

tumor is very sensitive to the effect of radiation. In recent years, the use of intraoperative radiotherapy has also spread to certain conditions in particular circumstances, which during the intervention allows to concentrate a greater dose of radiation in the area where the tumor had developed, reducing risk of recurrence. In other cases it is possible to place a permanent source of radiation in or near the area to be treated. We then talk about brachytherapy or internal radiotherapy. Brachytherapy can be used alone or in combination with other forms of treatment.

- Chemotherapy uses cytotoxic drugs, ones that are toxic to cells, as they block the division of rapidly replicating cells, without distinguishing between healthy cells and diseased cells. This is why chemotherapy has side effects on all rapidly changing tissues, such as mucous, hair and blood.
- Hormone therapy alters the balance of certain hormones in the body. It is mainly used to keep at bay so-called hormonal-sensitive tumors, such as those of the breast and prostate, in which such substances stimulate cell division.
- Targeted Therapy uses biological or molecular target drugs that are substances that can "recognize" the cancer cell and promote its destruction by the immune system. They can be antibodies that can target a drug within the diseased cell, causing its destruction. Other biologic drugs are so-called Kinase inhibitors, which interfere with chemical messengers used by cells to grow and reproduce.
- Immunotherapy consists of drugs capable of stimulating the immune system against cancer cells. In some cases the tumor may return even after one or more treatment cycles. Recurrence occurs at the same site where the disease first occurred and is due to the dormancy in quiescent form (i.e. "rest") of some malignant cells that have resisted surgery and radio- and chemotherapy treatments. When the disease reoccurs elsewhere in the body, it is metastases or secondary tumors. This is because some cancer cells have spread into the body.
- Stem cell transplants are procedures that restore blood-forming stem cells in cancer patients who have had theirs destroyed by very high doses of chemotherapy or radiation therapy.
- Precision medicine helps doctors select treatments that are most likely to help patients based on a genetic understanding of their disease.

### 2.2.4 Side effects of cancer treatment

Cancer treatments and cancer can cause side effects. Side effects are problems that occur when treatment affects healthy tissues or organs: anemia, appetite loss, bleeding and bruising (thrombocytopenia), constipation, delirium, diarrhea, edema (swelling), fatigue, fertility issues, flu-like symptoms, hair loss (alopecia), infection and neutropenia, lymphedema, memory or concentration problems, mouth and throat problems, nausea and vomiting, nerve problems (peripheral neuropathy), organ-related inflammation and immunotherapy, pain, sexual health issues, skin and nail changes, sleep problems, urinary and bladder problems. Side effects vary from person to person, even among those receiving the same treatment (Lalwani A.K., Harrison's Principles of Internal Medicine, 2018).

#### *Appetite loss and cancer treatment*

Cancer treatments and cancer-related fatigue may lower patients' appetite or change the way food tastes or smells. Side effects such as mouth and throat problems, or nausea and vomiting can also make eating difficult (National Cancer Institute).

Cancer treatments may cause dental, mouth, and throat problems. Radiation therapy to the head and neck may harm the salivary glands and tissues in mouth and/or make it hard to chew and swallow safely. Some types of chemotherapy and immunotherapy can also harm cells in mouth, throat, and lips. Drugs used to treat cancer and certain bone problems may also cause oral complications like: changes in taste (dysgeusia) or smell, dry mouth (xerostomia), infections and mouth sores, oral mucositis, sensitivity to hot or cold foods, swallowing problems (dysphagia), tooth decay (cavities) (Lalwani A.K., Harrison's Principles of Internal Medicine, 2018).

Mouth problems are more serious if they interfere with eating and drinking because they can lead to dehydration and/or malnutrition.

Foods may seem to have no taste or may not taste the way they used to, or food may not have much taste at all. Radiation therapy may cause a change in sweet, sour, bitter, and salty tastes. Chemotherapy drugs may cause an unpleasant chemical or metallic taste in patients' mouths.

Taste changes can lead to loss of appetite and weight loss (Drareni K. et al., Seminars in Oncology 2019). Also, it can cause a strong dislike of certain foods, also called food aversions. Relieving such

side effects is an important part of cancer care and treatment. This is called palliative or supportive care. There are several possible causes of taste changes related to cancer and its treatment.

Taste changes are a common side effect of chemotherapy (Van O. et al., Curr Opin Support Palliat Care 2018). About half of the people receiving chemotherapy have taste changes. But these taste changes usually stop about 3 to 4 weeks after treatment ends. The following types of chemotherapy are commonly known to cause taste changes: Cisplatin, Cyclophosphamide, Doxorubicin, Fluorouracil (5-FU, Efudex), Paclitaxel (Taxol), Vincristine (Oncovin, Vincasar PFS).

Radiation therapy to the neck and head can harm the taste buds and salivary glands, causing taste changes. It may also cause changes to the sense of smell. Changes to the sense of smell may affect how foods taste. Taste changes caused by radiation treatment usually start to improve 3 weeks to 2 months after treatment ends. Taste changes may continue to improve for about a year. If the salivary glands are harmed, then the sense of taste may not fully return to the way it was before treatment. Other causes of taste changes include: surgery to the nose, throat, or mouth, biological therapies, such as interleukin-2 (IL-2), called aldesleukin (Proleukin), damage to the nerves involved in tasting, mouth infections, dental or gum problems, nausea and vomiting, managing taste problems (Lalwani A.K., Harrison's Principles of Internal Medicine, 2018).

Usually, there are no specific treatments for taste problems. But sometimes treating the cause of the taste changes can help. For example, treating causes such as mouth infections, dry mouth, or dental or gum problems can improve taste changes.

Taste changes can make it hard for some people to eat healthy foods and maintain their weight.

Avoiding to eat 1 to 2 hours before chemotherapy and up to 3 hours after chemotherapy can help to prevent food aversions caused by nausea and vomiting. Zinc sulfate supplements may improve taste for some people.

## 2.3 Taste sensitivity in cancer disease

Changes in taste sensitivity give rise to severe consequences in humans, primarily because they are associated with an alteration of eating behavior with the risk of a nutritional deficit, also because they do not allow the recognition of potentially toxic substances for the individual. In addition to

this, often a taste deficit coexists with that of smell, resulting in anosmia, that is, the total loss of olfactory capacity, linked to a partial deficit of taste or secondary hypogeusia.

Taste changes are symptoms that are often reported by patients undergoing chemotherapy treatment; The literature shows that the percentage of these patients is between 45% and 84%, while patients who have only alterations in smell vary between 5% and 60% (Gamper E, J Pain Symptom Manage 2012; IJpma I, Cancer Treat Rev 2015; Zabernigg A. Oncologist 2010; Mosel DD, Oral Dis 2011). The problems of taste can be divided into two categories, qualitative and quantitative: the qualitative ones include dysgeusia, understood as an alteration of taste sensitivity given by foods which in the past were enjoyed and that have become unpleasant, and fantasy, a perception without stimuli; including ageusia (total deficit), hypogeusia and hypergeusia, which consist of the decrease and increase in taste sensitivity (IJpma I, Cancer Treat Rev 2015).

The mechanism by which chemotherapy alters the perception of taste and smell is not well known. Etiology seems to be sought in inhibition caused by cytostatic agents in the mitosis of olfactory and taste cells, which occurs in 10 and 30 days and therefore in both cases is very rapid. Since chemotherapy attacks cells with rapid cell division, this makes the olfactory and taste cells the targets of antiblastic therapy. After the course of chemotherapy, the cells resume their normal cycle (Gamper E, J Pain Symptom Manage 2012, Wickham RS, Oncol Nurs Forum 1999).

Changes in taste sensitivity give rise to severe consequences in humans, primarily because they are associated with an alteration of eating behaviour with the risk of a nutritional deficit, also because they do not allow a person to recognize potentially toxic substances. In addition to this, often a taste deficit coexists with that of smell, resulting in anosmia, that is, the total loss of olfactory capacity, linked to a partial deficit of taste or secondary hypogeusia.

# 3. PROBLEM STATEMENT

## 3.1 Side effects of chemotherapy

Among the various side effects induced by chemotherapy, those acting at the level of the gastro-intestinal tract are surely more related to the nutritional problems of cancer patients.
These include mucositis, which are mucosites that are created because soft tissues cannot replace cells damaged by antiblastic therapy. Pain causes difficulty in feeding and nutrients are not absorbed, thus increasing the risk of developing protein-calorie and vitamin deficits (Mosel DD, Oral Dis 2011).
Another adverse effect is oral caulking infections, caused both by mucosites and chemotherapy-induced immunodepression.
In addition, as mentioned above, the sense of nausea and the consequent vomiting lead to many problems: the patient has less appetite and, by eliminating the food ingested, loses the nutrients contained in it, also causing dehydration and electrolyte imbalance. As these two symptoms are very frequent, oncologists often prescribe antiemetic drugs in conjunction with antiblastic therapy.
Xerostomy, or dryness of the jaws, also leads to nutritional complications: the taste of foods is perceived with more difficulty and poor lubrication creates the basis for possible damage to the mucosa of the oral cavity. All these symptoms can lead to malnutrition in the cancer patient, a serious and current problem that should not be underestimated.

### 3.1.1 Malnutrition

According to the definition of the Council on Food and Nutrition of the American Medical Association, malnutrition refers to "a state of structural functional alteration and development of the organism, resulting from the discrepancy between requirements specific nutrients and essential nutrients."
Another broader and more complete definition given by Stratton et al., identifies it as a situation in which a deficit or excess (or an imbalance) of energy, proteins and other nutrients leads to

unwanted measurable effects on body composition or function and short/long-term prognosis (Stratton RJ, ed. CABI Publishing, 2003).

It is called primary food-derived malnutrition, which manifests itself without disease (poverty, famine, social isolation) and secondary to that which is associated with a pathological condition.

Being malnourished does not necessarily correspond to being in a state of undernutrition or nutritional deficiency: it is important to distinguish between nutritional situations by default or excess.

Protein Energy Malnutrition (PEM) is the typical form of malnutrition by default and is characterized by the reduction of lean mass and fatty tissue.

As stated by the International Classification of Diseases (ICD) there are two forms of PEM:

" Disease or cachexia type", due to chronic deficits of energy substrates. In this form we find the decrease of fat reserves and muscle masses, while the visceral protein component is minimally altered; " Kwashiorkor type" (or protein malnutrition or hypoalbuminemic), where we find a predominantly protein deficiency with sufficient energy intake. In this context, adipose tissue and somatic proteins are initially preserved, while visceral proteins and immune response are decreased; " Marasma-kwashiorkor type", which is the most frequent form of malnutrition and which includes the descriptions mentioned above.

### 3.1.2 Malnutrition and anorexia in cancer patients

Cancer is definitely the clinical condition that is most frequently associated with the concept of severe PEM, up to the so-called cachexia (Binetti P., Società Editrice Universo 2016).

It is important to distinguish actual malnutrition from cachexia: while the former is reversible and has a tendency to preserve muscle mass, with an adequate response to nutritional therapy, neoplastic cachexia, according to the literature and the international guidance lines, can be defined as a "multifactorial syndrome, characterized by progressive loss of muscle mass (with or without loss of fat mass) that cannot be completely corrected with conventional nutritional support and which leads to progressive functional damage" (Fearon K., Lancet Oncol 2011; Radbruch L., Aachen, Department of Palliative Medicine/European Palliative Care Research Collaborative 2010).

This syndrome, in brief, is characterized by decreased appetite and food intake, weight loss, metabolic alterations and reduction of muscle masses and adipose compartment. The diagnosis of cachexia should include other criteria besides sheer weight loss, such as loss of muscle mass, anorexia and inflammation. Thanks to these parameters we can classify the degree of severity. In fact, cachexia can evolve through various stages:

Pre-cachexia, where clinical-metabolic alterations (anorexia and reduced glucose tolerance) may precede weight loss (up 5%);

Cachexia, where patients have an involuntary weight loss >5% in the previous 6 months, or a BMI <20 with a weight loss of more than 2%, or sarcopenia with a concomitant weight loss of more than 2%; Refractory cachexia, which is an advanced or rapidly progressive disease and where antineoplastic therapy is no longer useful. This stage is related to a life expectancy of less than 3 months and a high non-reversible hyper-catabolism. In this case, the risks related to artificial nutritional support may outweigh the potential benefits (Fearon K., Lancet Oncol 2011).

Whereas in the past it was thought that the cancer patient was suffering from profound and irreversible nutritional alterations and the common treatments were palliative care (such as artificial nutrition in the terminal patient), today this vision is outdated. People affected by these problems are in fact a very heterogeneous group, with extremely different metabolic-nutritional needs that depend both on the type and the stage of the disease, as well as on the individual healing techniques.

In the literature we find numerous studies on this subject; for example, the "PreMiO" study, carried out in 2017 on almost 2000 patients at the first cancer control, showed that about half of these, 51%, had a high risk of malnutrition and 9% were frankly malnourished (Muscaritoli M., Oncotarget, 2017).

Malnutrition is present from 15% to 80% of cancer patients, of which 20-30% die from its effects.

The incidence of malnutrition likely depends on the location of the tumor, as demonstrated in the literature by Hébuterne X. et al., where 67% of patients with pancreatic cancer were malnourished, as well as 60% of patients with oesophagus and stomach cancer and 39% of those with colorectal cancer (Hébuterne X., JPEN, 2014).

Numerous studies have highlighted the consequences of malnutrition in patients with cancer, including the negative impact on health, survival and added health costs.

In fact, the presence and severity of PEM reduces total survival, leads to the onset of post-operative complications, increases the toxicity of radiotherapy and chemotherapy by reducing the sensitivity of cancer cells to antineoplastic treatment, decreases the response of the immune system and is a source of psychological stress for the patient and his/her family (Binetti P., Società Editrice Universo 2016).

Regarding the pathogenesis of malnutrition in the cancer patient, it is now widely believed that the tumor, in addition to the "theft" of nutrients operated by its cells, causes many changes in protein, lipid and glucid metabolism in the host, which lead to the dispersion of energy taken with food. Carbohydrate Metabolism: The main changes are increased gluconeogenesis and glucose intolerance. The latter, already known at the beginning of our century, has been confirmed by numerous studies, such as the one conducted by Rossi Fanelli F. et al, in which, in cancer patients undergoing oral load curves, 60% of them at diagnosis were shown to be glucose intolerant or even diabetic (Rossi Fanelli F., J Parenter Enteral Nutr 1991).

Lipid Metabolism: the increase in the mobilization and oxidation of lipids in the tissues of the host leads to the consumption of lipid deposits. Whereas in a healthy person oxidation is suppressed by glucose administration, in this case it is not possible. In addition, the action of hydrolysis of the exogenous lipids of lipoprotein-lipase undergoes a significant reduction (Muscaritoli M., Nutrition 1990).

Protein Metabolism: several studies have shown the reduction of gluconeogenetic amino acids in plasma, explained by increased liver gluconeogenesis. Branched chain amino acids (AACR), which in simple malnutrition are consumed in large numbers, are normal in this context. This confirms the profound difference between cancer cachexia and malnutrition. The increase in protein turnover and the consequent alteration of protein metabolism are definitely related to the decrease in lean mass.

Lately, the action of the proteasome ubiquitin system, a system that fulfills this task under physiological conditions, has been assumed as the cause of protein degradation. Several scientific studies have shown that its activity increases in the course of neoplasia (Bossola M., Am K Physiol Regul Integr Comp Physiol 2001; Bossola M., Ann Surg 2003).

Another problem frequently present and yet to be solved is neoplastic anorexia, defined as the loss of the desire to eat; this alone affects 20% of the indices that assess quality of life, negatively affecting the prognosis of each patient.

In cases of advanced cancer, anorexia reaches peaks of 60-65%, while in the earlier stages the prevalence varies between 10% and 50%.

Its pathogenesis is very complex and can be traced back to an alteration, at the hypothalamic level, of those mechanisms that regulate hunger and satiety.

Briefly we can say that the presence of pro-inflammatory cytokines in response to the growth of cancer, leads to a hypothalamic "resistance" to all energy stimuli that come from the periphery and that should signal an energy deficit. In this way the hypothalamus is tricked into giving answers such as loss of appetite (Binetti P., Società Editrice Universo 2016).

Among the various mechanisms, serotonin seems to play a key role in the pathogenesis of anorexia; this neurotransmitter is also involved in the regulation of satiety. Hypothalamic serotonin is produced from the tryptophan amino acid that overcomes the blood-brain barrier. Not having a negative feedback mechanism, more tryptophan reaches the brain and more serotonin is produced. Many scientific studies show that the amount of tryptophan, in conjunction with the tumor, increases considerably. Nevertheless, the increase in brain tryptophan is not enough to increase neurotransmission because, normally, the serotonin produced is degraded by the neuron. To witness an increase, you need a trigger, given by the action on serotonin neurons of cytokines (Laviano A., Lancet Oncol 2003; Laviano A., Nature Clin Pract Oncol 2005).

### 3.1.3 Nutritional approach to the cancer patients

From the previous paragraphs, it is clear that the nutritional approach to the neoplastic patient is fundamental; this must be developed in order to address and possibly resolve metabolic alterations, reduced caloric intake from neoplastic anorexia and specific nutritional demands of the tumor itself. There are numerous guidelines on this subject in international literature, such as the ESPEN (European Society for Clinical Nutrition and Metabolism) and ASPEN ones (American Society for Parental and Enteral Nutrition).

With regard to the Italy, we find the SINPE Guidelines (Italian Society of Artificial Nutrition and Metabolism), those of AIOM (Italian Association of Medical Oncology) and the Guidelines on the Nutritional Pathways in Oncology Patients, drawn up by a multidisciplinary working group of

representatives of the Ministry of Health, health companies, universities and representatives of scientific companies in the field.
In particular, a scientific review was carried out by Hunter R. et al., the AIOM-SINPE Practical Recommendations for Nutritional Support in the Oncology Patient, with the aim of giving clear indications for the nutritional approach in the oncology patient (Caccialanza R., J Cancer 2016).

### *Evaluation and screening*

The nutritional situation of the cancer patient was, for many years, assessed only at a later stage, often when cachexia had already occurred, making it impossible for the doctor to continue specific treatments. As specified in the previous paragraphs, neoplastic cachexia, depending on various factors including systemic inflammatory response, responds poorly to nutritional support, be it natural or artificial. Early recognition of this condition is therefore crucial. Many screening tools to identify patients at risk of malnutrition have been validated in the field of oncology, including the Nutritional Risk Screening 2002 (NRS 2002), the Malnutrition Universal Screening Tool (MUST), the Malnutrition Screening Tool (MST) and the Mini Nutritional Assessment (MNA) (Skipper A., JPEN 2012).
Screening should be done at the time of diagnosis and repeated regularly during the course of the disease, based on the type, stage and incidence of the tumor on the nutritional status.
With regard to nutritional assessment, this should include:

- the recording of height (m) and weight (kg);
- calculation of BMI, given by the ratio of weight (kg) to stature ($m^2$), an indicator of morbidity and mortality given by weight excess or weight defect;
- detection of waist circumference (cm), another indicator of mortality and independent morbidity of body mass index. The classification of the National Institutes of Health correlates this index with risk to health and the onset of chronic diseases; in particular, the risk is very high if the circumference is >102 cm in men and >88 cm in women;
- detection of arm circumference, a prognostic indicator in malnutrition by defect. Along with the tricepial plica, it allows the calculation of the muscle-adipose areas of the arm;

- Plicometry, especially tricepial, as explained above; calculation of weight loss compared to the usual one (MCP if >10% in the last 6 months);
- most relevant hematochemical parameters such as albumin and total serum proteins, VES and reactive C protein to assess inflammatory status;
- analysis of bioelectric impedance: recent scientific studies have highlighted the importance of body composition in cancer patients, which can be carried out through bioimpudiation, a tool that determines lean mass, fat mass and total water, therefore also the hydration state of the patient (Prado CM., Anticancer Agents Med Chem. 2013);
- objective examination aimed at assessing nutritional status;
- patients' food history.

### *Nutritional plan*

The nutritional condition should be evaluated and managed quickly in a targeted way in each patient, based on nutritional conditions, clinical status, expected treatments and expected outcomes.

Personalized nutritional counseling and the drafting of an appropriate nutritional plan, be it natural or artificial (enteral nutrition, by injection or with oral supplements), based on spontaneous and tolerated food intake and its effectiveness, are an integral part and the first steps of an adequate nutritional therapy.

With regard to nutritional counseling, a relational activity aimed at empowering the subject and overcoming the "descriptive" dietary approach, which is based on giving a qualitative and quantitative "technical support", we can to say that it is able to positively influence prognosis in the patient and improve, in general, the quality of life, proving even more useful than oral nutritional supplements (Ravasco P., Clin Oncol 2005).

If the calorie-protein intake is less than 75% of the required amount, then our patient is subjected to counseling: it should be remembered that if he/she is able to feed him/herself and meet the energy needs, the development of a personalized diet plan is recommended, prepared by competent staff such as a dietician, in agreement with the patient and re-evaluated according to the various individual needs. If physical activity is a part of lifestyle habits, the diet must provide 30-

35 kcal/kg/die and 1-1.2 g protein/kg/die, with a lipid share that can cover 30% to 50% of non-protein calories (Binetti P., Società Editrice Universo 2016).

If the intake is between 75% and 60%, together with counselling, oral nutritional supplements (ONS) are given, which are the first step in achieving a satisfactory energy intake and, if these are no longer sufficient, one must pass to artificial nutrition, first enteral and subsequently by injection.

Artificial nutritional support should be provided to all malnourished or malnourished patients, especially when energy intake is not sufficient (<60% of estimated calories) for more than 7 days (Bozzetti F., Clin Nutr 2009; Arends J., Clin. Nutr 2006; August DA., JPEN. 2009; French Speaking Society of Clinical Nutrition and Metabolism (SFNEP) Dig Liver Dis 2014).

Malnourished patients with planned surgery should receive at least 7 days of nutritional support to avoid or decrease post-operative complications (Weimann A., Clin Nutr 2006).

Enteral Nutrition (EN), defined as the administration of food through the placement of a probe in the patient's digestive system, should not be used in all patients but only in those who are malnourished or unable to eat for 7 days. This represents the first nutritional line in the peri-operative treatment of patients in need of artificial nutrition (Arends J., Clin. Nutr 2006; August DA., JPEN. 2009). Both American and European guidelines suggest NE with formulas containing arginine and omega 3 fatty acids in patients who need to undergo abdominal or head/neck operations, although other studies are being carried out regarding this topic (Arends J., Clin. Nutr 2006; August DA., JPEN2009; Weimann A., Clin Nutr 2006).

The use of Total Parental Nutrition (TPN), or the administration of nutrients via direct venous delivery, bypassing the digestive system, has been deeply discussed due to the risk of infection. The European and American Lines agree on its use in the cases of patients who receive chemotherapy treatment, are malnourished or have gone for more than 7 days without adequate sustenance, in which natural nutrition and EN are not effective. The routine use of TPN in these patients is strongly discouraged (Bozzetti F., Clin Nutr 2009; August DA., JPEN2009).

A period of 10-15 days of TPN is indicated in patients with acute and severe mucositis and vomiting, while long periods (more than 30 days) of TPN are recommended in those who have undergone extensive intestinal resection, suffer severe malabsorption, have a mechanical obstruction or are suffering from chronic or sub-acute enteritis from radiation (Bozzetti F., Clin Nutr 2009; August DA., JPEN 2009).

Home enteral nutrition nutrition (HENT) is a well-established extra-hospital therapy that certainly helps to lower the costs of hospitalization (Santarpia L., Clin. Nutr 2014). In addition, HENT can improve the prognosis of cancer patients and allow them to be more integrated in their family and society, leading to an improvement in their quality of life (Staun M., Clin Nutr 2009).

Given the complexity of its organization and related complications, HENT must be prescribed and monitored on a regular basis, using protocols shared between cancer specialists and clinical nutrition specialists.

Finally, the scientific world agrees on the importance of mandatory periodic monitoring in all cancer patients, with the aim of improving clinical outcomes.

### *Risk factors, diet and prevention*

The identification of cancer risk factors is certainly complex. Despite the significant amount of scientific research, cancer continues to be a killer worldwide. Statistical studies have shown that, in the United States, this is the second most frequent cause of death after cardiovascular disease cancer (Jemal A., CA Cancer J. Clin. 2007).

The scientific study by Anand et al., shows that only 5-10% of all cancer cases can be attributed to genetic defects, while the remaining 90-95% have their roots in the environment and lifestyles, first of all the diet (30-35%), followed by tobacco smoke (25-30%), infections (15-20%), obesity (10-20%), alcohol (4-6%) and other factors such as radiation, stress, physical activity and environmental pollution (10-15%) (Figure 10) (Anand P., Pharmaceutical Research, 2008).

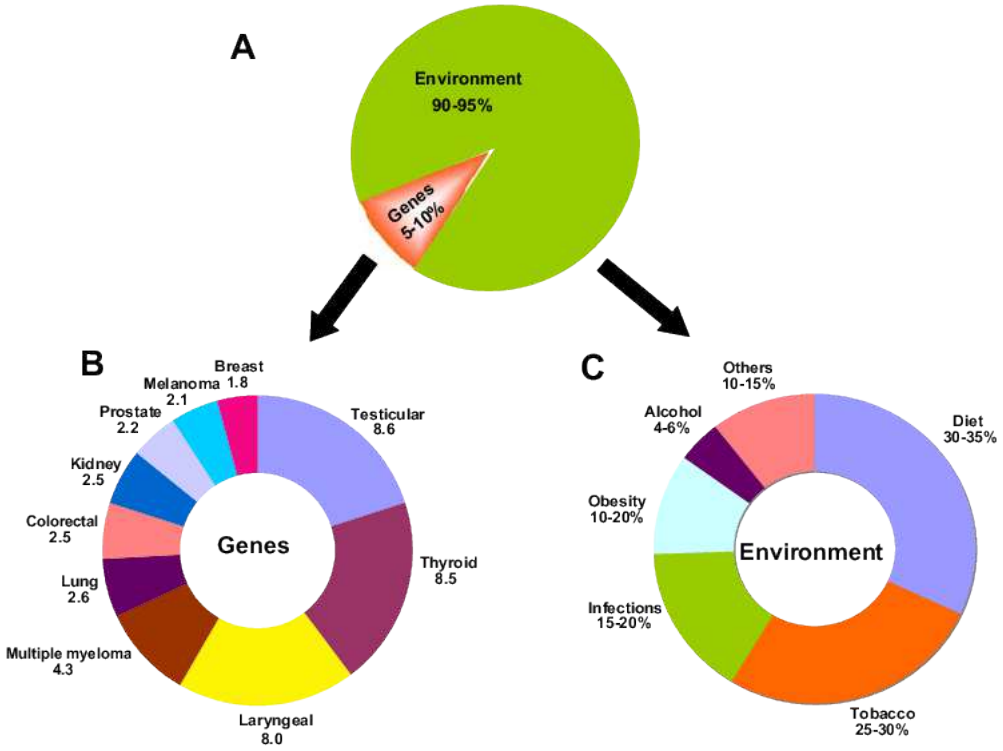


Figure 10. The role of genes and the environment in cancer development. (Anand P. et al., 2008)

While hereditary factors cannot be changed, lifestyle and environmental factors are potentially modifiable.

Regarding diet, the extent to which it contributes to cancer death varies greatly, even depending on the type of cancer (Willett W. C., Oncologist, 2000). For example, diet is linked for 70% to patients who die from colorectal cancer, 35% to patients with gastric cancer, 50% of those with pancreatic cancer and 50% to patients with breast cancer.

Some situations may be involved in tumorigenesis through the activation of procarcinogens: the first is the folic acid deficiency, the action of free radicals, nitrosamines and heterocyclic amines, the second by obesity, inflammation, saturated fats and alcohol.

Folic acid, or vitamin B9, found in certain foods such as lettuce, is crucial for the remethylation of homocysteine, a sulfide amino acid that is formed as a result of the loss of a methyl group by metionin, in the latter, which leads to physiological methylation of DNA and therefore to proper transcription. In the case of folate deficiency and of co-triggers such as alcohol, smoking and drugs,

homocysteine has an incorrect transformation into adenosyl-homocysteine, which leads to altered DNA methylation resulting in impaired repair, gene expression and loss of DNA integrity.

Free radicals, molecules determined by various metabolic processes and chemically very reactive due to the presence in the outermost orbital of one or more spaced electrons, lead to the peroxidation of PUFAs (polyunsaturated fatty acids), which are the constituents of cell membranes. Because of this problem, membranes fail to perform the correct functions of passing molecules from inside to outside the cell, and this results in reduced functionality, causing malignanites but also aging and cardiovascular disease.

Nitrosamines, organic compounds containing a nitroso group, are formed by the union of amines with nitrites, which in turn derive from nitrates, in the presence of factors such as heat, bacteria, long conversation, naturally present in food plants, animals and in the water. Nitrosamines are highly carcinogenic and their production should be minimized by using ascorbic acid (vitamin C), although scientific studies are testing the latter option.

By moving to indirect procarcinogen action, obesity plays a major role in the development of cancer; it has been associated with increased mortality in colon, breast, endometrial, kidney, esophagus, stomach, pancreas, prostate, gallbladder and liver cancer (Calle E. E., N Engl J Med. 2003).

Studies have shown that the common denominators between obesity and cancer are certain substances such as hormones, adipocytes, insulin resistance and inflammation (Hursting S.D., Cancer Drug Targets. 2007).

In obesity, in fact, the hypertrophic deposits of fatty tissue are characterized by a chronic inflammatory state, in which adipocytes and certain elements of the immune system, produce substances with pro-inflammatory, anti-apoptotic and procancerogenic activities, such as IL-6, PAI-1, TNF-, leptin and resistine.

In addition, one of the most confirmed hypotheses in the scientific world sees insulin resistance and the consequent hyperinsulinemia as a condition that can promote the development of cancer cell populations at the expense of different anatomical districts (Calle E.E Nature Reviews Cancer, 2004; Tsuganeand S, Cancer Science, 2010).

All this highlights the importance of inflammation for the advent of cancer; numerous studies have shown this correlation, others are developing and as early as 2010 it was evident that breaking down inflammation also meant slowing down the proliferation of cancer (Mantovani A., Curr Mol Med. 2010).

The human microbiota, the set of symbiotic microorganisms that coexist with the human organism without damaging it, is also involved in the development of cancer. The results of the study carried out by Ou J. et al., which aimed to examine the hypothesis that the influence of diet on the risk of colon cancer is mediated by the microbiota through its metabolites, supported the hypothesis that the risk of cancer is influenced by the balance between the microbial production of health-promoting metabolites such as butyrate and potentially carcinogenic metabolites such as secondary bile acids (Ou J., Am J Clin Nutr. 2013).

The protective action of nutrition on the development of cancer is given by individual food groups, such as fruits and vegetables, and also by safe/effective types of diets, first of all the Mediterranean diet and exercise. With regard to fruits and vegetables, numerous epidemiological studies show that their consumption protects from the onset of cancers especially of the oral cavity, esophagus, stomach and colorectal. This is due to inhibition of the absorption of mutagens/carcinogens through the high fiber content and the inhibition of endogenous production of nitrocomposites by vitamins (ascorbic acid,-tocoferol), S-compounds (cysteine, glutatione, N-acetylcisteina) and phenols.

In addition, fruits and vegetables cause the transformation of free oxygen radicals into non-radical and therefore toxic compounds, through vitamins, beta-carotene, ascorbic acid, tocopherols and flavonoids, and the maintenance of genomic stability with folic acid, vitamin B12, selenium, zinc, flavonoids, DNA repair and inhibition of the NF-KB system (cell differentiation) and the incentive of apoptosis through flavonoids (Anand P., Pharmaceutical Research, 2008).

As previously mentioned, the Mediterranean diet has a beneficial role in the prevention and remission of tumors, especially those of the gastro-intestinal tract. It can be defined as a nutritional model inspired by the eating habits widespread in the countries of the Mediterranean basin, especially those of Italy and Greece, and, since 2010, included by UNESCO (United Nations Educational Organization, Science and Culture) in the list of oral and intangible heritage of humanity. Among the features are plant-based foods (fruits, vegetables, bread and cereals, especially whole grains, potatoes, beans and other legumes, nuts, seeds), fresh, natural, seasonal, locally sourced; fresh fruit on a daily basis, while sweet products containing refined sugars or honey only a few times a week; olive oil as the main source of fats; (cheese and yoghurt) ingested every day in modest-moderate amounts; fish and poultry in moderate amounts, red meat in lower quantities and wine consumed in modest-moderate amounts, generally during the meal (Pitsavos C., Am J Clin Nutr, 2005).

Scientific studies confirm the preventive effects of the Mediterranean diet overall on all cancers, in particular stomach cancer, esophageal tumors, colon-rectal tumors, breast cancers. (Couto E., Br J Cancer, 2011; Reedy J., J Nutr, 2014; Buckland G., Am J Clin Nutr, 2010; Praud D., Int J Cancer, 2014; Li W.Q., Clin Gastroenterol Hepatol, 2013; Agnoli C., Int J Cancer, 2013; Fung TT., J Nutr, 2006).

Another anti-cancer action is exercise: in fact, there is extensive evidence suggesting that regular exercise could reduce the incidence of various cancers; In addition, physical inactivity has been correlated with increased risk of colon, pancreas, breast, prostate and melanoma cancer (Booth F. W., J. Appl. Physiol. 2002).

This could be due to the ability of exercise to reduce the action of inflammatory cytokines, but also to lead to a reduction in estrogen, IGF-1 and fatty tissue. All this combined with the improvement of the immune system determined by movement, a more effective peristalsis (reduced contact with the mucosa of harmful substances) and an insulin sensitivity, allows to reduce the inflammatory state, thus being a protective factor against carcinogenesis.

In order to arrive at evidence-based indications of many scientific studies on diet and cancer and to be able to answer the questions "Can we prevent cancer by eating better? And if so, how?" a systematic review of the entire scientific literature with standardized criteria was necessary. The World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) in 2007 made the following recommendations, which are regularly updated (WCRF, World Cancer Research Fund, AICR, American Institute for Cancer Research, 2018) (Figure 11).


Figure 11. Recommendations to prevent cancer. (WCRF and AICR, 2018)

- Keeping a healthy weight, in fact keeping in the range of normal-weight for life can be one of the best behaviors that can prevent cancer. In this context, BMI and the detection of the circumference of life are used to help us;
- Keep yourself physically active every day, using any kind of behavior that favors the use of your legs rather than machines in various daily activities. This allows an increase in energy expenditure, preventing weight gain, and at the same time promotes weight loss;
- Eating a diet rich in whole grains, vegetables and fruits, respecting the principles of the Mediterranean diet which, as described above, is a valid association against the onset of cancer;
- Limiting the consumption of "fast foods" and other processed foods rich in fat or sugar, in general all industrially refined, pre-cooked and pre-packaged foods;

- Limiting the consumption of red meat and avoiding the consumption of processed and stored meat, in general the data collected indicate that consumption below 500g per week of red meat is not a health hazard;
- Limiting the consumption of sugary drinks;
- Limiting alcohol consumption;
- Meeting nutritional requirements through diet and not with dietary supplements, unless there are serious deficiencies and confirmed by medical opinion;
- Breastfeeding for the first six months, as it protects against the onset of breast cancer for the woman, while breast milk is the best food for the baby up to 6 months;
- The recommendations for cancer prevention are also valid for those who have been diagnosed with cancer, in fact a healthy and varied diet that recalculates the Mediterranean model can control cancer growth.

# 4. PRESENTATION OF STUDIES

## 4.1 STUDY I

We focused our attention on how dysgeusia could be analyzed with a simple and inexpensive method in order to immediately intervene with a personalized diet to prevent the patients' malnutrition. This method not only allows to make a diagnosis of dysgeusia, but also to assess its degree and characteristics.

Thus, the aim of the present study was to analyze the taste alterations in a cancer patient population compared with healthy subjects as controls, also in relation to age and gender.

In this way, it could open up a new approach for a personalized diet to prevent and/ or reduce the taste alterations in cancer patients undergoing chemotherapy and so to prevent cases of malnutrition.

Those results were published in the June 2019 (Pugnaloni S. et al., Support Care Cancer, 2019).

### 4.1.1 Participants and methods

#### *Study population*

Our study was carried out on 45 cancer patients undergoing chemotherapy (18 males and 27 females) whose characteristics are summarized in Table 4. Thirty-two healthy subjects (14 males and 18 females) recruited from our previous studies (Vignini A, Dis Markers 2019) were defined as a historic control group.

| | Cancer Patients (N=45) | Control (N=32) |
|---|---|---|
| **Age (years), Mean (SD)** | 51.4 ±13.7 | 48.7 ± 9.4 |
| **Sex, No. (%)** | | |
| **Males** | 18 (40%) | 14 (44%) |
| **Females** | 27 (60%) | 18 (56%) |
| **Body mass index (kg/m2)** | 24.7 ± 3.1 | 22.9 ± 3.9 |
| **Cancer site** | | |
| **Breast** | 17 | |
| **Colonrectal** | 10 | |
| **Gastroesophageal** | 3 | |
| **Pancreatic** | 5 | |
| **Lung** | 3 | |
| **Genitourinary tract** | 5 | |
| **Other Cancer** | 2 | |
| **Chemotherapy Treatment** | | |
| **5-Fluoruracil (5-FU)** | 15 | |
| **Cisplatin** | 18 | |
| **Taxame-based** | 10 | |
| **Other CT** | 2 | |

Table 4. Clinical characteristics of the participants in the study

The historic control group consists in a subset of a larger group of healthy subjects assessed with the same method in a previous study conducted parallel to the present work. All the tests were performed by the same operators and in the same Hospital Nutrition Clinic. The 45 patients had a diagnosis of malignant neoplasia (breast, lung, pancreatic, colorectal, liver cancer). All patients performed oral hygiene with mouthwash and bicarbonate 3–4 times/day during chemotherapy. Salivation was not assessed, although none of the patients had mucositis according to the WHO diagnostic criteria. Demographic characteristics were similar in both groups. For each subject, body weight and height were determined.

*Taste sensitivity determination*

Taste sensitivity was evaluated by using the "taste strips" test (Landis BN, J Neurol 2009). Filter paper strips impregnated with a solution containing a tasting substance in four different concentrations for each of the four basic tastes (salty, sweet, sour, bitter) and pure rapeseed oil and deionized water were employed (Table 5). Rapeseed oil is a neutral oil and, unlike many other vegetable oils, it has a pale yellow color; it is almost odorless and tasteless, important aspects for the success of the experiment.

| Stimulus | Substance | Concentration |
|---|---|---|
| Sweet | Sucrose | • 0.05 g/ml<br>• 0.1 g/ml<br>• 0.2 g/ml<br>• 0.4 g/ml |
| Salty | Sodium chloride | • 0.016 g/ml<br>• 0.04 g/ml<br>• 0.1 g/ml<br>• 0.25 g/ml |
| Bitter | Quinine hydrochloride | • 0.0004 g/ml<br>• 0.0009 g/ml<br>• 0.0024 g/ml<br>• 0.006 g/ml |
| Sour | Citric acid | • 0.05 g/ml<br>• 0.09 g/ml<br>• 0.165 g/ml<br>• 0.3 g/ml |
| Fat | Rape oil | Pure |
| Neutral | Deionized water | Pure |

Table 5. Characteristics of taste stimuli

Previous research (Besnard P, Physiol Rev 2015) has shown that humans can detect free fatty acid, specifically oleic acid (C18:1), the most abundant fatty acid in the oil we used (the composition of the oil used was analyzed by our chemistry department). Olive oil has a specific texture and

increased volatility in the oral cavity making it easily recognizable, and we decided to not use it. Since gustatory stimulation also causes the activation of other sensory systems (e.g., touch receptors) (Mattes RD, Am J Clin Nutr 2009), the test should be performed in such a way as to minimize the activation of other receptors: the stimuli were applied just behind the anterior third of the tongue kept out of the mouth and subjects were required to wash their mouth with deionized water between samples to avoid carryover effects. Distilled water was used as solvent and taste solutions were freshly prepared at regular intervals. Umami was not included because the concept of this type of taste is difficult to explain and to understand in Western countries. Subjects were asked not to eat or drink anything other than plain water and not to chew gums or candies at least an hour before the beginning of test. Administration was randomized for each of the four levels of concentration. The enrolled subjects had to identify the taste by choosing from a list that included eight descriptions: “sweet, salty, bitter, sour, water, fat, nothing, I do not know” (forced multiple choice). The test took about 20 minutes.

The current study was performed in adherence to the guidelines of the Declaration of Helsinki as revised in 2001, after the protocol was approved by the Review Board of Università Politecnica delle Marche. Written informed consent was subscribed by all subjects enrolled in the study.

### *Statistical analysis*

Statistical analysis was performed using the SAS statistical package (Statistical Analysis System Institute, Cary, NC). The results were analyzed using appropriate statistical tests (Student’s t test, linear regression analysis, ANOVA) to assess the number of correct recognitions as a function of the stimulus (taste, concentration) and the other physiological (age and gender) and pathological characteristics.

Results are expressed as means ± SD. One-way ANOVA, followed by Tukey’s post hoc test, was used to analyze intergroup differences. Two-way ANOVA was used to analyze the effects of gender, type of stimulation, and presence of disease on taste sensitivity. Differences were considered significant with $p < 0.05$.

### 4.1.2 Results

The main result was a significant difference in taste sensitivity among patients undergoing chemotherapy compared with the historic control group (Figure 12).

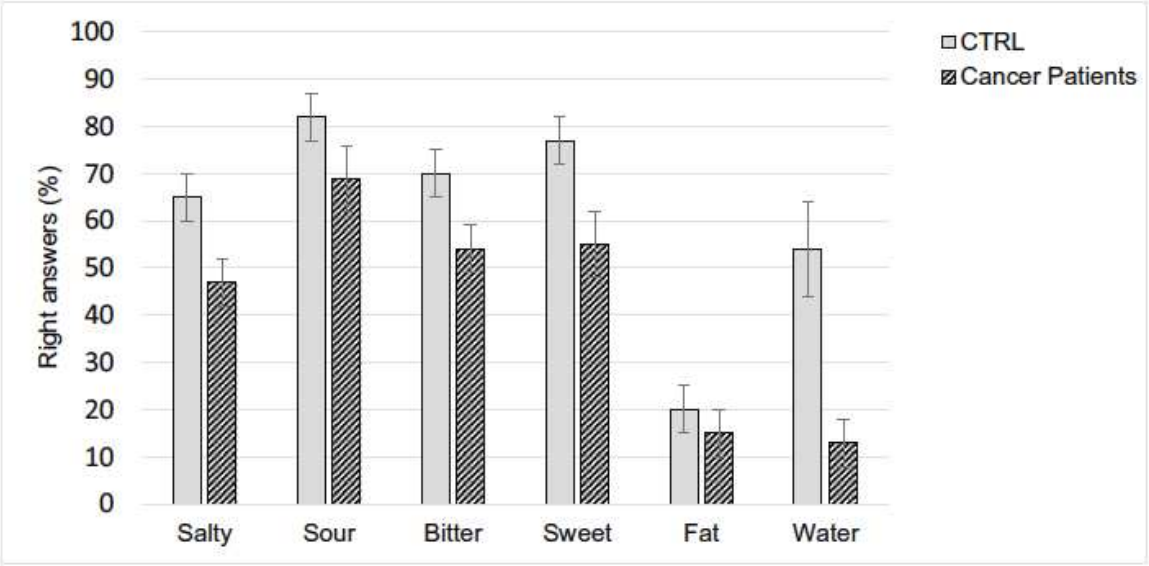


Figure 12. Difference in taste sensitivity between patients undergoing chemotherapy and historic control group

The most interesting and novel finding is the more marked difference for water sensitivity than for other tastes, which is more accentuated in males than in females (compare Figures 13a and 14).


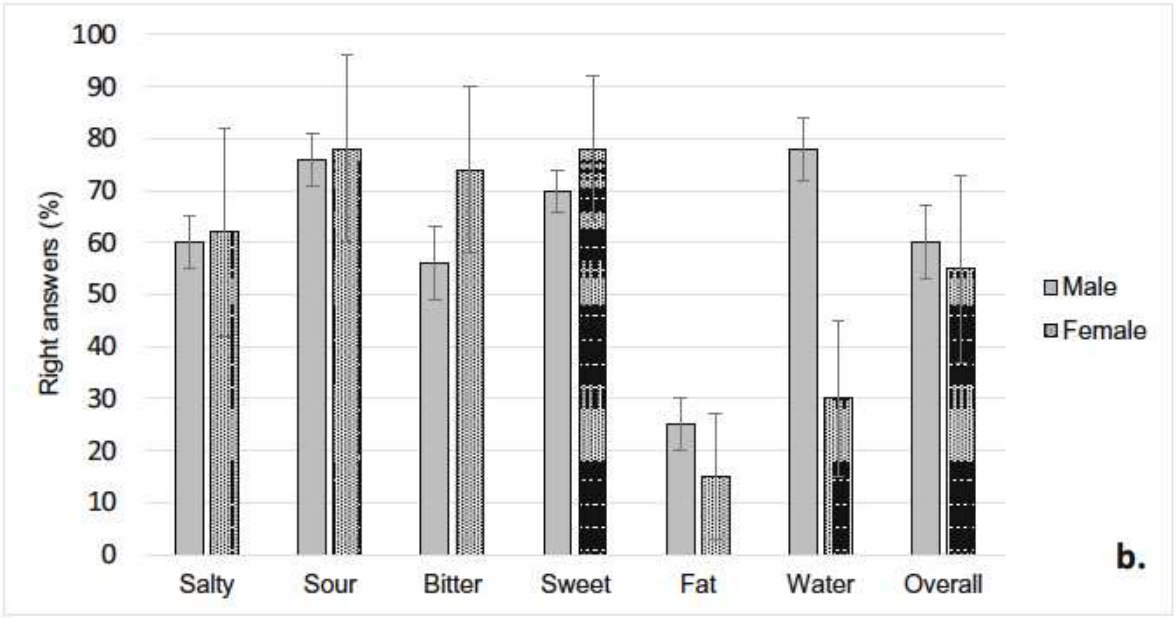


Figure 13. Interaction between type of stimulation and gender.

**a** Significant main effect for type of stimulation, $F(5,186) = 15{,}228$, $p < 0.001$, and no significant main effect for the gender factor $F(1,186) = 1.534$, $p > 0.05$.

**b** Significant interaction between gender and type of stimulation, $F(5,186) = 5.074$, $p < 0.001$

### *Historic controls*

Data from healthy people were collected in a previous research. They were reanalyzed for the present study in order to have a homogeneous way of describing and interpreting the results from controls and patients. The analysis of variance (ANOVA) on taste sensitivity tests performed on control subjects yielded significant variations among different types of stimuli ($F(5,185) = 15.306$, $p < 0.001$). The post hoc Tukey test showed that identification of all 4 basic tastes was significantly better than fat ($p < 0.001$) and water ($p < 0.05$) recognition. A two-way ANOVA was conducted on the percentage of correct answers among controls, with type of stimulation and gender as factors. The two-factor analysis of variance showed a significant main effect for type of stimulation, ($F(5,186) = 15,228$, $p < 0.001$) and no significant main effect for the gender factor ($F(1,186) = 1.534$, $p > 0.05$). Significant interaction between gender and type of stimulation ($F(5,186) = 5.074$, $p < 0.001$) was found. Females showed better taste sensitivity than males in relation to four basic tastes (salty, sweet, sour, bitter) while, on the contrary, perception of fat and water was better in males than in females, suggesting an interaction effect between taste perception and gender (Figure 13).

### *Patients*

The analysis of variance (ANOVA) on taste sensitivity tests performed on oncologic patients yielded significant variations between different types of stimuli ($F(5,264) = 24.656$, $p < 0.0001$). The post hoc Tukey test showed a better and significant sour taste perception in oncologic patients, compared with salty perception ($p < 0.001$) and not significant compared with bitter and sweet tastes perception. Identification of all four basic tastes was significantly better than fat ($p < 0.001$) and water ($p < 0.001$) recognition (Figure 12). A two-way ANOVA was conducted on percentage of correct answers among patients, with type of stimulation and gender as factors. The two-factor analysis of variance showed a significant main effect for type of stimulation ($F(5,258) = 23.211$, $p < 0.001$) and a significant main effect for the gender factor ($F(1,258) = 7.004$, $p < 0.01$). Interaction between gender and type of stimulation ($F(5,258) = 0.228$) was not significant.

Females showed better taste sensitivity than males in relation to four basic tastes (salty, sweet, sour, bitter) and also to fat and water, suggesting absence of interaction effect between taste perception and gender (Figure 14).


Figure 14. A two-way ANOVA conducted on percentage of correct answers among patients, with type of stimulation and gender

A simple linear regression analysis was calculated to predict taste sensitivity based on age. A significant regression equation was found ($F(1,43) = 48.225$, $p < 0.001$) with an $R2$ of 0.529 ($R = -0.727$) (Figure 15).

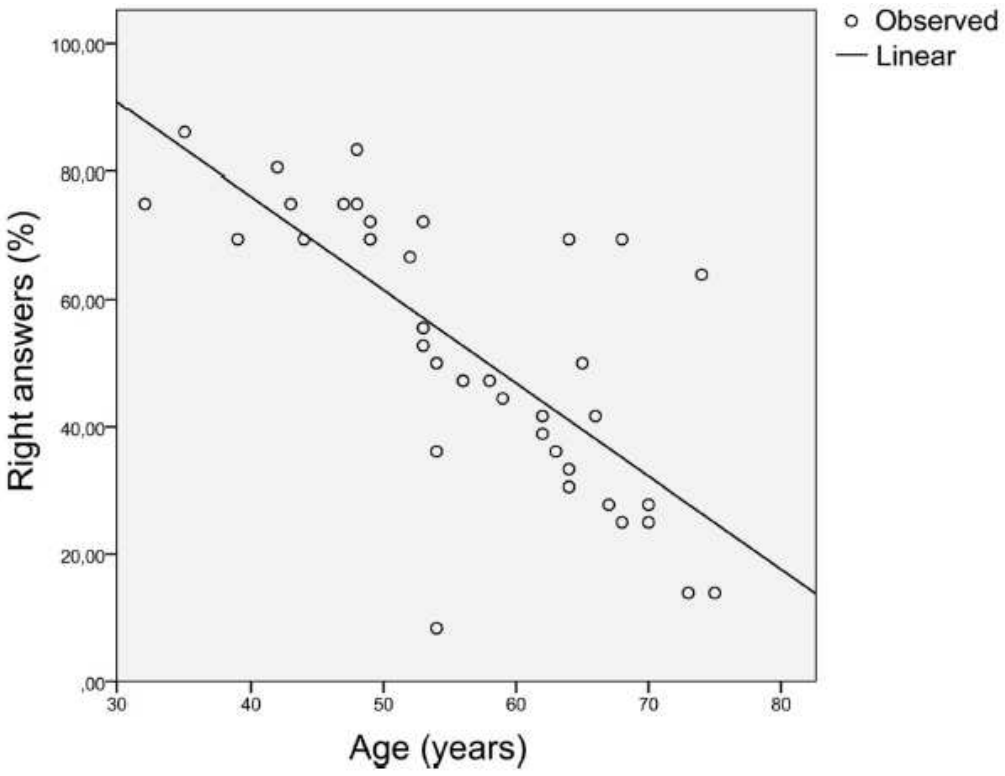


Figure 15. Correlation age–taste sensitivity: a simple linear regression calculated to predict taste sensitivity based on age in patients F (1,43) = 48.225, p < 0.001; R = −0.727

### *Historic controls and patients*

A two-way ANOVA analysis was conducted on the percentage of correct answers, with type of stimulation, subject's group (cases, controls), and gender as factors. The two factor analysis of variance showed a significant main effect for both the group factor (F (1,432) = 49,246; p < 0.001) and type of stimulation (F (5,432) = 33,699; p < 0.001), and no significant main effect for the gender factor (F (1,1) = 0.616; p > 0.05). Significant interactions between group and type of stimulation (F (5,432) = 3119; p < 0.01) and group and gender (F (1,432) = 7033; p < 0.01) were found. Cumulative interaction between groups, type of stimulation, and gender was significant (F (5,432) = 2650; p < 0.05). At variance with control group (where males and females showed similar taste sensitivity), taste perception in the patient group was better in females than in males, suggesting interaction effect between group and gender (Figure 14).

### 4.1.3 Discussion

In the present study, a simple and inexpensive method is described which has been used for the evaluation of taste sensitivity, with fat and water sensitivity analysis as well. A significant difference in taste sensitivity between patients undergoing chemotherapy compared with a historic control group was found, thus confirming the studies of patients with cancer-reported abnormalities in taste recognition (Nakazato Y, J Neurol Neurosurg Psychiatry, 2006; Schiffman SS, N Engl J Med 1983). However, up to date, no data are available on sensitivity alterations in chemotherapy-treated cancer patients to fat and water, recently described as basic tastes (Besnard P, Physiol Rev 2016; Breslin PA, Curr Biol 2008; Zocchi D, Nat Neurosci 2017).

In patients, a better and significant sour taste sensitivity in comparison with salty was observed, and not significant in comparison with bitter and sweet taste. In both control and patient groups, the identification of all four basic tastes was significantly better than fat and water. Specifically, fat stimuli were less strongly perceived by both groups. Patients feeling metallic taste often reported that they were annoyed by fatty foods— especially animal proteins sources, such as red meat—but this relationship is still not well interpreted (IJpma I, Nutr Cancer 2017). Moreover, a reduced response profile for salty stimuli in cancer patients compared to controls was found, while fewer differences between the two groups for sour stimuli were detected. Taste alterations in cancer patient population, compared with those in controls, were analyzed also in relation to age and gender. An interaction effect between group and gender was found. In controls, males did not show a significantly better taste sensitivity than females; taste perception in patients was generally better in females than in males. Many studies indicate that taste changes are more prevalent in females compared with those in males (Zabernigg A, Oncologist 2010; Ackerman BH, Pharmacotherapy 1997; Bernhardson BM, Support Care Cancer 2008), while other papers found no gender difference (Brisbois TD, J Pain Symptom Manag 2011). IJpma's research group (IJpma I, Nutr Cancer 2017) showed that females perceived metallic taste more than men. This sensation of metallic taste seems to be related with bitter taste, as supported by previous observations (IJpma I, Clin Nutr 2017). Even in the present study, we realized how common this experience was in patients undergoing chemotherapy but we did not observe gender differences. A previous paper on taste and smell alterations in patients with lung cancer showed that females more often report stronger sensations than males (McGreevy J, Support Care Cancer 2014). Although the reasons of gender differences in

taste alterations are still unknown, we can speculate that women's cognitive or emotional difference plays a role in sensorial behavior. However, as a general rule, females have greater taste and olfactory sensitivity than men (Doty RL, Physiol Behav 2009; Soter A, Laryngoscope 2008).

Our results demonstrated a more pronounced reduction of overall taste sensitivity related to age increase in patients than in controls. These results could be explained by a long-term impairment in cognitive function and brain activity alterations in regions involved with executive function (i.e., dorsolateral prefrontal cortex) and memory encoding (i.e., hippocampal regions) provoked by chemotherapy, as previously reported (de Ruiter MB, Hum Brain Mapp 2011). This may result in lower attention capacities and underlie the diffuse pattern of cognitive dysfunction observed in these patients, thus contributing to the progression of dysgeusia in older chemotherapy patients.

The more interesting and novel finding is an apparent marked reduction of water sensitivity, more than for other tastants, and more pronounced in men than in women. Since no data are available on changes in water recognition in chemotherapy-treated cancer patients, by also analyzing the wrong responses, we can only speculate that water is often felt to be bitter by cancer patients. Thus, rather than a reduction of water sensitivity, we could be in the presence of an enhanced bitter sensitivity. More research is needed to clarify the mechanisms of water taste (Zocchi D, Nat Neurosci 2017). In the future,it could be evaluated if a reduction of water sensitivity in cancer patients could influence the hydration state of patients. During cancer treatment, it is very important that patients are well hydrated (Price KA, Curr Opin Support Palliat Care 2010).

Thus, we would have a personalized therapy of dysgeusia resulting in an increased protein-energy intake and reduced malnutrition associated with the disease. Considerable importance will also be placed on palatability of foods, including not only the taste but also the color, the smell, and the combinations that best fit the individual patient. Sometimes cancer patients have difficulty chewing and swallowing and tend to prefer liquid preparations; other times, they suffer from nausea and prefer powder preparations.

Dysgeusia can be fought by means of prevention of malnutrition, combating the devastating effects of anticancer therapy and thus allowing to maintain a good nutritional status and

therefore a good QoL. Several limitations need to be addressed. First, the study population is heterogenous with respect to the types of cancer and the nature of chemotherapy regimens.

Moreover, poor oral conditions, e.g., disorders of salivation, as well as chemotherapy-related mucositis, could significantly impact on the feasibility of this method. For this reason, prior to the

application of this method in studies assessing dysgeusia as an endpoint, particular attention should be devoted to these conditions. With regard to our study, oral conditions of the patients were not assessed in-depth, although none of the subjects had mucositis according to the WHO diagnostic criteria. However, establishing associations between the degree of dysgeusia and various types or cancer and/or chemotherapy goes beyond the purpose of our study, which is mainly aimed at presenting an inexpensive, non-invasive, and objective method to assess dysgeusia in this particular category of patients. Secondly, the effects of patients' diet modification on malnutrition prevention and eventually also on taste sensitivity have not yet been studied. Finally, it would be advisable/advantageous, whenever possible, to test the patients before, during and after the cancer therapy treatment.

## 4.2 STUDY II

The purpose of this study was to analyze the taste sensitivity alterations in two types of cancer patients: gastro-intestinal cancer patients (in particular, bile tract and pancreas), and breast cancer patients. We wanted to study the patients in two moments: at the first cycle of chemotherapy (time 0) and after 2 months (time 1).

We also wanted to investigate whether there was a correlation between taste alteration, qualitative and quantitative diet modification and malnutrition.

The assessment of each patients' nutritional status was carried out through bioelectrical impedance analysis (BIA), anthropometry and the questionnaire on eating habits and lifestyle.

Finally, supported by the increasing importance that the professional figure of the nutritionist is acquiring in the field of oncology, we set out to implement in practice and subsequently to outline, the active role of the nutritionist in the multidisciplinary treatment of patients (de van der Schueren MAE, et al., Ann Oncol 2018). In this regard, it is necessary to take into account how proper nutrition plays a primary role in the overall treatment of cancer patients. As taste perception is closely related to the choice of foods introduced including quality and quantity, to the energy intake and substances necessary to maintain the electrolyte and acid-base balance and through the components of pleasantness and more generally, the quality of life and tone of mood, it could

interact significantly with the other factors mentioned above (K. Drareni, et al., Seminars in Oncology 2019).

To better investigate the correlation between oncological disease and malnutrition we added the umami taste, as it is considered a positive "signal" for our health because it is found in foods rich in proteins (even in the vegetable kingdom there are products rich in glutamate, including mushrooms, peas, corn and tomatoes and also some fruits), indispensable for our body and especially for cancer patients who risk malnutrition and sarcopenia. It is also thought that umami can help in cases of malnutrition and weight loss of cancer patients, and it is identified as new way to facilitate healthy eating without decreasing satisfaction with a meal (Federation of European Nutrition Societies).

### 4.2.1 Participants and methods

#### *Study population*

Our study was carried out on 32 cancer patients undergoing chemotherapy (9 males and 23 females) whose characteristics are summarized in Table 6.
Median age of admission was 60 years, with age range from 41 years to 78 years.
We divided the population in two groups based on cancer type:

- 17 ga cancer patients (17 females), after surgery, received adjuvant chemotherapy, not metastatic
- 15 gastrointestinal cancer patients (9 males and 6 females) with bile duct, stomach, colorectal, and pancreas, metastatic and/or locally advanced cancer who received chemotherapy.

No upper or lower age limits were established when entering the study.
For the gastrointestinal cancer group clinical stratification factors were sex (M vs F) and also metastatic disease vs non-metastatic disease, primary cancer site (stomach vs colon-rectal vs pancreas/bile ducts) and the type of treatment received (platinum vs non-platinum)

| **Cancer Patients** | **Gastrointestinal (N=15)** | **Breast (N=17)** |
|---|---|---|
| **Age (years), Mean (SD)** | 61.5 ± 11.5 | 58.8 ± 10.9 |
| **Sex, *No. (%)*** | | |
| **Males** | 8 (57%) | 0 (0%) |
| **Females** | 6 (43%) | 17 (100%) |
| **Body mass index (kg/m²)** | 26.0 ± 4.7 | 24.1 ± 5.7 |
| - **Underweight (< 18.9), *No.*** | 0 | 0 |
| - **Normal weight (19-24.9), *No.*** | 8 | 11 |
| - **Overweight (25-29.9), *No.*** | 3 | 5 |
| - **Obesity (>30), *No.*** | 4 | 1 |
| **Cancer site** | | |
| **Bile duct** | 2 | |
| **Colon-rectal** | 6 | |
| **Pancreatic** | 3 | |
| **Stomach** | 4 | |
| **Metastasis presence, *No.*** | 7 | 0 |

Table 6. Clinical characteristics of the participants in the study

All patients were tested on taste sensitivity.

BIA was used to estimate fat mass relative to lean body mass and total body water.

The questionnaire on eating habits and lifestyle, subsequently described, was admitted in two consecutive times (as for BIA analysis, anthropometry assessment and taste sensitivity test):

- Time 0, which was the same day as the start of chemotherapy
- Time 1, two months after time 0

All the tests were performed by the same operators and in the same Hospital Oncological Clinic. The 32 patients had a diagnosis of malignant neoplasia (breast or gastrointestinal cancer). All patients performed oral hygiene with mouthwash and bicarbonate 3–4 times/day during chemotherapy. Salivation was not assessed, although none of the patients had mucositis according to the WHO diagnostic criteria. Demographic characteristics were similar in both groups. For each subject, body weight, height, BMI, circumferences of waist and arm were determined (Table 6).

### *Questionnaire lifestyle and eating habits*

Each patient responded to a questionnaire to assess lifestyle and eating habits.
We administered two different questionnaires at time 0 and time 1. In particular, at time 1, the questionnaire asked if there had been any changes in diet and lifestyle compared to time 0 and the motivation of changes.
Both of them include two sections, the first of which allowed the collection of general information, while the second investigated the frequency of consumption for food groups (attachments 1 and 2). General information included age, gender, work activity, tumor description, smoking habits, type and frequency of physical activity.
In the second section, we asked how often foods, belonging to different groups, were eaten in a week: cereals and derivatives, cereal products, white meats, red meats, cured meats, fish, dairy products, fresh fruit, vegetables, legumes, eggs. Finally, a 24-h recall was reported.

### *Nutritional status assessment*

The assessment of the nutritional status was carried out by collecting parameters such as:

- Height (kilograms)
- Weight (meters)
- Body Mass Index, BMI: the measurement of body fat based on height and weight. It is calculated as (weight in kilograms)/(height in metres)$^2$.
- Waist and arm circumferences (centimeters)

- BIA (Bioelectrical Impedance Analysis)

BIA is based on the principle that different body tissues express a specific electrical conductivity. In BIA, a weak electric current flow through the body and the voltage is measured in order to calculate impedance (resistance) of the body. Most of human body water is stored in muscle. Therefore, if a body structure is more muscular there is also more body water, which leads to lower impedance. The resistance (Z) is measured by the instrument and depends on resistance (Rz) and reactance (Xc) according to the following equation:

$$Z = \sqrt{(Rz^2 + Xc^2)}$$

In the healthy human body, Rz contributes as much as 98% to Z, while Xc accounts for 2%. The Rz is high for fatty tissue and bone and low for water, which is an excellent conductor of electrical current; it is therefore inversely proportional to the amount of bodily fluids. Rz increases as the fat mass increases and body water decreases. Cells function like capacitors that accumulate and deplete the current: from them, and more precisely from the active cell mass ATM or Body Cellular Mass BCM, depends on the Xc.
BIA is a tri-compartmental model technique in that it identifies body fat mass (FM) and non-fat body mass (FFM), the latter further divided into extracellular body mass (ECM) and metabolically active tissue (ATM) or cellular body mass (BCM).
Among the parameters detected, we therefore have:

- FFM (free fat mass): consists of muscle mass, bone mass, body minerals and other non-fat tissues. It contains approximately 73% water, 20% protein, 7% minerals. It is divided into active tissue mass (ATM) and extracellular mass (ECM).
- ATM (active tissue mass) or BMC (cellular mass): constitutes the metabolically active tissue of the body such as organs, muscles and blood cells. Each person has a minimum BCM under which it is not recommended to go because it causes a decrease in both lean muscle mass and metabolism: it is an important indicator of a possible state of malnutrition.
- FM (fat mass): consists of external fatty tissues, identified as subcutaneous fat, and internal fatty tissues, identified as visceral fat.

- TBW (total body water: In a healthy adult it makes up about 60% of body mass, while the value is higher in children and lower in women and obese, due to the higher fat content. It is divided into two compartments: intracellular water and extracellular water.
- ICW (or ICF, intracellular water): consists of the set of intracellular fluids.
- ECW (or ECF, extracellular water): represents the volume of extracellular biological fluids, including liquor, plasma, saliva, lymphatic fluid, eye fluids and digestive juices.

Another important parameter is Phase Angle, a linear method for measuring the ratio of Rz to Xc detected by the BIA: it can be considered an excellent indicator of physical state and cellular integrity. In a body consisting only of limbs without fluids, the angle will be 90 degrees; if the body has an equal proportion between Rz and Xc you will have a 45-degree angle. In a healthy subject this value is between 6 and 7 degrees. Values below 5 degrees indicate a rupture of cell membranes or a build-up of extracellular fluids (water retention). A value less than 4 degrees is associated with a low Xc and is an important malnutrition index. Values above 10 degrees indicate strong dehydration or BCM above the norm, as in the case of professional sportsmen.
To perform the analysis, the subject must lie on a bed in a supine position, not in contact with metal surfaces, and wait a few minutes to allow the redistribution and stabilization of fluids. The legs are spread apart to form a 45-degree angle and the arms are abated, to form a 30-degree angle to the trunk. These angles may vary depending on the instrumentation. The parameters are measured by placing two pairs of electrodes (tetra-polar hand-foot technique), one on the back of the right hand by connecting the red tweezers in the distal position and the black ones in the next position. The other pair of electrodes is on the back of the right foot by connecting the red tweezers in the distal position and the black tweezers in the next position. Each pair of electrodes is 5 cm apart. The electrodes are connected, through clamps, to the measuring instrument and is turned on (Figure 16).

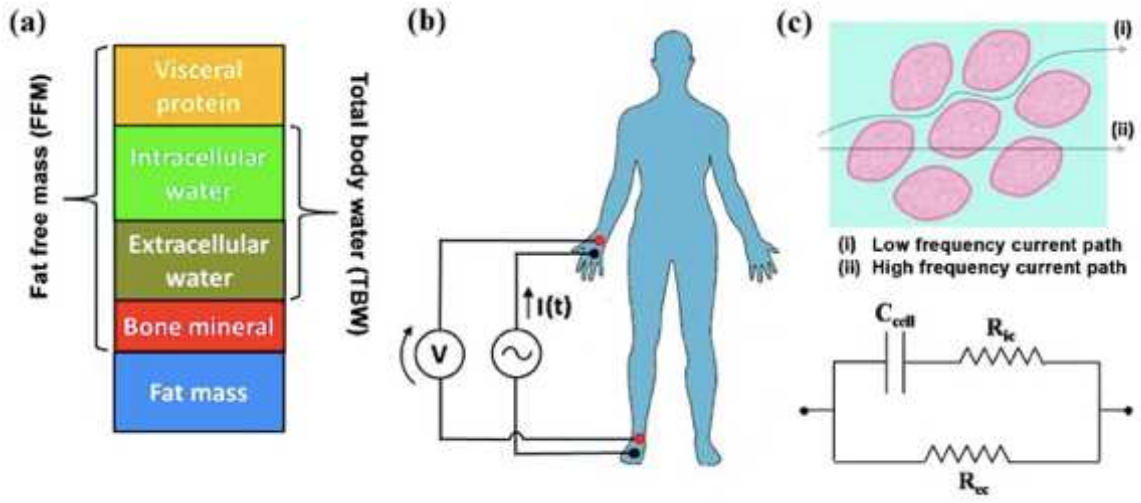


Figure 16. (a) The different compartments of the human body; (b) a typical four-electrode configuration for BIA measurements; (c) equivalent electrical circuit used to interpret measured data in BIA (Marco Grossi and Bruno Riccò, Electrical impedance spectroscopy (EIS) for biological analysis and food characterization: A review. 2017 DOI: 10.5194/jsss-6-303).

In our study, the tool used was the Impedimed DF 50 analyzer. It passes an alternating current, of very low intensity (400-800 -A) and high frequency (50 KHz) through the electrodes which, traveling along the body, will encounter different resistances depending on the composition of the various body districts: the instrument records the speed and its modifications and then directly detects the values of Rz and Xc, expressed in Ohm, calculating the Z of the tissues crossed. The Rz and Xc values are inserted into a special program and processed to obtain the graph called Biavector. Through the graph it is possible to assess the nutritional status of the subject, comparing the measured values with the reference values and to decide on any interventions of dietary therapy.

### *Taste sensitivity determination with umami*

The test used is the same as described earlier in *Taste sensitivity determination* in the Study I chapter.

In Study II we wanted to include the umami taste given its importance in protein intake and therefore malnutrition. For the umami taste, the concentrations (g/ml) used were as follows:

Monosodium Glutamate: 0.016, 0.04, 0.1, 0.25. For the other tastes, the concentrations reported in Table 4 were used.

The list in study II includes 9 descriptions (sweet, salty, bitter, umami, sour, water, fat, nothing, I don't know; forced multiple choice). Scores for each taste range from 0 to 4 and for water and fat from 0 to 1. A total taste score was derived by summing the scores of each taste and ranged from 0 to 20 for sweet, salty, bitter, umami and sour.

### *Statistical analysis*

Statistical analysis was performed using R Statistical Software version 3.6.1. The results were analyzed using appropriate statistical tests (Student's t test) to assess the difference of collected data at each time point, T0 and T1 (weight, BMI, waist and arm circumferences and taste sensitivity test score). Differences were considered significant with $p < 0.05$.

In order to identify patients who expressed taste modifications, we calculated the ratio in the minimal perceived concentration of the molecule used to detect that particular taste between the baseline (T1) and after therapy (T0). All patients who had a decrease in the minimal perceived concentration of the molecule were considered to be at increased sensitivity. All patients who had an increase in the minimal perceived concentration of the molecule were considered to have decreased sensitivity. All other instances were found to be invariant. All patients who were not able to discriminate a particular taste in both samplings were not considered in the taste analysis.

The association between categorical variables was estimated by Fisher exact test for binomial variables and by chi-square test for all other instances. Association between numerical variables was estimated by Mann-Whitney test.

For all analyses conducted, level of statistical significance $\alpha$ was set at 0.05.

### 4.2.2 Results

#### *Characteristics of the study population*

Patients with breast cancer who received adjuvant chemotherapy were consecutively enrolled. No upper or lower bounds for age at study entry were set.

Patients with either metastatic or locally advanced gastric/colorectal/pancreatic/bile duct cancer who received chemotherapy were consecutively enrolled. No upper or lower bounds for age at study entry were set. Clinical stratification factors were sex (M vs F), age (<70 or >70 years old at the time of study entry), metastatic vs not-metastatic disease, site of primary tumor (gastric vs colorectal vs pancreas/bile ducts), type of treatment received (platinum-based vs not).

All patients signed a written informed consent before study entry. Measurements were performed from February 2019 and July 2019. All patients completed all measurements up to T1.

The patients' clinical characteristics at baseline (T0) and after chemotherapy (T1) are summarized in Table 6. No statistical difference was found among different times for all the clinical characteristics (BMI, circumferences and BIA results). Body weight and BMI remained almost unchanged after the start of chemotherapy compared to baseline for all cancer patients. The same applied for the circumferences (see Table 7).

| **Cancer Patients** | **Gastrointestinal (N=15)** | | **Breast (N=17)** | | **All Cancer Patients (N=32)** | |
|---|---|---|---|---|---|---|
| **Age (years), Mean (SD)** | 61.5 ± 11.5 | | 58.8 ± 10.9 | | 60.1 ± 11.0 | |
| **Sex, *No. (%)*** | | | | | | |
| **Males** | 8 (57%) | | 0 (0%) | | 9 (28.1%) | |
| **Females** | 6 (43%) | | 17 (100%) | | 23 (71.9%) | |
| | **T0** | **T1** | **T0** | **T1** | **T0** | **T1** |
| **Body mass index (kg/m²)** | 26.0±4.7 | 25.8±4.7 | 24.2±5.7 | 24.1±5.3 | 25.0±5.3 | 24.9±5.0 |
| - **Underweight (< 18.9), *No.*** | 0 | 0 | 0 | 1 | 0 | 0 |
| - **Normal weight (19-24.9), *No.*** | 8 | 8 | 11 | 9 | 19 | 18 |
| - **Overweight (25-29.9), *No.*** | 3 | 3 | 5 | 6 | 8 | 9 |
| - **Obesity (>30), *No.*** | 4 | 4 | 1 | 1 | 5 | 5 |
| **Range []** | [19.5-34.9] | [19.1-33.1] | [19.1-42.9] | [18.6-40.9] | [19.1-42.9] | [18.6-40.9] |
| | **T0** | **T1** | **T0** | **T1** | **T0** | **T1** |
| **Waist circumference (cm), Mean (SD)** | 93.5±13.0 | 93.8±12.8 | 83.1±15.7 | 83.7±15.3 | 88.0±15.2 | 88.5±14.9 |
| **Range []** | [70.1-119.1] | [70.1-116.1] | [67.1-128.1] | [66.1-125.1] | [67.1-128.1] | [66.1-125.1] |
| **Arm circumference (cm), Mean (SD)** | 28.5±4.0 | 28.7±4.3 | 26.7±3.9 | 26.4±3.6 | 27.6±4.0 | 27.5±4.0 |
| **Range []** | [23.0-35.0] | [22.5-38.0] | [22.3-39.0] | [23.0-37.0] | [22.3-39.0] | [22.5-38.0] |
| **Bioelectrical impedance analysis (BIA)** | **T0** | **T1** | **T0** | **T1** | **T0** | **T1** |
| **Free Fat Mass (%), Mean (SD)** | 72.5±8.3 | 73.8±6.1 | 66.8±9.4 | 67.0±9.5 | 69.5±9.2 | 70.2±8.7 |
| **Fat Mass (%), Mean (SD)** | 27.5±8.3 | 25.8±6.6 | 32.8±9.6 | 35.6±10.5 | 30.3±9.3 | 31.1±10.1 |
| **Extracellular body water (%), Mean (SD)** | 51.9±5.3 | 53.0±8.9 | 48.5±3.5 | 50.1±5.5 | 50.1±4.7 | 51.4±7.3 |

Table 7. Patients' clinical characteristics at time point 0 and at time point 1.

*Body composition*

The total body composition of the patients was comparable among different cancer type groups at baseline (Table 6). The majority of patients were normal weight (59.4%) or overweight (25%) with a slight decrease after start of chemotherapy (from range [19.1-42.9] to range [18.6-40.9]). Breast cancer patients' BMI mean was in normal-weight BMI range but they showed higher mean value of fat mass (T0= 32.8±9.6; t1=35.6±10.5) compared to reference value (range 22-30% for women). Also for gastrointestinal cancer patients we found a higher mean value of fat mass (T0=27.5±8.3; T1=25.8±6.6) compared to the range value declared in international guidelines (range 18-24% for men).

Within breast cancer patients, the percentage of fat mass increased after chemotherapy (T1) compared to baseline (T0) while in the gastrointestinal cancer group the percentage of fat mass decreased (Table 7 and Figure 17).

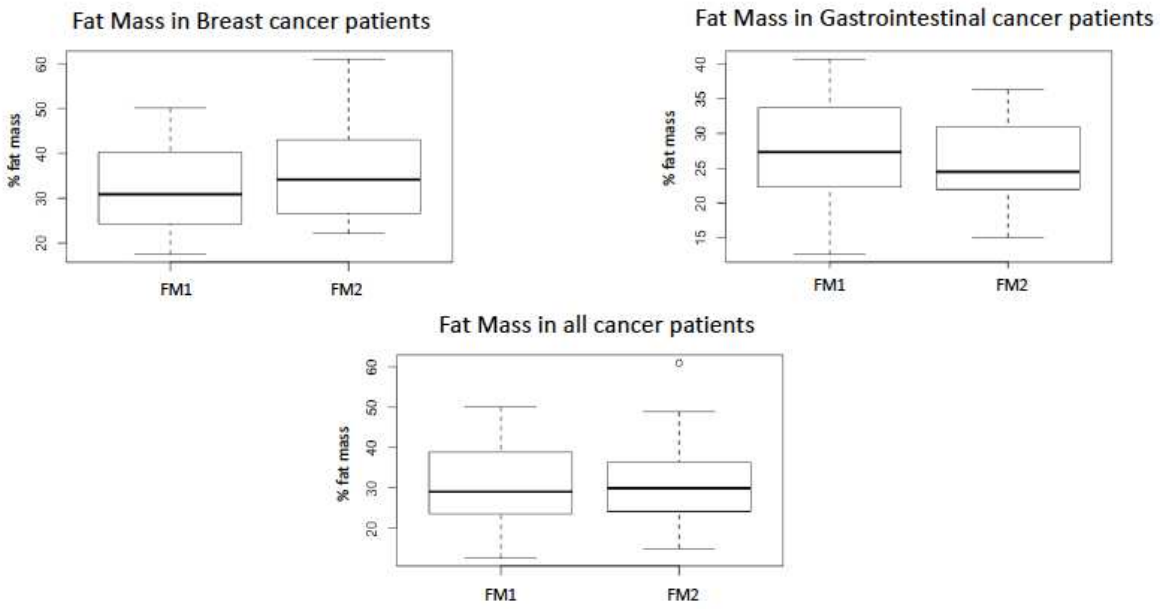


Figure 17. Patients percentage of Fat Mass at time point 0 (FM1) and at time point 1 (FM2).

In the breast cancer patients group the percentage of free fat mass decreased after chemotherapy (T1) compared to baseline (T0) while in the gastrointestinal cancer group the percentage of free fat mass increased (Table 7 and Figure 18).

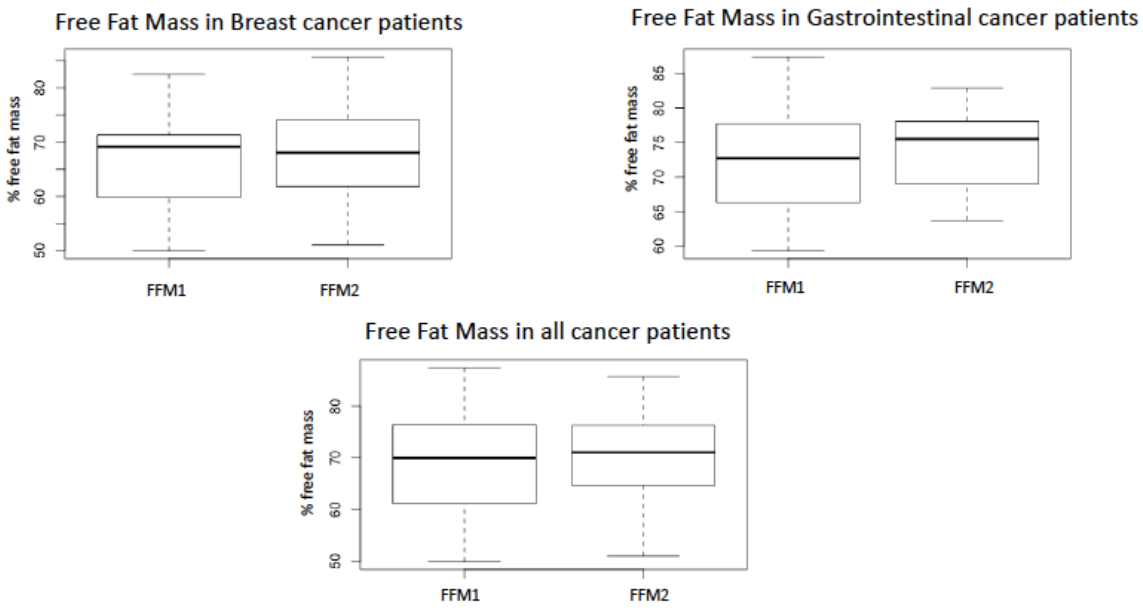


Figure 18. Patients' percentage of Free Fat Mass at time point 0 (FFM1) and at time point 1 (FFM2).

### *Weight and dietary intake*

A majority of patients had a weight change after the start of chemotherapy and weight loss was more common than weight gain. We found that 18.8% of patients analyzed had no change in weight, 28.1% of patients had an increase in body weight while 53.1% had a decrease in weight. The range of variation, both for the increase and decrease in weight, was 1-5 kg compared to the baseline (T0). We compared the change in weight between time 0 and time 1 with the modification of the caloric intake between time 0 and time 1. Patients who lost weight were more likely to report a decrease in appetite after the start of chemotherapy. Gastrointestinal cancer patients reported a decreased frequency of eating and drinking caused by taste changes more commonly than breast cancer patients. All patients reported more difficulties in eating immediately after chemotherapy caused by nausea and changes in taste sensitivity. Within all patients, no consistent differences in dietary intake were found over time. The comparison with the change in caloric intake led to interesting observations: in fact, 75% of patients with an increased weight showed an increase in caloric intake

while 25% of them did not report an increase in intake; in those with a weight gain, 50% also had a decrease in intake, 12.5% had no changes while 37.5% showed an increase in caloric intake despite a decrease in weight. This could be related to the problems that cancer patients (especially for those with gastrointestinal cancer) show, such as malabsorption, vomiting and diarrhea often declared by patients in the questionnaire given to each of them.

We found that at baseline more than after the start of chemotherapy all cancer patients, especially within the breast cancer group, preferred sweet foods. Compared to T0, choice of food rich in animal protein, especially red meat, was lower in all patients at T1. One of the main causes of this food preference change reported by patients was *"cause of the cancer disease".* Within gastrointestinal cancer patients, no substantial differences in food choice were found after chemotherapy compared to baseline, but they all limited intake of food rich in fiber.

### *Taste function*

The test was conducted on the total score of correct answers. Scores for each taste range from 0 to 4, and total taste scores range from 0 to 20, which is the sum of the five basic taste scores (bitter, sour, sweet, salty, umami). Higher scores indicate a better taste function. A low total taste score (less than 11.5 for women and 10.5 for men) was associated with hypogeusia.

We showed that identification of 4 basic tastes (sweet, sour, bitter, and salty) was better than fat and water recognition and was also better than umami. The results were tested for significant differences between time points, T0 and T1, using the Welch Two Sample t-test in R software.

For all patients (N= 32), the taste sensitivity test score for salty taste was significantly higher in T0 (mean ± sd: 2.31±1.12) compared to T1 (mean ± sd: 1.69±1.23; Welch Two Sample t-test, P=0.037). Compared to T0, breast cancer patients had a lower salty taste test score at T1 (P=0.007; Table 8).

| Cancer Patients | Gastrointestinal (N=15) | | Breast (N=17) | | All Cancer Patients (N=32) | |
|---|---|---|---|---|---|---|
| **Taste (Mean ± SD)** | **T0** | **T1** | **T0** | **T1** | **T0** | **T1** |
| - **Salty [0-4]** | 2.00±1.13 | 1.93±1.28 | 2.59±1.06 | 1.47±1.79 | 2.31±1.12 | 1.69±1.23 |
| | | | **P=0.007** | | **P=0.037** | |
| - **Sour [0-4]** | 2.07±1.44 | 2.27±1.44 | 3.23±1.09 | 2.88±1.41 | 2.69±1.38 | 2.59±1.43 |
| - **Bitter [0-4]** | 2.00±1.13 | 2.33±1.59 | 2.06±1.09 | 2.47±1.07 | 2.03±1.09 | 2.41±1.32 |
| - **Sweet [0-4]** | 1.73±1.16 | 1.93± 1.49 | 2.06±1.09 | 1.94±1.43 | 1.91±1.12 | 1.94±1.43 |
| - **Umami [0-4]** | 0.87±1.25 | 0.60±0.83 | 1.00±1.27 | 0.71±0.98 | 0.94±1.24 | 0.66±0.90 |
| **Total basic taste [0-20]** | 9.20±3.74 | 9.07±4.10 | 10.94±3.65 | 9.47±3.45 | 10.12±3.74 | 9.28±3.71 |
| **Taste (Mean ± SD)** | **T0** | **T1** | **T0** | **T1** | **T0** | **T1** |
| - **Fat [0-1]** | 0.13±0.35 | 0.20±0.41 | 0.41±0.51 | 0.29±0.47 | 0.28±0.46 | 0.25±0.44 |
| - **Water [0-1]** | 0.07±0.26 | 0.20±0.41 | 0.12±0.33 | 0.29±0.47 | 0.09±0.30 | 0.25±0.44 |

Table 8. Patients taste test score at time point 0 and at time point 1. P-values display significant differences in taste test score at time point 1 compared to baseline, T0. Only P-values < 0.05 are shown.

Breast cancer patients showed better taste sensitivity (higher taste test score) than gastrointestinal cancer patients in relation to all the tastes, at baseline but also at T1, except for salty taste at T1.

### *Taste sensitivity test results for gastrointestinal cancer patients*

Looking at the variation of the minimal concentration of the required molecule we found that: for sour taste 50% of patients had increased sensitivity to this taste, in 28% of patients it was unchanged, and in 7% of patients had diminished sensitivity. 14.3% of patients was not able to discriminate this taste in both time points.

For sweet taste 35.7% of patients reported increased sensitivity to this taste, 28.6% of patients reported decreased sensitivity, 28.6% of patients had unchanged sensitivity and 1 patient was censored due to not being able to discriminate this taste in both time points.
For salty taste 42.9% of patients reported increased sensitivity to this taste, 14.3% of patients had decreased sensitivity, 35.7% had unchanged sensitivity and 1 patient was censored due to not being able to discriminate this taste in both time points.
For bitter taste 50% of patients reported increased sensitivity, 28.6% of patients reported decreased sensitivity, 21.4% of patients had unchanged sensitivity.
For umami taste 42.9% of patients had decreased sensitivity, 14.3% of patients had increased sensitivity, 14.3% of patients had unchanged sensitivity and 28.6% of patients were censored.
As most of patients enrolled in the analysis were unable to discriminate water and fat, no further comparison was made between T0 and T1.
There was a statistically significant difference in terms of changes in taste sensitivity at the chi-square test between sour vs umami (P value=0.0319) whereas all other comparisons had p values greater than 0.05.

### *Taste sensitivity test results stratified by clinical factors for gastrointestinal cancer patients*

We conducted comparisons between patients who expressed changes in different tastes and their clinical characteristics. Among all analyses that were conducted, in those that were found to be statistically significant, or even if not statistically significant (perhaps due to the small sample size) amenable to further enquiry, there was a statistically significant association with increased sensitivity to bitter taste in patients affected by pancreatic cancer compared with gastric and colorectal cancer (p value=0.0463 and p value= 0.0205) (Figure 19).

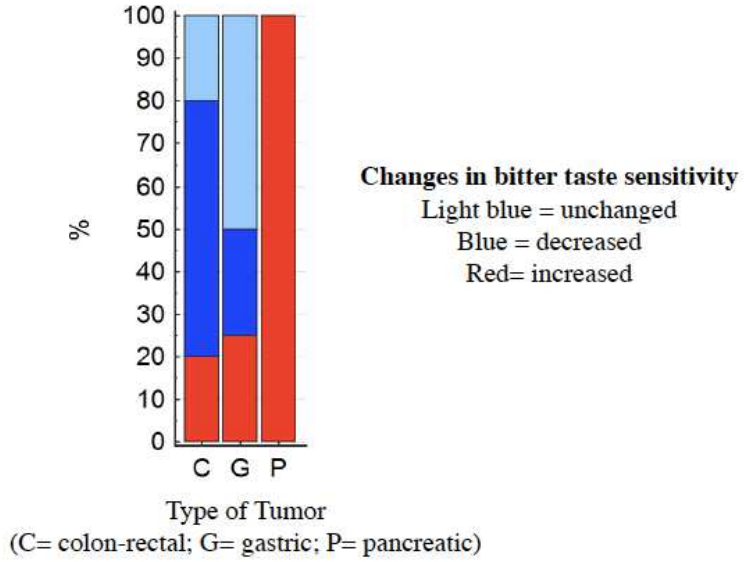


Figure 19. Variations in the perception of bitter taste by type of tumor.

Although not statistically significant, in males vs females there was a decreased sensitivity to sweet taste (p value=0.076) (Figure 20).

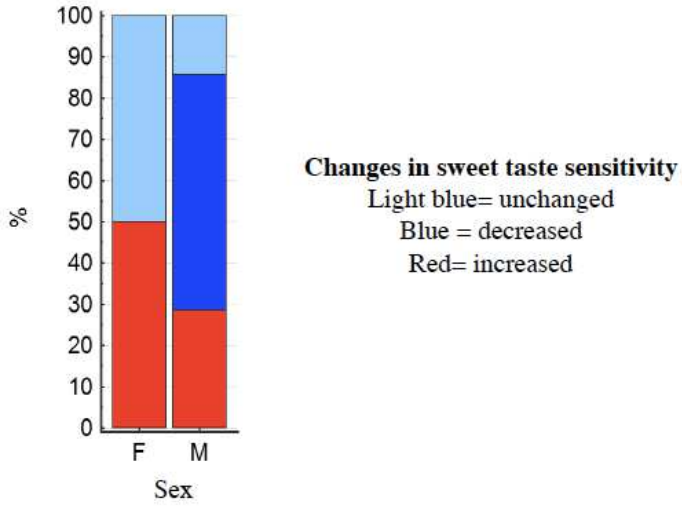


Figure 20. Changes in the perception of sweet taste by sex

In metastatic vs not-metastatic patients there was increased sensitivity to sour taste (p value=0.084). In patients who received platinum-based chemotherapy the taste that was found to be more reduced than other tastes was umami (50%), followed by sweet (37.5%), whereas sour (62.5%) increased in most patients. Due to the small number of patients who received chemotherapy without platinum-based compound, no further comparison was possible.

### *Taste sensitivity test results for breast cancer patients*

Looking at the variation of the perceived minimal concentration of the required molecule we found that: for sour taste 11.8% of patients had increased sensitivity to this taste, in 52.9% it was unchanged, 29.4% of patients had decreased sensitivity and 5.9% of patients were not able to discriminate this taste in both first and second time.

For sweet taste 47% of patients reported increased sensitivity to this taste, 41.2% of patients reported decreased sensitivity and 11.8% of patients had unchanged sensitivity.

For salty taste 5.9% of patients reported increased sensitivity to this taste, 52.9% of patients decreased sensitivity, 35.3% had unchanged sensitivity and 5.9% of patients was not able to discriminate this taste in both first and second time.

For bitter taste 52.9% of patients reported increased sensitivity, 23.5% of patients reported decreased sensitivity, 23.5% of patients unchanged sensitivity.

For umami taste 29.4% of patients had decreased sensitivity, 5.9% of patients had increased sensitivity, 17.6% of patients had unchanged sensitivity and 47.1% of patients were censored.

As most of patients enrolled in the analysis were unable to discriminate water and fat no further comparison was made between T0 and T1.

There was a statistically significant difference in terms of changes in taste at the chi-square test between bitter vs sweet (p=0.0153), salty vs sweet (p=0.0216), bitter vs salty (p=0.0129) and bitter vs sour (p=0.0395). There was a high concordance in changes in sweet/sour taste (58.8%) and in salty/acid (60%).

### 4.2.3 Discussion

In recent years, research has become increasingly interested in taste sensitivity; this sensory capacity can in fact face numerous alterations, both under physiological and pathological conditions, linked to taste modification with increased mortality in individuals affected by its modification (Devanand DP., The American Journal of Geriatric Psychiatry, 2016). The sensitivity of taste determines the choice of foods that, in turn, affect the nutritional status and body composition of the subject. The alteration of taste sensitivity can cause a change in eating behaviors (Garcia-Bailo B., OMICS J. Integr. Biol. 2009). This also affects the social life of individuals as food is an important mediator of interpersonal relationships.

We evaluated two groups of cancer patients: patients treated with chemotherapy for malignant tumors of the gastro-intestinal tract and patients treated with adjuvant chemotherapy for breast cancer. Among all tastes that were sampled, the taste that all patients were able to discriminate at baseline was bitter whereas the one that was more difficult to identify properly was umami.

After treatment, the tastes that experienced the most relevant changes were bitter and sour tastes (that increased in most patients), sweet taste (that decreased in most patients) and umami (which was the one that decreased the most in the majority of patients). In particular we found that there was a statistically significant correlation between loss of umami perception and increased sensitivity to sour.

In gastrointestinal cancer patients we found some interesting correlations between site of disease involvement (pancreatic-bile ducts vs stomach/colorectal) for bitter taste perception. This might be due to the primary organ involved and suggests that, although patients with gastric and colorectal cancer should have more intestinal problems (due to the concomitant obstruction/impaired function of the gastrointestinal tube caused by the tumor), they are actually at less risk of bitter taste compared to patients who have some kind of bile-ducts/pancreatic involvement.

Strangely enough we were not able to demonstrate a higher risk of changes in taste in patients who received platinum-based chemotherapy vs those who did not receive these drugs. This type of drugs is usually well-known as one of its relevant side effects is chemotherapy associated nausea in treated patients, but our analysis did not find any relevant association with changes in taste, albeit both sour and bitter taste were the ones that changed the most in this population. We think that it

is necessary to assess a greater number of patients (also including a higher number of patients who do not receive platinum-based chemotherapy).
In breast cancer patients, the tastes that almost patients were able to discriminate at baseline were salty and sour whereas the one that was more difficult to identify properly was umami.
As for the assessment of body composition, we found that most patients were at the upper limit of the normo-weight range for BMI. In reality, however, both groups, especially those of patients with breast cancer, had a higher than normal body fat percentage.
As obesity is a risk factor for the onset of cancer diseases and has a direct link to the size of waist circumference, we considered it appropriate to consider this criterion/factor. The detection of the change in the circumference of the arm between time 0 and time 1 did not yield significant results, as measuring the tricipital plica was found not to be useful in assessing the risk of malnutrition in patients.
To limit the problems related to changes in weight and energy intake described above, a key tool is the screening of malnutrition; in our study we used the MUST, which patients were subjected to undergo when entering the study and at T1.

Comparing patients with gastro-intestinal cancer and breast cancer patients, we found some differences and affinities.
In terms of sensitivity to bitter taste, it increased in both pathologies in more than 50% of the patients analyzed. Taking into account the sweet taste, it is necessary to make a gender distinction with regard to the gastro-intestinal tract since, in men compared to women, there was a decrease in the sensitivity of this taste. In breast cancer it was not possible to carry out this stratification due to gender homogeneity; however, comparisons between women with gastro-intestinal tract cancer and breast cancer were found to show an increase in sensitivity to sweet taste in both groups.
Sensitivity to sour taste increased in gastrointestinal cancer patients, while in patients with breast cancer it decreased. In both groups the umami taste decreased, although the difference is not statistically significant. We noticed that at T1 umami was often confused with salty. At the same time, we noticed from food frequency questionnaires that most patients reported decreased meat consumption, and in general protein consumption, compared to T0. It would be interesting to evaluate a larger number of patients to understand whether this decrease in sensitivity to umami taste can be linked to a decrease in protein intake.

The biggest difference found is sensitivity to salty taste, which statistically decreased in all patients but particularly in the group of breast cancer patients. In the group of patients with gastrointestinal cancer we did not find a significant difference in the perception of salty taste, not even in the female gender group (p-value=0.804), so we can say that it does not appear to be related to gender.

In reference to the nutritional status of the two groups of patients and from the analysis of BMI at time 0, we noticed that half of the subjects were normal.

An interesting difference is the prevalence of overweight and obesity in the two groups; In fact, in women with breast cancer the percentage of overweight (29.4%) was greater than that of obesity (6%), confirming that clinicians' attention must also be extended to mild forms of overweight, which already expose the individual to the risk of cancer. This did not occur in patients with gastrointestinal tract cancer, where we found an almost fair distribution of subjects in the two weight classes (20% overweight and 26.7% obese). We can therefore confirm the association between being overweight, obese and at risk of developing many types of cancer, especially those examined by us.

This helps to corroborate what has just been said about waist circumference detection at time 0, used to stratify patients based on the risk of the onset of chronic diseases. We found that waist circumferences of all patients were greater than 94 cm in men or greater than 80 cm in women, so higher than current guideline threshold for increased metabolic risk.

We found gender differences in weight values in gastrointestinal cancer patients and comparing them with those of women with breast cancer, it emerged that they mirrored the above BMI weight classes. With regard to the effects of therapy on the variation of body weight, very similar percentages in the two groups were found: in fact, weight loss prevails in both samples (42% in women with breast cancer and 57% in patients with tract cancers followed by a fair percentage of both patients who had weight gain (29%), those in which the latter remained unchanged (29% in women with breast cancer and 14% in patients with gastro-intestinal tract tumors).

This is linked to the importance of screening for malnutrition in all patients but especially in cancer patients, who have a higher risk than the general population.

Comparing our data, we found a further difference between the two sample populations: women with breast cancer were at lower risk of malnutrition at baseline (17.6% in middle risk and no one in high risk classes) than those with gastrointestinal tumor, who showed higher percentages in the middle- and high-risk classes (33.3% in middle risk and 6.7% in high risk classes).

Despite of this, we found that by comparing the results of the MUST questionnaire between T0 and T1, the group of patients with gastrointestinal cancer was not shown to change the risk status for malnutrition (33% of patients at middle risk and 6.7% of patients with high risk, at both T0 and T1). In breast cancer patients, on the other hand, we found a worsening of the MUST score between T0 and T1: from 17.6% of patients with middle risk (score 1) to 35.3% post chemotherapy treatment.
Assessment of the variation in patients' waist circumference between time 0 and time 1 showed that 35.5% had maintained the same measurement, in 28% of patients it had increased while in 35.5% it had fallen.
To fully understand the mechanisms that lead to the alteration of taste and, above all, to identify, as far as possible, the main variations, could be useful for the design of an adequate nutritional therapy, which responds to the many needs of people with cancer. To clarify this topic, further studies are needed on larger patient populations, possibly differentiated for both the type of cancer and the chemotherapy treatment used.

# 5. CONCLUSIONS

The sensory properties of food, including taste, play an important role in food preference and intake. Recently studies explored taste function and perception in order to enquire if modifications of taste sensitivity can be associated with changes in dietary intake (Y.C. de Vriesa, Food Quality and Preference, 2018; IJpma I, Clin Nutr, 2017; Sze-Yen Tan and Robin M. Tucker, Nutrients, 2019).

Each taste quality is associated with specific nutrients that are important to health and well-being: sweet taste widely thought to help detect sources of carbohydrate, sour taste is related to the presence of vitamins, salty taste controls the steady state of essential electrolytes, and umami is a marker of protein (which is made up of amino acids, which are essential for life) and bitter taste helps as a warning against potentially dangerous compounds (Paul A.S. Breslin. Current Biology, 2013). If these purported functions are accurate, then positive associations between taste function modifications and food preference and related nutrient intake should exist. Taste sensitivity changes affect the patient's daily life, both from a physical point of view, leading to alterations in nutritional status to malnutrition, and psychologically, leading to problems of social interaction and, summarily, to a decrease in the quality of life.

In study I we described a simple testing method which allowed to demonstrate a significant difference in taste sensitivity in patients undergoing chemotherapy in line with previous similar studies (IJpma I, Clin Nutr 2017; Cohen J, Curr Pharm Des 2016).

In study II we evaluated a homogenous patient population regarding types of cancer and treatment and, using simple tools such as the eating habits questionnaire, we demonstrated interesting differences regarding dysgeusia and its link to the nutritional status of patients.

With this thesis I wanted to highlight the importance that the nutritionist has throughout the whole nutritional process, in such a complex and different area such as oncology, starting first of all from the screening, mentioned in all the world guidelines but, in fact, little used in clinical practice.

Since alterations in taste sensitivity influence food preferences and appetite, an evaluation of taste sensitivity could be useful to modify diet composition in such a way as to guarantee the necessary nutritional intake. More research is needed to clarify the character, frequency, and duration of taste modifications and their healthy implications, and the correlations between taste alterations, food consumption, and malnutrition in cancer patients undergoing chemotherapy.

In the future it could be interesting to perform intervention studies examining the impact of combined programs involving both evaluations of taste sensitivity and dietary and lifestyle (such as physical activity) modifications on body composition and pathological conditions like the metabolic syndrome, and cardiovascular disease. I believe that performing a complete nutritional management of individuals with chemosensory disorders, through clinical evaluation and recommendation of appropriate dietary-intake measurements could be really important to increase the quality of life of cancer patients. This type of analysis can allow the patients themselves to evaluate the changes in thresholds for different taste modalities. Consequently, they can adopt appropriate appetizing strategies and, based on that, change their feeding habits.

# REFERENCES


- Abdoul-Azize S., Selvakumar S., Sadou H., Besnard P., Khan N.A., Ca2+ signaling in taste bud cells and spontaneous preference for fat: unresolved roles of CD36 and GPR120. Biochimie. 2014 96:8-13.
- Ackerman B.H., Kasbekar N., Disturbances of taste and smell induced by drugs. Pharmacotherapy. 1997 17(3): 482–496
- Adler E., Hoon M.A., Mueller K.L., Chandrashekar J., Ryba N.J., Zuker C.S., A novel family of mammalian taste receptors. Cell. 2000 100(6):693-702.
- Agnoli C., Grioni S., Sieri S., Palli D., Masala G., Sacerdote C., Vineis P., Tumino R., Giurdanella MC., Pala V., Berrino F., Italian Mediterranean Index and risk of colorectal cancer in the Italian section of the EPIC cohort., Int J Cancer. 2013 132(6):1404-11.
- Anand P., Kunnumakara AB., Sundaram C., Harikumar KB., Tharakan ST., Lai OS., Sung B, Aggarwal BB., Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharm Res. 2008 25( 9):2097-116.
- Antinucci M., Risso D.,A Matter of Taste: Lineage-Specific Loss of Function of Taste Receptor Genes in Vertebrates. Front Mol Biosci. 2017 28 4:8.
- Arends J., Bodoky G., Bozzetti F., at al. ESPEN guidelines on enteral nutrition: non-surgical oncology. Clin. Nutr. 2006; 25: 245-259.
- Assadi-Porter F.M., Radek J., Rao H., Tonelli M.,Multimodal Ligand Binding Studies of Human and Mouse G-Coupled Taste Receptors to Correlate Their Species-Specific Sweetness Tasting Properties. Molecules. 2018 23(10):2531-2546.
- August DA., Hummann MB., American Society for Parental and Enteral Nutrition (ASPEN) Board of Directors. ASPEN clinical guidelines: nutrition sypport therapy during adult anticancer treatment and in hematopoietic cell transplantation. JPEN. 2009; 33: 472-500.
- Avenet P., Lindemann B.,Amiloride-blockable sodium currents in isolated taste receptor cells. J Membr Biol. 1988 105(3):245-55.
- Behrens M., Brockhoff A., Kuhn C., Bufe B., Winnig M., Meyerhof W.,The human taste receptor hTAS2R14 responds to a variety of different bitter compounds. Biochem Biophys Res Commun. 2004 319(2):479-85
- Behrens M., Meyerhof W.,Gustatory and extragustatory functions of mammalian taste receptors. Physiol Behav. 2011 30;105(1):4-13
- Bernhardson BM, Tishelman C, Rutqvist LE Self-reported taste and smell changes during cancer chemotherapy. Support Care Cancer 2008 16(3):275–283
- Besnard P., Passilly-Degrace P., Khan N.A.,Taste of Fat: A Sixth Taste Modality? Physiol Rev. 2016 96(1):151-76
- Binetti P., Marcelli M., Baisi R., Manuale di nutrizione clinica e scienze dietetiche applicate. Società Editrice Universo 2016, 279-287.

- Blonde G.D., Spector A.C.,An Examination of the Role of L-Glutamate and Inosine 5'-Monophosphate in Hedonic Taste-Guided Behavior by Mice Lacking the T1R1 + T1R3 Receptor. Chem Senses. 2017 42(5):393-404.
- Booth F. W., Chakravarthy M. V., Gordon S. E., Spangenburg E.E., Waging war on physical inactivity: using modern molecular ammunition against an ancient enemy. J. Appl. Physiol. 2002 93: 3-30
- Bossola M., Muscaritoli M., Costelli P., et al.: Increased muscle ubiquitin mRNA levels in gastric cancer patients. Am J Physiol Regul Integr Comp Physiol. 2001 280(5):R1518-23.
- Bossola M., Muscaritoli M., Costelli P., et al.: Increased muscle proteasome activity correlates with disease severity in gastric cancer patients. Ann Surg 2003 237(3): 384-9,.
- Boughter J.D., Pumplin D.W., Yu C.,Christy R.C. and Smith D.V. .Differential expression of α-gustducin in taste bud populations of the rat and hamster, J Neurosci. 1997 17(8):2852-8.
- Bozzetti F., Arends J., Lundholm K., et al. ESPEN guidelines on parental nutrition: non-surgical oncology. Clin Nutr. 2009 28: 445-54.
- Breslin P.A., Spector A.C. Mammalian taste perception. Curr Biol Curr Biol. 2008 26;18(4):R148-55
- Brisbois T.D., de Kock I.H.,Watanabe S.M., Baracos V.E.,Wismer.W.V. Characterization of chemosensory alterations in advanced cancer reveals specific chemosensory phenotypes impacting dietary intake and quality of life. J Pain Symptom Manag 2011 41(4):673–683
- Buckland G., Agudo A., Luján L., Jakszyn P., Bueno-de-Mesquita HB., Palli D., Boeing H., Carneiro F., Krogh V., Sacerdote C., Tumino R., Adherence to a Mediterranean diet and risk of gastric adenocarcinoma within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study. Am J Clin Nutr 2010 91(2): 381-90.
- Caccialanza R., Pedrazzoli P., Cereda E., Gavazzi C., Pinto C., Paccagnella A., Berretta GD., Nardi M., Laviano A., Zagonel V. Nutritional Support in Cancer Patients: A Position Paper from the Italian Society of Medical Oncology (AIOM) and the Italian Society of Artifical Nutrition and Metabolism (SINPE), J Cancer. 2016 7(2):131-5.
- Calle E. E., Rodriguez C., Walker-Thurmond K., Thun MJ., Overweight, obesity and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med. 2003, 348: 1625-1638.
- Calle E.E and Kaaks R., "Overweight, obesity and cancer: epidemiological evidence and proposedmechanisms," Nat Rev Cancer. 2004 4(8):579-9.
- Calò C., Padiglia A., Zonza A., Corrias L., Contu P., Tepper B.J., Tomassini Barbarossa I., Polymorphisms in TAS2R38 and the taste bud trophic factor, gustin gene co-operate inmodulating PROP taste phenotype. Physiol Behav. 2011 104(5):1065-71
- Cecchini M.P., Cardobi N., Sbarbati A., Monaco S., Tinazzi M., Tamburin S., Post-traumatic taste disorders: a case series. J Neurol. 2018 265(4):836-844.
- Chandrashekar J., Hoon M.A., Ryba N.J., Zuker C.S.,The receptors and cells for mammalian taste. Nature. 2006 444(7117):288-94.
- Chandrashekar J., Kuhn C., Oka Y., Yarmolinsky D.A., Hummler E., Ryba N.J., Zuker C.S., The cells and peripheral representation of sodium taste in mice, Nature 2010 464(7286):297-301.

- Chandrashekar J., Mueller K.L., Hoon M.A., Adler E., Feng L., Guo W., Zuker C.S., Ryba N.J.,T2Rs function as bitter taste receptors. Cell. 2000 100(6):703-11.
- Chang R.B., Waters H., Liman E.R., A proton current drives action potentials in genetically identified sour taste cells. PNAS 2010 107(51):22320-5.
- Chaudhari N., Landin A.M., Roper S.D.,A metabotropic glutamate receptor variant functions as a taste receptor. Nature Neuroscience. 2000 3(2):113-9.
- Chaudhari N., Roper S.D.,The cell biology of taste. J Cell Biol. 2010 190(3):285-96.
- Cohen J.,Wakefield C.E., Laing D.G. Smell and taste disorders resulting from cancer and chemotherapy. Curr Pharm Des 2016 22(15):2253–2263
- Couto E., Boffetta P., Lagiou P., Ferrari P., Buckland G., Overvad K., Dahm CC., Tjønneland A., Olsen A., Clavel-Chapelon F., Boutron-Ruault MC., Mediterranean dietary pattern and cancer risk in the EPIC cohort., in Br J Cancer 2011 104(9):1493-9.
- Cummings T.A., Kinnamon S.C., Apical K+ channels in Necturus taste cells. Modulation by intracellular factors and taste stimuli. J Gen Physiol. 1992 99(4):591-613.
- Curtis K.S., Stratford J.M., Contreras R.J., Estrogen increases the taste threshold for sucrose in rats. Physiol Behav. 2005 86(3):281-6
- Damak S., Rong M., Yasumatsu K., Kokrashvili Z., Varadarajan V., Zou S., Jiang P., Ninomiya Y., Margolskee R.F.,Detection of sweet and umami taste in the absence of taste receptor T1r3. Science. 2003 8;301(5634):850-3.
- de Ruiter M.B., Reneman L., Boogerd W., Veltman D.J., van Dam F.S., Nederveen A.J. Cerebral hyporesponsiveness and cognitive impairment 10 years after chemotherapy for breast cancer. Hum Brain Mapp 2011 32(8):1206–1219
- de van der Schueren M.A.E., Laviano A., Blanchard H., Jourdan M., Arends J., Baracos V.E. Systematic review and meta-analysis of the evidence for oral nutritional intervention on nutritional and clinical outcomes during chemo(radio)therapy: current evidence and guidance for design of future trials. Ann Oncol. 2018 May 1;29(5):1141-1153.
- Devanand DP., Olfactory Identification Deficits, Cognitive Decline, and Dementia in Older Adults. The American Journal of Geriatric Psychiatry, 2016, 24(12):1151-1157
- DeVere R., Disorders of Taste and Smell. Continuum. 2017 23(2): 421-446.
- Doty R.L., Measurement of chemosensory function. World J Otorhinolaryngol Head Neck Surg. 2018 28;4(1):11-28.
- Doty R.L., Cameron E.L. Sex differences and reproductive hormone influences on human odor perception. Physiol Behav 2009 97(2):213–228
- Dutta Banik D., Martin L.E., Freichel M., Torregrossa A.M., Medler K.F.,TRPM4 and TRPM5 are both required for normal signaling in taste receptor cells. PNAS 2018 23;115(4):E772-E781.
- Fearon K., Strasser F., Anker SD., Bosaeus I., Bruera E., Fainsinger RL., Jatoi A., Loprinzi C., MacDonald N., Mantovani G., Davis M., Muscaritoli M., Ottery F., RadBruch L., Ravasco P., Walsh D., Wilcock A., Kaasa S., Baracos VE. Definition and Classification of cancer cachexia: an international consensus. Lancet Oncol 2011 12: 489-95.

- Finger T.E., Bottger B., Hansen A., Anderson K.T., Alimohammadi H., Silver W.L., Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration, PNAS 2003 22;100(15):8981-6.
- Fletcher M.L., Ogg M.C., Lu L., Ogg R.J., Boughter J.D. Jr.,Overlapping Representation of Primary Tastes in a Defined Region of the Gustatory Cortex. J Neurosci. 2017 9;37(32):7595-7605.
- Fox A.F., The relationship between chemical constitution and taste. PNAS 1932 18:115-20.
- Frank M.,An Analysis of Hamster Afferent Taste Nerve Response Functions, J Gen Physiol. 1973 61(5):588-618.
- Frank M.E., Lundy R.F.Jr, Contreras R.J.,Cracking taste codes by tapping into sensory neuron impulse traffic. Prog Neurobiol. 2008 86(3):245-63
- French Speaking Society of Clinical Nutrition and Metabolism (SFNEP). Clinical nutrition guidelines of the French Speaking Society of Clinical Nutrition and Metabolism (SFNEP): Summary of recommendations for adults undergoing non-surgical anticancer treatment. Dig Liver Dis. 2014 46: 667-74.
- Frings S., The sour taste of a proton current. Proc Natl Acad Sci U S A. 2010 107(51):21955-6.
- Fuller J.L., Single-locus control of saccharin preference in mice. J Hered. 1974 65(1):33-6
- Fung TT., Hu FB., McCullough ML., Newby PK., Willett W.C., Holmes MD., Diet quality is associated with the risk of estrogen receptor-negative breast cancer in postmenopausal women. J Nutr 2006 36(2):466-72.
- Gamper E-, Zabernigg A, Wintner LM, Giesinger JM, Oberguggenberger A, Kemmler G, et al. Coming to your senses: Detecting taste and smell alterations in chemotherapy patients. a systematic review. J Pain Symptom Manage 2012 44(6):880-895.
- Garcia-Bailo B., Toguri C., Eny K.M., El-Sohemy A., Genetic variation in taste and its influence on food selection. OMICS J. Integr. Biol. 2009, 13:69–80
- Gilbertson TA, Yu T, Shah BP. Gustatory Mechanisms for Fat Detection In: Montmayeur JP, le Coutre J, editors. Fat Detection: Taste, Texture, and Post Ingestive Effects. Boca Raton (FL): CRC Press/Taylor & Francis; 2010. Chapter 3.
- Gilbertson T.A.,Gustatory mechanisms for the detection of fat. Curr Opin Neurobiol. 1998 8(4):447-52.
- Gulbransen B.D., Clapp T.R., Finger T.E., Kinnamon S.C., Nasal solitary chemoreceptor cell responses to bitter and trigeminal stimulants in vitro. J Neurophysiol. 2008 99(6):2929-37.
- He W., Yasumatsu K., Varadarajan V., Yamada A., Lem J., Ninomiya Y., Margolskee R.F., Damak S.,Umami taste responses are mediated by alpha-transducin and alpha-gustducin. J Neurosci. 2004 24(35):7674-80.
- Hébuterne X., Lemarié E., Michallet M., De Montreuil C.B., Schneider S.M., Goldwasser F., Prevalence of malnutrition and current use of nutrition support in patients with cancer. J Parenter Enteral Nutr. 2014 38(2):196-204.
- Heck G.L., Mierson S., DeSimone J.A., Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway., Science 1984 223: 403–405.
- Heckmann S.M., Hujoel P., Habiger S., Friess W., Wichmann M., Heckmann J.G., Hummel T., Zinc gluconate in the treatment of dysgeusia--a randomized clinical trial. J Dent Res. 2005 84(1):35-8..
- Hoon M.A., Adler E., Lindemeier J., Battey J.F., Ryba N.J., Zuker C.S.,Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell. 1999 96(4):541-51.

- Huang A.L., Chen X., Hoon M.A., Chandrashekar J., Guo W., Tränkner D., Ryba N.J., Zuker C.S.,The cells and logic for mammalian sour taste detection. Nature. 2006 442(7105):934-8.
- Hummel T, Landis BN, Hüttenbrink K. Smell and taste disorders. GMS Curr Top Otorhinolaryngol Head Neck Surg. 2011;10:Doc04.
- Hursting S.D., Lashinger M., Colbert H., Rogers C.J., Wheatley K.W., Nunez N.P., Mahabir S., Barrett J.C., Forman M.R., Perkins S.N, Energy balance and carcinogenesis:underlying pathways and targets for interventation. Curr. Cancer Drug Targets. 2007, 7: 484-491
- IJpma I., Renken R.J., Gietema J.A., Slart R.H.,Mensink M.G., Lefrandt J.D. (2017) Changes in taste and smell function, dietary intake, food preference, and body composition in testicular cancer patients treated with cisplatin-based chemotherapy. Clin Nutr 36(6):1642–1648
- IJpma I, Renken RJ, ter Horst GJ, Reyners AKL. Metallic taste in cancer patients treated with chemotherapy. Cancer Treat Rev 2015 41(2):179-186.
- IJpma I., Timmermans E.R., Renken R.J., Ter Horst G.J., Reyners A.K. Metallic taste in cancer patients treated with systemic therapy: a questionnaire-based study. Nutr Cancer. 2017 69(1):140-145.
- Ikeda K.,New seasonings. Chemical Senses. 2002 27(9):847-9.
- Ishimaru Y., Inada H., Kubota M., Zhuang H., Tominaga M., Matsunami H.,Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. Proc Natl Acad Sci U S A. 2006 103(33):12569-74.
- Ishimaru Y.,Molecular mechanisms underlying the reception and transmission of sour taste information. Biosci Biotechnol Biochem. 2015 79(2):171-6
- Iwasaki K., Kasahara T., Sato M.,Gustatory effectiveness of amino acids in mice: behavioral and neurophysiological studies. Physiology & Behaviour. 1985 34(4):531-42.
- Jemal A., Siegel R., Ward E., Murray T., Xu J., Thun MJ., Cancer statistics, 2007. CA Cancer J. Clin. 2007 57:43-66.
- Ji M., Su X., Su X., Chen Y., Huang W., Zhang J., Gao Z., Li C., Lu X.,Identification of novel compounds for human bitter taste receptors. Chem Biol Drug Des. 2014 84(1):63-74..
- Kandel E.R., Schwart J.H., Jessel. T.M., Dodd J., Castellucci V.F., Principi di neuroscienze, 2 edizione Milano Ed. Ambrosiana 2005
- Kawai T., Fushiki T.,Importance of lipolysis in oral cavity for orosensory detection of fat. Am J Physiol Regul Integr Comp Physiol. 2003 285(2):R447-54
- Kwamura Y., Kare M.R, Umami:a basic taste-physiology, biochemistry, nutrition, food science. Marcel Dekker, New York 1987, 649.
- Kenza Drareni, Anestis Dougkas, Agnes Giboreau, Martine Laville, Pierre-Jean Souquet, Moustafa Bensafib, Relationship between food behavior and taste and smell alterations in cancer patients undergoing chemotherapy: A structured review. Seminars in Oncology. 2019 June; 46(2).
- Kershaw J.C., Mattes R.D., Nutrition and taste and smell dysfunction. World J Otorhinolaryngol Head Neck Surg. 2018 4(1):3-10
- Khan N.A., Besnard P., Oro-sensory perception of dietary lipids: New insights into the fat taste transduction. Biochim Biophys Acta 2009 1791(3):149-55.

- Kikut-Ligaj D., Trzcielińska-Lorych J.,How taste works: cells, receptors and gustatory perception. Cell Mol Biol Lett. 2015 20(5):699-716.
- Kim U.K., Drayna D.,Genetics of individual differences in bitter taste perception: lessons from the PTC gene. Clin Genet 2005 67(4):275-80.
- Kimura K., Beidler L.M. Microelectrode study of taste receptors of rat and hamster. J Cell Comp Physiol. 1961 58:131-9
- Kindleysides S., Beck K.L., Walsh D.C.I., Henderson L., Jayasinghe S.N., Golding M., Breier B.H.,Fat Sensation: Fatty Acid Taste and Olfaction Sensitivity and the Link with Disinhibited Eating Behaviour. Nutrients. 2017 Aug 15;9(8):879-900
- Kinnamon S.C., Neurosensory transmission without a synapse: new perspectives on taste signaling. BMC Biol. 2013 11:42-46
- Kitagawa M., Kusakabe Y., Miura H., Ninomiya Y., Hino A.,Molecular genetic identification of a candidate receptor gene for sweet taste. Biochemical and Biophysical Research Communication. 2001 283(1):236-42.
- Kulkarni B.V., Mattes R.D.,Lingual lipase activity in the orosensory detection of fat by humans. American Journal of Physiology Regulatory Integrative and Comparative Physiology. 2014 306(12):R879-85.
- Kunishima N., Shimada Y., Tsuji Y., Sato T., Yamamoto M., Kumasaka T., Nakanishi S., Jingami H., Morikawa K.,Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. Nature. 2000 407(6807):971-7.
- Kurihara K., Umami the Fifth Basic Taste: History of Studies on Receptor Mechanisms and Role as a Food Biomed Res Int. 2015;2015:189402 pp1-10.
- Lahiri S., Forster R.E. 2nd.,CO2/H(+) sensing: peripheral and central chemoreception. Int J Biochem Cell Biol. 2003 Oct;35(10):1413-35.
- Laird, A.K. et al., Dynamics of normal growth, Growth. 1965 29(3):233-48.
- Laird, A.K. Laird, Dynamics of tumour growth, Br J Cancer. 1964 13:490-502.
- Lalwani A.K., Fauci A.S., Braunwald E., Kasper D.L., Hauser S.L., Longo D.L., Jameson J.L:, Loscalzo J., Harrison Principi di Medicina Interna 17 ediz Milano McGraw-Hill 2009:196-9.
- Landis B.N., Welge-Luessen A., Brämerson A., Bende M., Mueller C.A., Nordin S., Hummel T., "Taste Strips" - a rapid, lateralized, gustatory bedside identification test based on impregnated filter papers. Journal of Neuroogy. 2009 256(2):242-8.
- Larry Jameson, Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, Dan L. Longo, Joseph Loscalzo, Harrison's Principles of Internal Medicine. McGraw Hill Higher Education, Twentieth Edition (Vol.1 & Vol.2), 2018
- Laugerette F., Passilly-Degrace P., Patris B., Niot I., Febbraio M., Montmayeur J.P., Besnard P.,CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. The Journal of Clinical Investigation. 2005 115(11):3177-84.
- Laviano A., Meguid MM., Inui A., et al.: Therapy insight: cancer anorexia-cachexia syndrome – when all you can eat is yourself. Nature Clin Pract Oncol 2005 2: 158-165.
- Laviano A., Meguid MM., Rossi Fanelli F: Cancer anorexia: clinical implications, pathogenesis, and therapeutic strategies. Lancet Oncol 2003 4: 686-694.

- Lee R.J., Hariri B.M., McMahon D.B., Chen B., Doghramji L., Adappa N.D., Palmer J.N., Kennedy D.W., Jiang P., Margolskee R.F., Cohen N.A..Bacterial d-amino acids suppress sinonasal innate immunity through sweet taste receptors in solitary chemosensory cells. Sci Signal. 2017 10: 495-503
- Li W.Q., Park Y., Wu JW., Ren JS., Goldstein AM., Taylor PR., Hollenbeck AR., Freedman ND., Abnet CC., Index-based dietary patterns and risk of esophageal and gastric cancer in a large cohort study., in Clin Gastroenterol Hepatol. 2013 9:1130-1136.
- Li X., Li W., Wang H., Cao J., Maehashi K., Huang L., Bachmanov A.A., Reed D.R., Legrand-Defretin V., Beauchamp G.K., Brand J.G.,Pseudogenization of a sweet-receptor gene accounts for cats' indifference toward sugar. PLoS Genet. 2005 1(1):27-35.
- Liman E.R., Zhang Y.V., Montell C.,Peripheral coding of taste. Neuron. 2014 81(5):984-1000.
- Lin W., Burks C.A., Hansen D.R., Kinnamon S.C., Gilbertson T.A.,Taste receptor cells express pH-sensitive leak K+ channels. J Neurophysiol. 2004 92(5):2909-19.
- Liu D., Archer N., Duesing K., Hannan G., Keast R.,Mechanism of fat taste perception: Association with diet and obesity. Prog Lipid Res. 2016 63:41-9.
- Liu D., Liman E.R., Intracellular Ca2+ and the phospholipid PIP2 regulate the taste transduction ion channel TRPM5. PNAS. 2003 100(25):15160-5.
- LopezJimenez N.D., Cavenagh M.M., Sainz E., Cruz-Ithier M.A., Battey J.F., Sullivan S.L.,Two members of the TRPP family of ion channels, Pkd1l3 and Pkd2l1, are co-expressed in a subset of taste receptor cells. J Neurochem. 2006 98(1):68-77.
- Lush I.E., Holland G., The genetics of tasting in mice., V. Glycine and cycloheximide. Genet Res. 1988 52(3):207-12.
- Lyall V., Alam R.I., Malik S.A., Phan T.H., Vinnikova A.K., Heck G.L., DeSimone J.A.,Basolateral Na+-H+ exchanger-1 in rat taste receptor cells is involved in neural adaptation to acidic stimuli. J Physiol. 2004 556(Pt 1):159-73
- Mace O.J., Affleck J., Patel N., Kellett G.L., Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. J Physiol. 2007 582(Pt 1):379-92.
- Macht M., Mueller J. Increased negative emotional responses in PROP supertasters. Physiol Behav. 2007 90(2-3):466-72.
- Maina I.W., Workman A.D., Cohen N.A.,The role of bitter and sweet taste receptors in upper airway innate immunity: Recent advances and future directions. World J Otorhinolaryngol Head Neck Surg. 2018 4(3):200-208.
- Matsunami H., Montmayeur J.P., Buck L.B.,A family of candidate taste receptors in human and mouse. Nature. 2000 404(6778):601-4.
- Mattes R.D.,Is there a fatty acid taste? Annu Rev Nutr. 2009;29:305-27.
- Mattes R.D., Popkin B.M. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. Am J Clin Nutr 2009 89(1):1–14.
- McGreevy J., Orrevall Y., Belqaid K., Wismer W. Tishelman C., Bernhardson B.M. (2014) Characteristics of taste and smell alterations reported by patients after starting treatment for lung cancer. Support Care Cancer. 2014 22(10):2635-44

- Melis M., Grzeschuchna L., Sollai G., Hummel T., Tomassini Barbarossa I.,Taste disorders are partly genetically determined: Role of the TAS2R38 gene, a pilot study. Laryngoscope. 2019 129(9):E307-E312.
- Meyerhof W., Batram C., Kuhn C., Brockhoff A., Chudoba E., Bufe B., Appendino G., Behrens M., The molecular receptive ranges of human TAS2R bitter taste receptors. Chem Senses 2010 35(2):157-70.
- Mosel DD, Bauer RL, Lynch DP, Hwang ST. Oral complications in the treatment of cancer patients. Oral Dis 2011 17(6):550-559.
- Mueller K.L., Hoon M.A., Erlenbach I., Chandrashekar J., Zuker C.S., Ryba N.J.,The receptors and coding logic for bitter taste. Nature. 2005 434(7030):225-9.
- Muscaritoli M., Cangiano C., Cascino A., et al.: Plasma clearance of exogenus lipids in patients with malignant disease. Nutrition 1990 6(2):147-51.
- Muscaritoli M., Lucia S., Farcomeni A., Lorusso V., Saracino V. et al., Prevalence of malnutrition in patients at first medical oncology visit: the PreMiO study. Oncotarget, 2017 8:79884-7896.
- Nakajima K., Morita Y., Koizumi A., Asakura T., Terada T., Ito K., Shimizu-Ibuka A., Maruyama J., Kitamoto K., Misaka T., Abe K., Acid-induced sweetness of neoculin is ascribed to its pH-dependent agonistic–antagonistic interaction with human sweet taste receptor. FASEB Journal. 2008 22(7):2323-30.
- Nakazato Y, Imai K, Abe T, Tamura N, Shimazu K Unpleasant sweet taste: a symptom of SIADH caused by lung cancer. J Neurol Neurosurg Psychiatry 2006 77(3):405–406
- National Cancer Institute (NCI), the U.S. government's principal agency for cancer research; www.cancer.gov
- Nelson G., Chandrashekar J., Hoon M.A., Feng L., Zhao G., Ryba N.J., Zuker C.S.,An amino-acid taste receptor. Nature. 2002 416(6877):199-202.
- Nelson G., Hoon M.A., Chandrashekar J., Zhang Y., Ryba N.J., Zuker C.S.,Mammalian sweet taste receptors. Cell. 2001 106(3):381-90.
- Nelson T., LopezJimenez N.D., Tessarollo L., Inoue M., Bachmanov A., Sullivan S.L., Taste function in mice with a targeted mutation of the Pkd1l3 gene. Chem Senses. 2010 35(7):565-77.
- Oka Y., Opening a "Wide" Window onto Taste Signal Transmission. Neuron. 2018 98(3): 456–458.
- Ou J., Carbonero F., Zoetendal E.G., DeLany J. P., Wang M., Newton K., Gaskins H. R., O'Keefe S. JD., Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. Am J Clin Nutr. 2013 98(1):111-20.
- Ozdener M.H., Subramaniam S., Sundaresan S., Sery O., Hashimoto T., Asakawa Y., Besnard P., Abumrad N.A., Khan N.A.,CD36- and GPR120-mediated $Ca^{2+}$ signaling in human taste bud cells mediates differential responses to fatty acids and is altered in obese mice. Gastroenterology. 2014 146(4):995-1005.
- Patel N.N., Workman A.D., Cohen N.A.,Role of Taste Receptors as Sentinels of Innate Immunity in the Upper AirwayJ Pathog. 2018 2018(9541987):1-8.
- Paul A.S. Breslin. Review: An Evolutionary Perspective on Food and Human Taste. Current Biology, 2013, Volume 23, Issue 9, Pages R409-R418
- Pepino MY, Love-Gregory L, Klein S, Abumrad NA, The fatty acid translocase gene, CD36, and lingual lipase influence oral sensitivity to fat in obese subjects. J Lipid Res. 2011 52(12):2332-40.
- Peterschmitt Y., Abdoul-Azize S., Murtaza B., Barbier M., Khan A.S., Millot J.L., Khan N.A.,Fatty Acid Lingual Application Activates Gustatory and Reward Brain Circuits in the Mouse. Nutrients. 2018 6;10(9):1246-1256.

- Pfaffmann C., Gustatory afferent impulses., J Cell Physiol 1941 17:243-258.
- Pfaffmann C., Gustatory nerve impulses in rat, cat and rabbit., J Neurophysiol. 1955 18:429-440.
- Pin J.P., Acher F.,The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. Curr Drug Targets CNS Neurol Disord. 2002 1(3):297-317.
- Pitsavos C., Panagiotakos DB., Tzima N., Chrysohoou C., Economou M., Zampelas A., Stefanadis C., Adherence to the Mediterranean diet is associated with total antioxidant capacity in healthy adults: the ATTICA study., Am J Clin Nutr 2005 82(3):694-9.
- Prado C.M., Maia YL., Ormsbee M., et al. Assessment of nutritional status in cancer – the relationship between body composition and pharmacokinetics. Anticancer Agents Med Chem. 2013 13: 1197-203.
- Praud D., Bertuccio P., Bosetti C., Turati F., Ferraroni M., La Vecchia C., Adherence to the Mediterranean diet and gastric cancer risk in Italy., Int J Cancer 2014 134(12):2935-41.
- Price K.A. Hydration in cancer patients. Curr Opin Support Palliat Care 2010 4(4):276–280
- Pritchard T.C., Scott T.R., Amino acids as taste stimuli. I. Neural and behavioural attributes., Brain Res 1982 253:81–92.
- Pronin A.N., Xu H., Tang H., Zhang L., Li Q., Li X.,Specific alleles of bitter receptor genes influence human sensitivity to the bitterness of aloin and saccharin Curr Biol. 2007 17(16):1403-8.
- Pugnaloni S., Vignini A, Borroni F, Sabbatinelli J, Alia S, Fabri M, Taus M, Mazzanti L, Berardi R., Modifications of taste sensitivity in cancer patients: a method for the evaluations of dysgeusia. Support Care Cancer. 2019
- Rać M.E., Safranow K., Poncyljusz W., Molecular basis of human CD36 gene mutations. Mol Med. 2007 13(5-6):288-96.
- Radbruch L., Elsner F., Trottenberg P., Strasser F., Fearon K., Clinical practise guidelines on cancer cachexia in advanced cancer patients. Aachen, Department of Palliative Medicine/European Palliative Care Research Collaborative; 2010.
- Ravasco P., Monteiro-Grillo I., Vidal PM., et al.: Dietary counseling improves patient outcomes: a prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy. Clin Oncol 2005; 23(7): 1431-8.
- Ravi K.,Sweet proteins – Potential replacement for artificial low calorie sweeteners. Nutrition Journal. 2005 4: 5-10.
- Reed D.R., Nanthakumar E., North M., Bell C., Bartoshuk L.M., Price R.A.,Localization of a gene for bitter-taste perception to human chromosome 5p15. American Journal of Human Genetics. 1999 64(5):1478-80.
- Reedy J., Krebs-Smith SM., Miller PE., Liese AD., Kahle LL., Park Y., Subar AF., Higher diet quality is associated with decreased risk of all-cause, cardiovascular disease, and cancer mortality among older adults., in J Nutr 2014 144(6):881-9
- Richter T.A., Dvoryanchikov G.A., Chaudhari N., Roper S.D.,Acid-sensitive two-pore domain potassium (K2P) channels in mouse taste buds. J Neurophysiol. 2004 92(3):1928-36.
- Rolls E.T.,Brain mechanisms underlying flavour and appetite. Philos Trans R Soc Lond B Biol Sci. 2006 361(1471):1123-36.
- Rolls E.T.,The representation of umami taste in the taste cortex J Nutr. 2000 130(4S Suppl):960S-5S

- Roper S.D., Signal transduction and information processing in mammalian taste buds. Pflugers Archiv. 2007 454(5):759-76.
- Rossi Fanelli F., Cascino A., Muscaritoli M: Abnormal substrate metabolism and nutritional strategies in cancer management. JPEN J Parenter Enteral Nutr. 1991 15(6):680-3.
- Roudnitzky N., Bufe B., Thalmann S., Kuhn C., Gunn H.C., Xing C., Crider B.P., Behrens M., Meyerhof W., Wooding S.P., Genomic, genetic and functional dissection of bitter taste responses to artificial sweeteners. Hum Mol Genet. 2011 20(17):3437-49
- Rozengurt E., Taste receptors in the gastrointestinal tract. I. Bitter taste receptors and alphagustducin in the mammalian gut. Am J Physiol Gastrointest Liver Physiol. 2006 291(2):G171-7.
- Ruiz C.J., Wray K., Delay E., Margolskee R.F., Kinnamon S.C., Behavioral evidence for a role of alpha-gustducin in glutamate taste. Chem Senses. 2003 28(7):573-9.
- Sanematsu K., Kitagawa M., Yoshida R., Nirasawa S., Shigemura N., Ninomiya Y., Intracellular acidification is required for full activation of the sweet taste receptor by miraculin. Sci Rep. 2016 6:22807-14.
- Santarpia L., Pagano MC., Pasanisi F., et al., Home artificial nutrition: An update seven years after the regional regulation. Clin. Nutr. 2014; 33: 872-8.
- Sbarbati A., Bramanti P., Benati D., Merigo F.,The diffuse chemosensory system: exploring the iceberg toward the definition of functional roles. Progr Neurobiol. 2010 91(1):77-89.
- Sbarbati A., Osculati F.,The taste cell-related diffuse chemosensory system. Progr Neurobiol 2005 75(4):295-307.
- Schiffman S.S., Influence of medications on taste and smell. World J Otorhinolaryngol Head Neck Surg 2018 4(1):84-91.
- Schiffman S.S..Perception of taste and smell in elderly persons. Crit Rev Food Sci Nutr. 1993;33(1):17-26.
- Schiffman SS, Taste and smell in disease (first of two parts). N Engl J Med, 1983 308(21):1275–1279
- Schiffman SS, Gatlin CA. Clinical physiology of taste and smell. Annu Rev Nutr, 1993 13:405-36.
- Shigemura N., Shirosaki S., Sanematsu K., Yoshida R., Ninomiya Y., Genetic and molecular basis of individual differences in human umami taste perception. PLoS One. 2009 4(8):e6717-25.
- Skipper A., Ferguson M., Thompson K., et al. Nutrition screening tools: an analysis of the evidence. JPEN. 2012; 36: 292-8.
- Smith D.V., Van Buskirk R.L., Travers J.B., Bieber S.L.,Coding of taste stimuli by hamster brain stem neurons. Journal of Neurophysiology. 1983 Aug;50(2):541-58.
- Soter A, Kim J, Jackman A, Tourbier I, Kaul A, Doty RL (2008) Accuracy of self-report in detecting taste dysfunction. Laryngoscope 118(4):611–617
- Spector A.C., Kopka S.L.,Rats fail to discriminate quinine from denatonium: implications for the neural coding of bitter-tasting compounds. J Neurosci. 2002 22(5):1937-41..
- Staun M., Pironi L., Bozzetti F., Baxter J., Forbes A., Joly F., et al., ESPEN guidelines on parental nutrition: home parental nutrition (HPN) in adult patients. Clin Nutr. 2009 28: 467-79.
- Stevens D.R., Seifert R., Bufe B., Müller F., Kremmer E., Gauss R., Meyerhof W., Kaupp U.B., Lindemann B.,Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. Nature. 2001 413(6856):631-5.

- Stone L.M., Barrows J., Finger T.E., Kinnamon S.C., Expression of T1Rs and gustducin in palatal taste buds of mice. Chemical Senses. 2007 Mar;32(3):255-62.
- Stratton RJ, Green CJ, Elia M: Disease-related malnutrition. An evidence based approach to treatment. ed. CABI Publishing, 2003.
- Sze-Yen Tan and Robin M. Tucker, Sweet Taste as a Predictor of Dietary Intake: A Systematic Review. Nutrients, 2019, 11(1): 94.
- Tepper B.J., Koelliker Y., Zhao L., Ullrich N.V., Lanzara C., d'Adamo P., Ferrara A., Ulivi S., Esposito L., Gasparini P.,Variation in the bitter-taste receptor gene TAS2R38, and adiposity in a genetically isolated population in Southern Italy. Obesity 2008;16(10):2289-95.
- Tsuganeand S, Inoue M., "Insulin resistance and cancer: epidemiological evidence," Cancer Sci. 2010 101(5):1073-9
- Ugawa S., Minami Y., Guo W., Saishin Y., Takatsuji K., Yamamoto T., Tohyama M., Shimada S.,Receptor that leaves a sour taste in the mouth. Nature. 1998 395(6702):555-6.
- Van Oort S, Kramer E, de Groot JW, Visser O. Taste alterations and cancer treatment. Curr Opin Support Palliat Care 2018 12(2):162-167.
- Vandenbeuch A., Clapp T.R., Kinnamon S.C., Amiloride-sensitive channels in type I fungiform taste cells in mouse. BMC Neuroscience. 2008 9: 1-14.
- Vígh B., Manzano e Silva M.J., Frank C.L., Vincze C., Czirok S.J., Szabó A., Lukáts A., Szél A.,The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. Histol Histopathol. 2004 19(2):607-28.
- Vignini A, Borroni F, Sabbatinelli J, Pugnaloni S, Alia S, Taus M, Ferrante L, Mazzanti L, Fabri M (2019) General decrease of taste sensitivity is related to increase of BMI: a simple method to monitor eating behavior. Dis Markers. 2019 2019:2978026-34.
- Waldmann R., Champigny G., Bassilana F., Heurteaux C., Lazdunski M.,A proton-gated cation channel involved in acid-sensing. Nature. 1997 386(6621):173-7.
- Walliczek-Dworschak U., Schöps F., Feron G., Brignot H., Hähner A., Hummel T., Differences in the Density of Fungiform Papillae and Composition of Saliva in Patients With Taste Disorders Compared to Healthy Controls. Chem Senses. 2017 42(8):699-708.
- WCRF, World Cancer Research Fund, AICR, American Institute for Cancer Research, Recommendations and public health and policy implications. CUP, Continuous Update Project, 2018.
- Weimann A., Braga M., Harsanyi L. et al., ESPEN guidelines on Enteral Nutrition: surgery including organ transplantation. Clin Nutr. 2006; 25: 224-44.
- Wellendorph P., Johansen L.D., Bräuner-Osborne H.,Molecular pharmacology of promiscuous seven transmembrane receptors sensing organic nutrients. Mol Pharmacol. 2009 76(3):453-65.
- Wickham RS, Rehwaldt M, Kefer C, Shott S, Abbas K, Glynn-Tucker E, et al. Taste changes experienced by patients receiving chemotherapy. Oncol Nurs Forum 1999;26(4):697-706.
- Willett W. C., Diet and Cancer. Oncologist, 2000, 5: 394-404.

- Wooding S., Bufe B., Grassi C., Howard M.T., Stone A.C., Vazquez M., Dunn D.M., Meyerhof W., Weiss R.B., Bamshad M.J.,Independent evolution of bitter-taste sensitivity in humans and chimpanzees. Nature. 2006 440(7086):930-4.
- Y.C. de Vriesa, R.M. Winkelsa, M.M.G.A. van den Berga, C. de Graafa, C.S. Kelfkensa, J.Th.C.M de Kruifc, E. Gökerd, S. Grosfeldd, D.W. Sommeijere, H.W.M. van Laarhovene, E. Kampmana, S. Boesveldta, Altered food preferences and chemosensory perception during chemotherapy in breast cancer patients: A longitudinal comparison with healthy controls. Food Quality and Preference, 2018; 63:135-143
- Yamaguchi S.,The synergistic taste effect of monosodium glutamate and disodium 5'- inosinate., Journal of Food Science 1967 32:473–478.
- Yang H., Wanner I.B., Roper S.D., Chaudhari N.,An optimized method for in situ hybridization with signal amplification that allows the detection of rare mRNAs. J Histochem Cytochem. 1999 (4):431-46.
- Yasumatsu K., Manabe T., Yoshida R., Iwatsuki K., Uneyama H., Takahashi I., Ninomiya Y., Involvement of multiple taste receptors in umami taste: analysis of gustatory nerve responses in metabotropic glutamate receptor 4 knockout mice. J Physiol. 2015 593(4):1021-34
- Yasuo T., Kusuhara Y., Yasumatsu K., Ninomiya Y.,Multiple receptor systems for glutamate detection in the taste organ. Biol Pharm Bull. 2008 31(10):1833-7
- Yin W., Hewson L., Linforth R., Taylor M., Fisk I.D.,Effects of aroma and taste, independently or in combination, on appetite sensation and subsequent food intake. Appetite. 2017 114: 265–274.
- Yoshida R., Miyauchi A., Yasuo T., Jyotaki M., Murata Y., Yasumatsu K., Shigemura N., Yanagawa Y., Obata K., Ueno H., Margolskee R.F., Ninomiya Y., Discrimination of taste qualities among mouse fungiform taste bud cells. J Physiol. 2009 587(Pt 18):4425-39
- Yoshida R., Ninomiya Y.,Taste information derived from T1R-expressing taste cells in mice. Biochem J. 2016 473(5):525-36
- Yoshida R., Noguchi K., Shigemura N., Jyotaki M., Takahashi I., Margolskee R.F., Ninomiya Y., Leptin Suppresses Mouse Taste Cell Responses to Sweet Compounds. Diabetes. 2015 64(11):3751-62.
- Zabernigg A., Gamper E.M.,Giesinger J.M., Rumpold G.,Kemmler G., Gattringer K., Sperner-Unterweger B., Holzner B. Taste alterations in cancer patients receiving chemotherapy: a neglected side effect? Oncologist 2010 15(8):913–920.
- Zhang Y., Hoon M.A., Chandrashekar J., Mueller K.L., Cook B., Wu D., Zuker C.S., Ryba N.J.,Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell. 2003 112(3):293-301.
- Zhao G.Q., Zhang Y., Hoon M.A., Chandrashekar J., Erlenbach I., Ryba N.J., Zuker C.S., The receptors for mammalian sweet and umami taste. Cell. 2003 115(3):255-66.
- Zocchi D, Wennemuth G, Oka Y The cellular mechanism for water detection in the mammalian taste system. Nat Neurosc 2017 20(7):927–933.

# ATTACHMENTS

1. Questionnaire "Lifestyle and eating habits" at the time 0

The questionnaire was anonymous and the data was used in accordance with the privacy law.

**INDAGINE NUTRIZIONALE NEL PAZIENTE ONCOLOGICO**

**TEMPO 0**

Pz. n° : ___

SESSO : M / F

ETÀ :

☐ 18 – 34 anni

☐ 35 – 54 anni

☐ 55 – 75 anni

☐ > 76 anni

Professione: _____

DESCRIZIONE DEL TUMORE _____

Tempo trascorso dalla diagnosi_____

Presenta familiarità per la patologia?

☐ No

☐ Sì, parente di primo grado

☐ Sì, parente di secondo grado

☐ Sì, parente di terzo grado

Comorbidità _____

Fuma? SI / NO
- Per un corretto svolgimento dello studio è indispensabile che il soggetto non sia un fumatore !

Beve alcol ?

☐ No
☐ Sì, 1 bicchiere/die
☐ Sì, < 1 bicchiere/die
☐ Sì, > 1 bicchiere/die

**VALUTAZIONE DELLO STATO NUTRIZIONALE**

**TEMPO 0**

Altezza_____

Peso attuale_____

BMI_____

Circonferenza braccio_____

**BIA**:

- Resistenza (RX) : _____
- Reattanza (XC) : _____

- Massa magra (FFM): _____
- Massa grassa (FM): _____
- Massa muscolare (MM): _____

**PLICOMETRIA**:

_____

_____

_____

**PUNTEGGIO MUST:**

☐ 0, rischio basso

☐ 1, rischio medio

☐ 2 o più, rischio alto

Pratica attività fisica?

☐ No

☐ Sì, < 3 die/settimana

☐ Sì, 3 die/settimana

☐ Sì, > 3 die/settimana

Ha notato variazioni dell'alvo?

☐ No

☐ Sì, alvo stitico

☐ Sì, alvo diarroico

Aveva notato dei cambiamenti prima della diagnosi che l' avevano allarmata sul suo stato di salute? SÌ / NO

Se sì, quali?_____

Ha notato variazioni dell' appetito?

☐ No

☐ Sì, è diminuito

☐ Sì, è aumentato

Mangia volontariamente?

☐ Sì
☐ No, _____

Ha riscontrato problemi nella masticazione e/o infiammazioni del cavo orale? (mucosite, secchezza mucose ecc..)?

☐ No

☐ Sì, senza infiammazione

☐ Sì, con infiammazione

Ha riscontrato variazioni della percezione dei sapori e/o odori dei cibi e dell'acqua ?

☐ No

☐ Sì, solo ai sapori

☐ Sì, solo agli odori

☐ Sì, ad entrambi

Se presenti, a cosa attribuisce questi cambiamenti?

☐ Effetti collaterali della terapia
☐ Basso tono dell'umore
☐ Indicazioni mediche
☐ Altro _____

Quanta acqua beve durante la giornata?

☐ < 1 L
☐ Da 1 L a 1,5 L
☐ > 1,5 L

Descriva una giornata alimentare tipo:

| | ORARIO | ALIMENTI | QUANTITÀ | KCAL |
|---|---|---|---|---|
| **Colazione** | | | | |
| **Spuntino** | | | | |
| **Pranzo** | | | | |
| **Merenda** | | | | |
| **Cena** | | | | |
| **Spuntino serale** | | | | |

Intake calorico stimato: Kcal _____ **Fabbisogno calorico stimato: Kcal _____**

Frequenze alimentari settimanali :

Carne bianca ___ v/sett

Carne rossa ___ v/sett

Pesce ___ v/sett

Uova ___ v/sett

Legumi ___ v/sett

Insaccati __ v/sett

Formaggi ___ v/sett

Cereali ___ v/die

Frutta ___ v/die

Verdura ___ v/die

2. Questionnaire "Lifestyle and eating habits" at the time 1

The questionnaire was anonymous and the data was used in accordance with the privacy law.

**INDAGINE NUTRIZIONALE NEL PAZIENTE ONCOLOGICO**

**TEMPO 1**

Pz. n° :

Terapia svolta:

☐ Intervento chirurgico

☐ Chemioterapia _____

☐ Radioterapia

Fuma? SI / NO
- Per un corretto svolgimento dello studio è indispensabile che il soggetto non sia un fumatore !

Beve alcol ?

☐ No
☐ Sì, 1 bicchiere/die
☐ Sì, < 1 bicchiere/die
☐ Sì, > 1 bicchiere/die

**VALUTAZIONE DELLO STATO NUTRIZIONALE**

**TEMPO 1**

Altezza_____

Peso attuale_____

BMI_____

Circonferenza braccio_____

**BIA**:

- Resistenza (RX) : _____
- Reattanza (XC) : _____

- Massa magra (FFM): _____
- Massa grassa (FM): _____
- Massa muscolare (MM): _____

**PLICOMETRIA**:

_____

_____

_____

**PUNTEGGIO MUST:**

☐ 0, rischio basso

☐ 1, rischio medio

☐ 2 o più, rischio alto

Ci sono state variazioni del peso corporeo dall'inizio della terapia?

☐ Sì

☐ No

Se presenti, a cosa le attribuisce?

☐ Effetto collaterale della terapia

☐ Basso tono dell'umore

☐ Altro _____

Ha dovuto modificare l'attività fisica dall'inizio della terapia?

☐ No

☐ Sì _____

Ha notato variazioni dell'alvo?

☐ No

☐ Sì, alvo stitico

☐ Sì, alvo diarroico

È cambiato il suo modo di mangiare?

☐ No

☐ Sì

Se sì, in che modo?

_____

_____

_____

Ha notato variazioni dell' appetito?

☐ No

☐ Sì, è diminuito

☐ Sì, è aumentato

Mangia volontariamente?

☐ Sì

☐ No, _____

Ha riscontrato problemi nella masticazione e/o infiammazioni del cavo orale? (mucosite, secchezza mucose ecc..)?

☐ No

☐ Sì, senza infiammazione

☐ Sì, con infiammazione

Ha riscontrato variazioni della percezione dei sapori e/o odori dei cibi e dell'acqua ?

☐ No

☐ Sì, solo ai sapori

☐ Sì, solo agli odori

☐ Sì, ad entrambi

Se presenti, a cosa attribuisce questi cambiamenti?

☐ Effetti collaterali della terapia

☐ Basso tono dell'umore

☐ Indicazioni mediche

☐ Altro _____

Quanta acqua beve durante la giornata?

☐ < 1 L

☐ Da 1 L a 1,5 L

☐ > 1,5 L

C'è qualche alimento che prima della diagnosi mangiava e ora non riesce più a mangiare?

☐ No

☐ Sì _____

Ci sono determinati alimenti che ha iniziato a limitare - evitare - o che ha introdotto rispetto alle sue abitudini alimentari prima della diagnosi? (es. perché fanno bene/male per il tumore)

☐ No
☐ Sì _____

| | ORARIO | ALIMENTI | QUANTITÀ | KCAL |
|---|---|---|---|---|
| **Colazione** | | | | |
| **Spuntino** | | | | |
| **Pranzo** | | | | |
| **Merenda** | | | | |
| **Cena** | | | | |
| **Spuntino serale** | | | | |

Intake calorico stimato: Kcal _____ **Fabbisogno calorico stimato: Kcal _____**

Frequenze alimentari settimanali attuali:

Carne bianca ___ v/sett

Carne rossa ___ v/sett

Pesce ___ v/sett

Uova ___ v/sett

Legumi ___ v/sett

Insaccati __ v/sett

Formaggi __v/sett

Cereali ___ v/die

Frutta ___ v/die

Verdura ___ v/die