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Umami and Umami Compounds

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Abstract:

Since the discovery of umami, the fifth basic taste, and the general ambition for healthy nutrition the amount of research on umami has increased in the last years. Changes in lifestyle, including diet can lead to all kind of diseases, like obesity, diabetes and asthma. Therefore, scientiests try to find a way to make food more palatable and healthy by using well known umami compounds, such as monosodium glutamate (MSG) and umami taste-enhancer, like inosine 5′-monophosphate (IMP), as well as looking for new umami compounds. This paper covers the most recent scientific literature regarding umami, including the influence of MSG on the brain, side effects of MSG and ways of reducing them, umami taste transduction pathways and receptors, the influence of umami taste on gastric secretion and duodenal mucosa, compounds which have an effect on the umami aftertaste. In the past four years scientists made a huge process in discovering more about the umami taste and collecting a lot of new evidence in this field of research.

Keywords: umami, MSG, duodenal mucosa, gastric secretion, obesity, mGluR1/mGluR4, umami aftertaste, brain activation, umami compounds, metallic taste

Abstract:

Seit der Entdeckung von Umami, dem fünften Geschmacksrezeptor, und das generelle Bestreben nach gesunder Ernährung, nahm die Anzahl an Recherchen bezüglich Umami in den letzten Jahren beträchtlich zu. Veränderungen im Lebensstil, auch Ernährung miteingeschlossen, können zu verschiedenen Krankheiten führen, wie Übergewicht, Diabetes und Asthma. Daher versuchten Forscher einen Weg zu finden Nahrung schmackhaft und gesund zu machen, indem sie schon bekannte Umami-Verbindungen, wie Mononatriumglutamat und Umami-Geschmacksverstärker, Inosin 5´-Monophosphat, einsetzen und auch nach neuen Umami Verbindungen suchen. Diese Arbeit schließt die neusten wissenschaftlichen Studien bezüglich Umami ein, miteingeschlossen der Einfluss von Mononatriumglutamat auf das Gehirn, die Nebenwirkungen von Mononatriumglutamat und Wege diese zu mindern, Umami-Geschmackstransduktion und Rezeptoren, der Einfluss von Umami auf die Magensekretion und Duodenum Mukosa und Verbindungen die einen Effekt auf den Umami-Nachgeschmack haben. In den letzten vier Jahren machten Forscher einen großen Fortschritt hinsichtlich Umami-Geschmack und sammelten viele neue Hinweise in diesem Bereich.

Introduction

Nutrition and healthy food as a research field is getting more and more attention in the past few years, because poor nutrition can lead to various diseases like obesity, hypertension, diabetes and asthma. So the main goal of scientists is to make food more palatable and healthy by finding compounds that have influence on energy intake, eating habits and also on the enhancement of taste. Umami was discovered as the fifth basic taste and since then a lot of research was conducted to determine the role of umami taste and umami compounds in nutrition. The umami taste is responsible for the palatability of food and induces pleasure while eating. The first identified umami compound was monosodiumglutamate which led to a lot of researches for determining which receptor is responsible for umami taste, possible negative effects and effects on the brain. Scientist also tried to find other umami compounds besides monosodiumglutamate and discovered rubemamine and rubescenamine for example which trigger a umami taste, furthermore different peptides in various food products can lead to umami taste, which are described in this paper. Monosodiumglutamate and other umami compounds are used as food additives to enhance the palatability of food, but it is elemental to know if they are harmful over a long period of time. So a lot of studies were conducted to exclude this assumption. It was also identified that glutamate has plenty effects on the gastrointestinal tract, like on the gastric mucosa, gastric emptying, motility and HCO_3^- secretion. Since taste gained more importance in correlation with nutrition and healthy food in the course of time, scientist tried to find undiscovered mechanisms and new receptors. One example is the not so well known metallic taste, which was identified in various foods and salts. Also the artificial sweeteners like acesulfam-K and cyclamate can induce a metallic taste in high doses. Furthermore cancer patients often complain about metallic taste, which is a side effect of the therapy. In general taste and smell dysfunction is a big issue concerning cancer patients and also elderly people leading to a decreased quality of life. Therefore finding the right treatment and medication is important, because patients are seeking persistent for medical help. This paper covers all the topics mentioned above.

Umami in general

Since the discovery of umami, which is identified as the fifth basic taste, a lot of

research and studies were carried out to identify the effect of umami taste, to discover umami compounds and furthermore how to use this knowledge to make food healthier and tastier. Nowadays food-induced diseases like diabetes, high blood pressure and obesity occurs more frequently. Thus, the food industry tries to find a remedy for consumers to stay healthy without losing taste quality. [1, 2] Kikunae Ikeda, professor of physical chemistry at the university of Tokyo, was the first realizing that there might be an undiscovered taste while he was interested in the exclusive taste of kelp (=Konbu) and meat.

Knowing the four basic tastes sour, sweet, bitter and salty he assumed that a fifth taste is existing. His main interest was on seaweed, also named kelp, because of the high level of familiarity of this nutrition in Japan. He extracted the taste component in dried seaweed in 1907 trying to identify it and in the following year he described the sodium salt of glutamic acid as this taste component, naming it “Umami”. The meaning of umami in Japanese is “savoury”, “yummy” or “delicious”. $C_5H_9NO_4$ was revealed as a molecular formula after combustion analysis. Besides it is very impressive that Ikeda finished his work in 1908 one year after he began his investigation. [3]

The taste of glutamic acid was described autonomously by Ritthausen and Fischer, firstly as sour and later enveloping a insipid taste. The chemical structure was already identified by both. Forty years later Ikeda discovered the sodium salt of glutamic acid and described it as a umami taste enhancer. At neutral pH glutamic acid occurs as Na, K or Ca salt.[3] The main focus in the following studies was to determine the importance of monosodium glutamate (MSG), the synergizing ribonucleotides guanosine monophosphates (GMP) and inosine (IMP) and [5'-inosinate and 5'-guanylate, salt of IMP and GMP], because of the potential and use of these substances as food additives. [4] The food industry is using the free form of glutamate in the L-configuration, because that configuration is responsible for the flavor-enhancing characteristics. [5]. The ribonucleotide compounds IMP and GMP, specifically disodium 5´-monoinosinate and disodium 5`-monoguanylate, monosodium glutamate, monoammonium glutamate and monopotassium glutamate are flavor enhancers and can be added to all kind of food.[6]

In a study it was shown that glutamate increased the savory character and the enjoyment of a soup. The test persons who participated in this study, used to eat more soup with glutamate added than without. Also a rise in hunger was induced by the soup with glutamate as an additive. Glutamate increases the umami taste quality when adding to different foods and also the food’s acceptability.[7]

Rubemamine and Rubescenamine triggering umami taste

To increase the amount of flavor molecules activating the umami taste, a lot of studies determined savory flavor constituents like phthalides and also amino acids and nucleotide-type compounds. They discovered molecules for umami taste like, 2*E*,6*Z*-nonadienoic acid *N*-ethylamide, *N*-neomenthylamide, *N*-(heptan-4-yl)benzo[*d*] [1.3]dioxole-5-carboxamide, *N*-benzyl-*N*-(2-pyridylethyl)oxalic acid amides and *N*-cyclopropylamide, but no modulators from natural origin were known until lately. Important for the umami-taste-modulating characteristics of synthetic compounds is a central amide group or diamide with two medium-sized, nonpolar substituents. The N-cinnamoyl derivatives of aromatic amines show a congeneric structure and are suggested to have a umami-taste-modulating effect. To clarify if the natural N-cinnamoyl derivatives have an effect on the umami-taste sensing, a test by a sensory panel was conducted and also an in vitro TAS1R1-TAS1R3 receptor assay was conducted to cover the biological basis and furthermore to examine the activity together with MSG. Most of these *N*-cinnamoyl derivatives lead to a bitter taste, except for rubemamine and rubescenamine which triggered an umami taste stimulus. Only the homologue *N*-3,4-dimethoxycinnamoyl-4-methoxyphenethylamine was found in natural sources and that the 3,4-dimethoxycinnamic acid part was needed for an intensive umami activity. Some umami-taste-modulating metabolites discovered in plants were, e.g. theogallin, a polyphenol in green tea, and morelid in mushrooms. The problem is that these substances occur in very low concentrations in the plants, like rubescenamine for example which was discovered in concentrations of 10 ppm in *Zanthoxylum piperitum* (Rutaceae) and rubemamine in concentrations of 6 mg/kg in *Chenopodium album* (Amaranthaceae). Furthermore, only rubemamine and rubescenamine stimulated the heterologous hTAS1R1-rTas1r3 receptor from all tested N-cinnamoyl amines via calcium imaging. Results showed that rubemamine elicits a umami taste stronger than MSG. In comparison rubescenmine is not so efficient but is capable to stimulate the receptor in lower concentrations, approximately a 200 times higher potency than MSG. Both compounds were able to modulate the receptor response to MSG in a positive way. Furthermore, rubemamine also had an impact on the IMP-enhancing receptor responses to MSG. Further studies will be useful to determine the exact binding site and binding mode in the umami receptor. [16]

Natural sources of umami compounds

As new umami compounds two active peptides, namely Ser-Ser-Arg-Asn-Glu-Gln-Ser-Arg (SSRNEQSR, 963.9 Da) and Glu-Gly-Ser-Glu-Ala-Pro-Asp-Gly-Ser-Ser-Arg (EGSEAPDGSSR, 1091.1 Da) were discovered via MALDI-TOF/TOF MS in peanut hydrolysate, which comes from a protease extract from *Aspergillus oryzae*. These low molecular weight compounds produce an intensive umami and umami-enhancing effect. The two active peptides were obtained by purifying two fractions of the peanut hdrolysate with a reverse-phase high-performance liquid chromatography (RP-HPLC) and gel filtration chromatography.[17]

Food which contains free glutamate for example is seaweed, Parmesan cheese, potatoes, tomatoes, Chinese cabbage, sardines, prawns, soybeans, green tea, and clams. Also ripe tomatoes contain a lot of free Glu (Glutamate) and have the characteristic umami taste, but much higher concentrations are found in Parmesan cheese. So, humans get used to umami taste very early in life.[3]

Macronutrients, such as starch vegetable proteins and fat have a high molecular weight and are not able to bind on taste receptors. These macronutrients are not inducing any taste. The low molecular substances, like carbohydrates, sugars, fatty acids, fats, proteins and amino acids that co-exist with these macronutrients provoke a taste signal by binding on taste receptors on the tongue.[18]

Effects of glutamate on the gastrointestinal tract

1. Glutamate receptors on the gastric mucosa

The receptors that interact with these low molecular substances also exist on the mucosal epithelium in the digestive tract conciliating chemical perception for dietary nutrients in the gut. α-Gustducin (GTP binding protein) is particular for taste cells and its expression of α-Gustducin takes place on the brush cell in the pyloric antrum and duodenum. In addition, T1R receptors and calcium-sensing receptors, which sense amino acids and GPR120 which senses fatty acids are extensively spread in the gastrointestinal epithelium. GLP-1 (glucagon like peptide-1) controls body glucose utilization and stimulates the insulin synthesis. It was shown that this gut hormone is

released by the sweet taste receptor T1R2/T1R3 complexes, which occur in the endocrine cells of the intestinal epithelium. In 2007 it was shown that Glu receptors occur on gastric mucosa and that there is a chance of luminal Glu-sensing via the vagal afferent pathway in the stomach conveyed by the glutamate receptors.[18]

The assumption that for the communication between the vagus nerve endings and mucosal cells in rat gastric mucosa NO and 5-HT is needed, comes from experiments showing that due to the lack of serotonin (5-HT) and inhibition of nitric oxide (NO) synthase or 5-HT Type 3 ($5\text{-}HT_3$) the luminal Glu response was halted. Also luminal perfusion of a NO donor (sodium nitroprusside) showed that the afferent response was imitated and that the inhibition of $5\text{-}HT_3$ repealed the afferent activation induced by the NO donor. The muscosal 5-HT from enterochromaffin cells (EC), which appear in the GI mucosa, exhibits a paracrine function and identifies Glu in the lumen of the stomach. Luminal Glu binds on mucosal receptors (mGluRs or T1Rs) which activate the mucosal 5-HT/ NO secretion subsequently activating the 5-HT3 receptors at the nerve endings of gastric vagal afferents.[19,20]

Some studies showed that serotonin may be involved in the gastric Glu sensing. After the application of 150 mmol glutamate into the stomach, the concentration of serotonin and its metabolite 5-hydroxy acetic acid (5-HIAA) were determined to see if there is a correlation between glutamate sensing and serotonin. The mucosal serotonin was enhanced, whereas the blood serotonin stayed the same. The constant blood serotonin concentration is maybe the result of mobilization and metabolism of mucosal serotonin, which prohibits the passage of the bioactive substance into the bloodstream. Also the numerous monoamine oxidases in the gastric mucosa or the blood platelet uptake can decrease the mucosal serotonin. Thus, gut mucosal serotonin might play an important role for gut nutrient sensing, besides initiating diarrhea and vomiting in critic reactions, because more than 90 percent of the body serotonin is located in the gastrointestinal mucosa.[19]

Recent experiments showed that excitatory responses in vagal celiac afferent activity (VCA) are induced through intraintestinal infusion of MSG, lysine, leucine, and other amino acids, whereas methionine, glycine and some other amino acids suppressed the afferent nerve activity.[20]

2. Brain activation via glutamate

After electrophysiological experiments it was shown that gastric and celiac vagal afferents are responsible for sensing 20 dietary amino acids. Luminal glycine, for example, inhibits the celiac branch of the vagus nerve activity in the small intestine and luminal Glu (glutamate), aspartate and tryptophan increase the activity. The gastric proteolytic enzyme cannot convert protein to every amino acid and has a mechanism that can only recognize Glu, whereas all amino acids can be recognized by the celiac afferents. There is also the potential that the stomach is a taste organ due to the appearance of specific chemoreception for Glu, which manages gastric exocrine functions and enhances the gastric protein digestion. So, the stomach does not only represent a storehouse for food but also a functional organ sensing free Glu.[18]
The activation of the Dorsal Vagal Nucleus (DVN) and Nucleus of the Solitary Tract (NTS), which are brain nuclei were discovered with the functional MRI (fMRI) after gastric Glu cognition. Also the activation of each nucleus of the hypothalamus, which mediates body temperature control and feeding behavior, was observed. To show that the brain response initiated by luminal Glu is transmitted by the vagal afferent pathway, the brain activation was reduced by cutting the vagus nerve. Also the gastric effects of Glu were diminished by cutting the vagal nerve innervation to the stomach. In an experiment the scientists applied free Glu directly into the stomach to investigate the effect of free Glu on gastric acid secretion, using an enteral liquid diet containing amino acids and nutrients digested from protein.[18] They used the Pavlov pouched dog. This model was described by Pavlov, a Russian scientist. He split a dogs stomach surgically and separated the fundus and upper corpus from the main stomach, so that the solutions did not have any interaction with the mucosa.[21] Seventeen amino acids, namely Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, His, Arg, Ala, Asp, Gln, Gly, Pro, Ser, Tyr and micronutrients, vitamins and carbohydrates were enclosed in this liquid, but the liquid diet did not enclose any glutamic acid. As an outcome a slight gastric secretion was observed after applying the amino acid diet. In comparison the gastric secretion was much higher when free glutamate was applied intra-gastric in combination with the liquid diet in a dose-dependent way, whereas without the liquid diet the glutamate solution did not provoke any gastric acid secretion. This experiment showed that Glu has only an positive effect on the gastric secretion in combination with the amino acid diet, furthermore Glu affects directly the stomach and induces the digestion of dietary protein by stimulating the vagal nerve. The assumption that free Glu can lead to gastric

and duodenal ulcer is related to the digestion of protein with pepsin and gastric acid induced by Glu. As in experiments shown, luminal Glu solution enhances the duodenal mucus layer and has an effect on the gastric mucosa as well. Due to this findings further research showed that the addition of Glu to the diets (1% to 5%) reduces Non-steroid Anti-inflamatory Drugs (NSAIDs)-induced gastroduodenal ulcers in rats. In fact, free Glu has a protective function on the gastrointestinal mucosa against pepsin and gastric acid impact via mucus secretion.[18,19]

Besides the autonomic reflexes the process in the forebrain which registers the effects of ingested nutrients, is important to identify whether food is good or not good, and afterward determines further steps of feeding behavior. An intragstric application of isocaloric (60mM) glucose and 60 mM MSG solution induces forebrain activation, examined with a functional magnetic resonance imaging (fMRI) technique. Brain regions like the lateral hypothalamus, medial preoptic area, dorsomedial hypothalamus and the amygdala were extensively stimulated by intragastric application of MSG, whereas brain regions like the nucleus accumbens, amygdala, insular cortex and ventromedial hypothalamus are stimulated by glucose. It was also shown that the ingestion of glutamate or glucose can influence flavor preference in rats, because of positive post-ingestion effects. In the nucleus accumbens the dopamine release is triggered by sugar which can lead to addiction. In contrast Glu does not stimulate the nucleus accumbens, which suggests that the post-ingestion effects of Glu are diverse from those of lipids and sugar. Altogether it was shown that Glu has an effect on several physiological functions indicating an important role for dietary Glu in body homeostasis.[20]

Health problems, like functional dyspepsia, irritable bowel syndrome and obesity are often related to eating habits. Because of that, the nutritional information from the gastrointestinal tract which affects the regulation of food digestion and nutrient absorption gains more importance as it also has an effect on eating behaviors.[19]

3. Glutamate and gastric emptying

Other studies also showed that free glutamate in a high protein diet has a positive effect on delayed gastric emptying and abdominal unpleasantness after eating. This effect was

determined with a pure dextrin diet and a high protein liquid diet, which contained 50 percent casein and 50 percent dextrin. Additional free glutamate (0.5%) in the dextrin diet exhibited no improvement in the gastric emptying rate, whereas the addition of free glutamate to the high protein diet showed an improved gastric emptying. The same high protein liquid diet was used to determine the effect on post-ingestive abdominal uneasiness. No effect was observed in young adults (20-39 years), whereas adults over 40 years old showed a response to the protein diet containing free glutamate (0.5%) and showed an improvement in view of fullness and heavy stomach. The acceleration of the gastric emptying rate and improvement of abdominal unpleasantness is related to the increased gastric secretion, induced by free glutamate in a high protein diet as mentioned before. A different study revealed that patients with chronic atrophic gastritis who consumed hospital meals, containing free glutamate, showed an enhancement in Maximal Acid Output, Basal Acid Output (BAO) and appetite. Also the level of consciousness and nutritional parameters, like plasma albumin and peripheral circulating lymphocytes were increased by the consumption of hospital food.[18]
Some experiments showed that the preferableness for umami taste specifically increases glutamate when the body needs to metabolize ingested dietary protein. In this research a diet containing purified egg protein was given to rats and the preference for three solutions, namely glycine, NaCl and glutamate were exhibited. A protein content from 0-5 % shows a preference for sweet taste (glycine solution) and salty taste (NaCl solution) rather than umami taste (monosodium glutamate solution) in rats, whereas by a protein content about 20 % the preference changes and the rats prefer an umami solution rather than a glycine or NaCl solution. This shows that due to the enhancement of gastric protein digestion triggered by glutamate as mentioned before, the preference for umami taste increases when the diet contains a higher amount of protein. In addition the nutrient intake and the body amino acid homeostasis is managed by the amino acid sensing abilities of the gut, which can alter feeding behavior. [19]

4. Motility in the distal colon influenced by umami

In a study the motility in the distal colon was tested with activation of amino acid-sensing receptors, suggesting that T1R1/T1R3 (umami taste receptor) may cause changes in motility. The L-amino-acid sensing T1R1/T1R3 receptors were determined in the mucosal cells of rat, human, guinea and mouse colon with immunohistochemical analysis of colonic cryosections and these showed the attendence of T1R1 in certain

cells of the mucosal layer. In addition some T1R1-positive cells were found on the surface epithelium with the same stretched structure of brush cells, which have an approach to the intestinal lumen and its contents. MSG increases the release of calcitonin gene-related peptide (CGRP) above the basal levels, furthermore the release of CGRP is much higher when additional IMP was applied. Inosine 5′-monophosphate (IMP) enhances T1R1/T1R3 activation and therefore IMP is used to identify if T1R1/T1R3 receptors are also responsible for changes in GI motility and if the MSG effect is T1R1/T1R3-specific. The sensory neurons mediate the peristaltic reflex and are stimulated by CGRP. It was also shown that the contraction ascended (measured in the orad compartment) and relaxation descended (measured in caudad compartment) the limbs of the peristaltic reflex, after MSG+IMP was applied to the central compartment of a rat colonic flat-sheet preparation. A statistically significant change in ascending contraction (AC) and descending relaxation (DC) was observed at a concentration of 10 mM MSG. The combination of MSG and IMP induced a higher effect which suggests that the effect is induced by T1R1/T1R3 activation. Furthermore, this was confirmed in other studies, showing that MSG did not induce AC and DC in T1R1/T1R3 knockout mice. MSG also increases the velocity of fecal pellet propulsion which was again enhanced by IMP, but no effect was observed when IMP alone was applied. Control studies, where only NaCl was applied, indicated that there was no increase on pellet velocity due to additional sodium and higher osmolarity and that the effect of MSG was related to glutamate. In addition L-cysteine enhanced pellet velocity, whereas L-tryptophan did not increase pellet velocity because L-tryptophan activates CaSR (calcium-sensing receptor), which is a extracellular amino acid sensor and a class C G-protein-coupled receptor, and not T1R1/T1R3 receptors. This also proposes that CaSR is not involved in inducing colonic motility. There are a lot of pathways that induce peristalsis and the obstruction of one of those pathways does not hinder initiation of peristalsis and fecal pellet propulsion. For example initiation of the peristaltic reflex and the colonic motility are only reduced, due to excision of the mucosa and the loss of 5-HT in the enteric neurons, not fully lost. This stimulation of colonic motility by the activation of T1R1/T1R3, can be used to deal with motility disorders such as diarrhea and constipation. In a normally functioning gut the amino acids may not be able to penetrate into the colon, whereas in case of inflammatory gut disorders or prohibited absorption a larger amount of amino acids can penetrate. As shown in this study, the fecal pellet propulsion is enhanced by some amino acids, suggesting that this could lead to a diarrhea-like syndrome when a large amount of amino acid content reaches the

colonic lumen. Furthermore, this increased colonic motility enhances the removal of amino acids and decreases the high concentration in the lumen, which has an positive effect on gut health, because the fermentation of the amino acids by colonic bacteria (phenolic compounds, NH_3 and H_2S) are harmful for the gut.[22]

5. Effect of glutamate on HCO_3^- secretion

The gut hormones usually control the procedure of digestion after meal ingestion and are produced in the endocrine cells, which are than mixed in the gut epithelium. In previous studies it was shown that the secretion of the protective duodenal epithelial HCO_3^- is induced by postprandial luminal H^+ and pCO_2, which can also lead to a luminal ATP release by stimulating submucosal H^+ chemosensors. The assumption that other activators of HCO_3^- secretion, besides the gastric postprandial H^+ secretion, may exist, was the main reason for determining if the activation of the umami taste receptors could induce duodenal HCO_3^- secretion. A lot of nutrient-sensing G-protein-coupled receptors were identified on endocrine cells which leads to the theory that these receptors could trigger a hormone release all over the entero-endocrine cell basolateral membrane. This furnishes a stimulation of the HCO_3^- secretion from adjacent epithelial cells. Due to the expression of T1R1/T1R3 (umami taste receptor) on entero-endocrine cells, a study tried to determine whether the infusion of 1-Glu+IMP in the duodenum of rats is stimulating HCO_3^- secretion trough a hormone release mechanism and is inducing other mucosal defense mechanisms. The three hormones responsible for the enhancement of duodenal HCO_3^- secretion are glucagon-like peptide (GLP)-1, which is unleashed by oral glutamate, secondly GLP-2 which assimilates anion secretion and thirdly glucose-dependent insulinotropic peptide (GIP) which is unleashed from K-cells (identified in small intestine).[23] The K-cells were discovered in small intestine showing that these cells are an important origin for the circulating GIP. In addition, high-fat diet and obesity can enhance the density of K-cells and furthermore stands in connection with plasma GIP levels. [24] GLP-2 enhances HCO_3^- secretion by the secretion of vasoactive intestinal peptide (VIP), as VIP has an approved secretagogue activity for HCO_3^- secretion. Besides the vasodilation of the enteric smooth muscle is induced by VIP and nitric oxide (NO). The study showed actually that actviation of umami taste by 1-Glu+IMP induced GLP-2 release from L-cells, resulting in a higher HCO_3^- secretion and induced GLP-1 release into portal vein (PV). Due to the intestinotrophic effects of GLP-2 and the insulinotropic effects of GLP-1 an additional

positive effect on the duodenal mucosa and the glycemic control can be related to umami receptor activation. The umami receptor activation by luminal amino acids leads to secretion of the insulinotropic GLP-1 and intestinotrophic GLP-2 as mentioned before and induces HCO_3^- secretion which improves the protection and the repair mechanism of the duodenal mucosa while improving glycemic control.[23] On the other hand some indications lead to the hypothesis that lowering the GIP production or activation may be a way to diminish obesity. As mentioned before, the K-cells release GIP meal-dependent and can be a resource for producing glucose-lowering agents (insulin) or satiety factors genetically. It was determined that transgenic mice show a resistance to induced diabetes by beta-cell toxin. The incretine hormone GIP enhances insulin release from the beta cells of the pancreas and has an effect on insulin secretion, nutrient absorption and glucose homeostasis like other hormones unleashed by K- and L-cells also have an effect on this factors.[24,25] One study showed that GIP has detrimental effects in obesity and diabetes and the incretin effect is not pre-existing in diabetes. So the most effective way in medicating obesity and diabetes is to keep the GLP-1 secretion from L-cells high and instead reduce the GIP secretion from K-cells. [26] It seems that GIP and K-cells have a very important function in health and disease and the enhanced GIP release by umami activation should be considered controversial as it protects and repairs the gastric mucosa on the one hand but has detrimental effects in obesity and diabetes on the other.

Compounds binding on the metabotropic glutamate receptor and also some amino acids, do not enhance the HCO_3^- secretion but the co-perfusion of an amino acid with IMP synergistically increases HCO_3^-. This synergism was also shown when additional IMP was added to a concentration of 10 or 50 mM 1-Glu, which enhanced HCO_3^- secretion, whereas the application of 100 mM 1-Glu without IMP was needed to see an obvious effect on HCO_3^- secretion. This synergism underlines the hypothesis that HCO_3^- secretion is enhanced by umami taste activation, because of the cooperative binding site for 1-Glu and IMP on the Venus flytrap domain of T1R1. So in fact the GLP-2 release and GLP-2R activation and furthermore VIP and NO release, triggered by 1-Glu/IMP, leads to an enhancement of duodenal HCO_3^- secretion and leads to the conclusion that the umami receptor may protect the intestinal mucosa.[23]

In fact another study tried to determine if the addition of MSG to soup has an effect on energy intake and food selection in obese women without eating disorders. The

additional MSG in the vegetable soup increased the perception of creaminess and satisfaction. In comparison the MSG soup preload before lunch or a mid-afternoon snack decreased the energy intake and leads to a reduction of the total energy intake in obese women without eating disorders than the soup without MSG added. In addition to that, the consumption of high-fat savory foods was lower at snack time, lunch and in total when a vegetable soup with MSG was consumed. Although the second MSG soup did not decrease the energy intake substantial at snack time, the test persons did not compensate the lower energy intake after lunch as there is a synergistic effect of MSG on the feeling of satiety. But it was also shown that during the intervention period the soup with MSG enhanced hunger ratings, although this did not induce a higher energy intake. In fact other studies also reported the assumption that there is no significant correlation between hunger ratings and energy intake, furthermore that lower hunger ratings did not necessarily lead to lower energy intake and vice versa. Entero-endocrine cells in the gut wall produce glutamate signalling molecules and the administration of glutamate excite the afferent vagal fibres of the gastric branch and furthermore stimulates certain forbrain regions via vagal nerves. Aditionally in rodents cholecystokinin (satiety-related hormone) increased after glutamate administration to intestinal tissue and after MSG ingestion and in humans the glucagon-like peptide-1 (GLP-1) increased. As well it is possible that the MSG stimulation in the oral cavity is participating in the energy intake after ingestion of food with MSG, but for this more studies are required to determine the mechanism. One matter in question this study had was that the soup was served twice a day 10 min before lunch and snack time, which is not a normal setting. So more studies are required with a protocol more related to normal daily eating behaviors such as eating soup just at lunch. The second issue was that this was tested in a short period of time and actually more studies with a long term determination are needed to see if MSG has a long term effect on energy intake and obesity.[27]

Glutamate and asthma

Western countries like USA, Canada and Australia show a higher percentage of adults having asthma (10%) than in developing countries. A lot of hypotheses and reasons were suggested to clarify the higher percentage of people having asthma in developed

countries than in developing countries. Reasons like enhanced hygiene and antibiotic usage, obesity, less indoor allergens exposure and low intake of dietary antioxidants were carried out. Some studies suggest that nutrition may also contribute to the higher percentage of asthma in developed countries and that a correlation between asthma and dietary pattern exists. The connection between asthma attacks and ingestion of a Chinese meal was first reported 1981, suggesting that MSG, which is commonly used in Asian cooking in high amounts, is responsible for asthma. Since then inconsistent results were observed and a lot of studies were carried out with poorly research methods. In the latest review it was reported that there is no need for adults with chronic asthma to avoid MSG, because of the lack of evidence. Furthermore, a study determined with ovalbumin(antigen)-sensitized mice the effect of MSG and concluded that there is no correlation between constant ingestion of MSG and developing asthma. So in conclusion, recent studies and investigations suggest that the intake of MSG in high amounts do not lead to asthma, but in fact more studies with larger samples and younger subjects are needed to confirm this hypothesis.[28]

Umami-bitter taste interaction

The taste-taste interaction between umami and bitter can also have an effect on eating behavior. A team of scientists studied on the umami-bitter taste interaction in bitter taste receptors (TAS2Rs). They used hTAS2R16-expressing cells in order to determine the influence of umami on Ca^{2+}-flux signaling assay in combination with the bitter substance (salicin). The hTAS2R16- cells were used due to high expression and extensive activation by different ligands compared to other hTAS2Rs. Umami peptides, like Glu-Asp, Glu-Glu, Glu-Ser, Asp-Glu-Ser, Glu-Gly-Ser decreased the intracellular calcium influx induced by salicin, whereas Gly-Gly which is a tasteless peptide did not decrease it. MSG in addition with salts of 5′-ribonucleotides or adenosine monophosphate also inhibits bitter tastes and decreases the response of C57BL/6J mice to quinine hydrochloride (bitter substance). Surprisingly, Glu-Glu showed a higher suppression of hTAS2R16 than probenecid, which is an antagonist specific for hTAS2R16. The umami peptides bind directly in the binding region against orthosteric ligand site and inhibit the effect of hTAS2R agonists like salicin. This suggests that umami peptides can have a high impact on bitter taste suppression via bitter taste receptors. The importance of this suppression is related to the negative effect of bitter on

food intake and the general goal of enhancing the intake of healthy food without the loss of taste pleasantness. The interaction between umami and bitter was shown in the parabrachial nucleus (PbN) and the chorda tympani (CT). In this study bitter taste receptors were used which induce the activation of G-protein and phospholipase C (PLC), after binding bitter substances. Diacylglycerol (DAG) and inositol triphosphate (IP_3) are produced after hydrolysis of Phosphatidylinositol-4,5-bisphosphate. Those are second messengers of G-protein-coupled receptor signaling. For the declaration of inhibitory efficacy of bitterness and the correlation between the mixture of amino acids in glutamyl peptides, as well to gain information about cognitive interaction and cell-to-cell interaction further studies will be needed.[29]

Effect of umami taste on the brain

The taste signal, which has its origin in the taste receptor cells, is transferred to the brain via cranial nerves, the facial nerve, glossopharyngeal nerve, and the the vagal nerve. In different studies the primary gustatory cortex in the anterior insula and the orbitofrontal cortex, which is the secondary taste area, where analyzed constantly. For people who are not familiar with the umami taste such as Germans and Norwegians, the recognition is very variable. The main goal of the study (*Cerebral processing of umami: A pilot study on the effects of familiarity)* [30], was to test the effects of familiarity to the umami taste and which impact this exerts on brain activation. The authors used a functional magnetic resonance imaging (fMRI) to analyze the brain activity before and after familiarizing with the umami taste. After the training sessions the results from pleasantness, intensity and familiarity revealed that the participants got acquainted with the umami taste so that the familiarity increased ($p<0.001$, paired T-test=23.2, df=9). The impression of intensity and hedonicity remained the same.

The substance used in this study was MSG which elicits the umami taste. Participants where chosen who where not familiar with the MSG taste, so they could test if there are any differences in cortical representation of gustatory function before and after familiarization with the new taste. The results showed that there are no major differences, but there was an additional activation in the parahippocampal gyrus. The parahippocampal gyrus is connected to effects of perceptual training and the memory processing.

The team tested the capability of the participants to detect the umami taste in three tests.

In the first test they wanted to be sure if the participants can taste glutamate, because previous studies showed that if there are SNP´s (Single nucleotide polymorphisms) in the subunits of T1R1 and T1R3, which belong to the glutamate taste receptor, the ability of tasting glutamate was not pre-existing. A sample of water and liquid of MSG (50mM) was given to the participants. They were requested to characterize the samples and additionally to give a comment to familiarity, hedonicity and intensity to the tastant. In the second test, liquid solutions of salt (NaCl 50mM), sugar (sucrose 50mM) and MSG (50mM) were given to the participants. The ulterior motive was to identify whether the participants could distinguish between the three tastants, which all of them could. In the last and third test four different concentrations of umami solutions were arranged randomly namely 10mM, 25mM, 50mM and 75mM. According to the concentration, all participants managed to place the samples in the right order from 10mM to 75mM, after training. This study shows that there is an involvement of the anterior cingulate cortex and the rolandic operculum in taste sensing and there are no changes in the response at the level of taste primary areas when umami taste was submitted over a short period of time, but interestingly the parahippocampal gyrus was activated.[30]
The Japanese physiologist Takashi Hayashi suggested that the neurotransmitter glutamate builds up human memory. But his hypothesis was declined with following outcomes. Firstly, nearly all free glutamate is depleted as an energy source in the intestinal mucosa and just 5 percent is transmitted to the bloodstream. Secondly, the blood-brain barrier inhibits the diffusion of plasma glutamate into brain tissue. Thirdly, glutamate is not taken from peripheral blood, but is synthesized in the brain for neuronal function.[18]
To determine if umami taste has a significant effect on the parahippocampal gyrus and effects perceptual training and memory processing, further studies will be needed.

Negative effects of MSG

Some studies presented the negative effects that MSG can exert in animals and humans in high doses. High concentrations of MSG in stages of brain development lead to damage of the hypothalamus causing neuroendocrine trouble.[31] The neurotoxic effect is related to the neonatal period, because the blood-brain barrier is not fully developed and thus MSG can easily penetrate then the blood-brain barrier.[32] This influence on the hypothalamus can than lead to behavioral disorders in adulthood, like hyperexcitability, depressive and anxiogenic behaviors, obesity, impairment of memory,

pain-sensitivity and changes in analgesic responses.[31] It was shown that the anxiogenic- and depressive-like behaviors are induced by dysfunction in the serotonergic system after exposure of newborn rats to subcutaneous injection of MSG. The deregulation in the HPA axis and the enhancement of 5-HT uptake in cerebral cortices lead to this dysfunction in the serotonergic system. The anxiogenic-like behavior was assumed due to minimized time of grooming during locomotor activity and enhanced freezing time, furthermore the amount of fecal pellets, vocalization and urine excretion increased in rats. For today's society anxiety and depression are diseases which should not be neglected because they are often related to higher mortality and morbidity. A lot of studies exhibit that the serotonergic system and changes in the 5-HT neurotransmission are involved in these diseases. In addition it was shown that rats who became MSG had a higher concentration of serum corticosterone and ACTH suggesting a deregulation of the HPA axis which is related to behavioral changes and is also observed in depressive patients. All these results together suggest that a subcutaneous injection of MSG leads to anxiogenic-like and depressive-like behaviors. [32]
The enhancement of extracellular glutamate levels happens due to enhanced release of glutamate and decreased glutamate uptake. The glutamate receptors regulate pain hypersensitivity and excitatory synaptic transmission by binding glutamate. In some studies the results showed that due to accumulation of glutamate in the hippocampus, the glutamate uptake in rats was decreased causing excitotoxicity and suggesting that the enhancement of glutamate in the hippocampus is induced by nociception caused by MSG. Also the expression of NMDAR subunits changes when the glutamate concentration increases. Furthermore, the gene expression of NMDAR subunits in the hippocampus is enhanced with MSG neonatal. The release of pro-inflammatory cytokines like IL-1β, INF-γ, IL-6 and TNF-α is enhanced by MSG, whereas the anti-inflammatory IL-10 is decreased. These cytokines are only recognizable under pathological events (excitotoxicity and neuro inflammation) in the central nervous system. Pro-inflammatory cytokines are also able to increase extracellular glutamate levels, because it was shown that IL-1β inhibits the glutamate uptake and enhances neuronal excitability. In addition, the release of mediators like bradykinin, substance P, histamine and nitric oxide is triggered by IL-1β, TNF-α, IL-6 and INF-γ leading to the appearance of pain. Thus it is suggested that MSG leads to nociception, but the pathophysiological mechanism was not determined precisely. [31]

Headache and craniofacial pains like temporomandibular disorders (related to

dysfunction of the muscles of mastication causing pain) are often connected with the intake of MSG. Therefore, a study was conducted designed as a double-blind, randomized and placebo-controlled, to determine if ingestion of MSG has an effect on muscle pain sensitivity. One possible cause that the application of Glu intramuscular can simulate symptoms of myofascial temporomandibular disorders is that patients with myofascial TMD showed increased Glu concentrations. Furthermore the oral application of 150 mg/kg MSG in healthy patients induced peri-cranial muscle tenderness and enhanced headache. In addition, 150 mg/kg MSG is enhancing the Glu concentration in the masseter muscle about 750% over the baseline concentration. The intake of high amounts of MSG is one possible way of enhancing Glu concentration. This study tries to determine if oral applied MSG can lead to enhanced pain and mechanical sensitivity compared to placebo.[33] Some previous studies showed that the systolic blood pressure increases due to oral ingestion of 150 mg/kg MSG by 5-10%, whereas the heart rate decreases and the effect on the diastolic blood pressure is more differential.[34] The intramuscular injection of Glu into the temporalis muscle and the masseter shows a substantial enhancement of the systolic and diastolic blood pressure. It is actually not clear if the pain, caused by the Glu injection, increased the systolic and diastolic blood pressure or if there is also a postive inotropic effect of Glu on the heart. For example it was shown that the Glu receptor agonist NMDA enhances blood pressure through a nociceptive input and a positive inotropic effect on the heart. This inotropic effect was observed when NMDA reached the systemic circulation after the clearance from the muscle. Some results of recent studies suggest that the muscle pain sensitivity is not increased by the intake of a high amount of oral MSG, whereas others suggest a connection between nociception and MSG. Furthermore, there is a correlation between systemic MSG administration and increased blood pressure. [33]

A complex of symptoms namely weakness, palpitation and numbness at the back of the head after consumption of a Chinese meal was firstly reported by Kwok and he also suggested that MSG may be involved in causing these symptoms. These symptoms were firstly called Chinese Restaurant Syndrome and afterward the Federation of American Societies for Experimental biology (FASEB) introduced the term MSG symptom complex to reveal that these symptoms can occur after MSG intake. The intake of more than 5 g leads to these above mentioned symptoms. It was shown that some lactic acid bacteria species have positive effects on health and have nutritional benefits, like better digestion of lactose and the control of serum cholesterol levels, intestinal infections and even some types of cancer. These lactic acid bacteria are

present in cheese, yoghurt and in the gut microbiotica. Furthermore, some lactic acid bacteria metabolize MSG to gamma-aminobutyric acid (GABA) and are used to generate food with high GABA concentrations, because of its diverse physiological effects. One kimchi-derived (kimchi: traditional fermented Korean side dish) lactic acid bacterium namely *Lactobacillus brevis* G-101 was isolated and it was shown that this specific *Lactobacillus* decreased the blood concentration of MSG in mice that were orally fed. In addition, the results showed that *L. brevis* G101 was not significantly converting MSG to GABA, but rather suppressing the absorption of MSG from the intestine into the blood. A double-blind, placebo-controlled study, consisting 30 Korean test persons who reported themselves as MSG-sensitive, tried to determine whether the oral application of *L. brevis* G101 can diminish the MSG symptom complex using maltodextrin as placebo. A exclusion criteria was the use of medicaments. After ingestion of rice with black soybean sauce which contains 6g of MSG (RBSM: is the rice with black soybean sauce containing 6 g MSG), the test persons conveyed there condition and the team examined the intensity of the MSG symptom complex. The subjects were split into two groups. One group took a capsule containing *L. brevis* G101 and the other group one capsule maltodextrin orally for five days. After these five days RBSM was applied and the subjects characterized the intensity of the MSG symptom complex over 6h. After this test the subjects switched the groups and the same test was executed for five days. The difference between the two groups was 23.3%, 43.3% characterized a strong MSG symptom complex in the placebo group, whereas 20.0% characterized a strong MSG symptom complex in the *L. brevis* G101 group. There was a greater difference among female subjects than among male subjects, suggesting that female are more sensible to the MSG symptom complex. The oral threshold for MSG-induced symptoms in 36 test persons was determined to be between 1.5 to 12 g, furthermore the intensity of the symptoms was enhanced when the oral dose was increased. The negative effects induced by application of RBSM were drowsiness, thirstiness, indigestion, weakness, headache and nausea. The daily intake amount of MSG and oral intake of more than 6g of MSG is crucial for inducing side effects. One potential way to reduce the side effects was the intake of vitamin C. The scientists looked for other options to reduce the side effects of MSG and determined the effect of *L. brevis* G101, which decreased the MSG complex substantially and shortened the disappearance time of the MSG symptom complex.[35]

The Umami taste receptor

To taste salt, sweet and umami the release of ATP from the taste bud cells is required. ATP plays the role of a neurotransmitter and is necessary for the activation of afferent neural gustatory pathways. The role of the calcium homeostasis modulator 1 in the ATP-releasing process could be shown recently [36]. It is a voltage-gated ion channel and is essential for the ATP release from taste bud cells. The expression of CALHM1 occurs in the type II taste bud cells which are generated for sweet, bitter and umami-sensing. The knockout of CALHM1 in mice hindered the recognition of these tastes, whereas the recognition of the tastes sour and salty stayed the same. The tastes sour and salty are sensed with the type III taste bud cells. The difference between the type II bud cells and type III bud cells is that type III bud cells have synaptic contacts and express synaptic vesicles, whereas type II cells lack classical synaptic structures. So type III cells are able to transfer signals directly to the nervous system. Type II cells need to release the neurotransmitter ATP to transmit the information to gustatory neurons.[36]
The scientists used knockout mice, where the loss of CALHM1-expression was generated in the taste bud cells to define the purpose of CALHM1. The absence of the CALHM1-signal in taste buds of knockout mice and also the results of the reverse-transcription PCR of isolated type III and type II cells and also individual taste cells, showed that the expression of CALHM1 occurs specifically in type II taste bud cells. In response of a reduced extracellular Ca^{2+}-concentration, the CALHM1-ion-channel can be activated. The activation of the ATP release from human CALHM1-expressing COS1-cells (fibroblast-like cell line, from monkey kidney tissue), *Xenopus oocytes* and HeLa cells (human cell line, cells taken from cervical cancer) was induced due to reduction oft he Ca^{2+}-concentration from 1.9mM to nearly zero (17nM). Also a membrane polarization can induce the CALHM1-ion-channel activation. CALHM1 links the taste receptor activation and the generation of Na^{+}-action potentials and is an important element in the signal-transduction cascade in type II cells. The involvement of other CALHM isoforms, pannexin 1 and connexins in ATP release cannot be ruled out, because they are also present in taste bud cells and possibly fullfiling there function parallel or in combination with CALHM1. [36]

One big issue in finding out which receptors play a main role in umami taste transduction is that glutamate also serves as a neurotransmitter. Some receptors have already been identiefied in taste bud cells, namley T1R1/T1R3 a heterodimer,

mGluR1, mGluR2, mGluR3 and mGluR4 metabotropic glutamate receptors, and ionotropic glutamate receptors like NMDA (*N*-methyl-D-aspartic acid or *N*-methyl-D-aspartate) and kainate receptors.[8,9,10] The kainate receptor responses to glutamate which plays also a role as a neurotransmitter and is classified as a non-NMDA ionotropic receptor. With the activation by the agonist kainate which was extracted from the red algae *Digenea simplex* this receptor was first analyzed.[11]
Since the knockout of T1R1/T1R3 was analyzed in studies and the genetic data showed that this knockout disabled the responses to glutamate, the question appeared if this heterodimer receptor was the only umami sensing receptor. The metabotropic glutamate receptors also showed a high potential to be involved in the umami sensing pathway.[8]

It was shown that in T1R3 knockout mice the capability to recognize umami stimuli was only partially lost and furthermore in wild-type mice the diverse umami stimuli were registered in separate afferent neurons or populations of taste bud cells. These different taste cells which can sense umami compounds, suggest that there are more than just one single molecular receptor. In addition, a study determined that umami sensing is working despite the absence of the T1R1+T1R3 heterodimer. It was determined what happens in mice when the alleles which are encoding the T1R1 subunit are deactivated and how this affects the taste transduction from two nerves responsive for taste information. In these two nerves, namely the chorda tympani nerve and the glossopharyngeal nerve, the stimulation from MSG was not disturbed, although the T1R1 subunit was not pre-existing. These findings suggest that there are also other umami receptors bedsides the T1R1+T1R3 receptor.[37]

One study tried to find out if the brain-expressed mGluR4 and mGluR1 are involved in the taste transduction of umami. The authors [38] used a combination of conditioned taste aversion (CTA) and detection threshold methods in mice. With glutamate agonists and antagonists they tried to examine the involvement of mGluR4 and mGluR1 which are expressed in the brain but where also found in taste papillae. These brain-type mGluR´s showed higher sensitivities to glutamate. Mice detected the mGluR agonist L(+)-2-amino-4-phosphonobutyrate (L-AP4) at a concentration of 0.0009–0.0019 mM. Correspondingly at 0.0001 mM and more the mice that have been conditioned showed an aversive response to MSG or MPG restrained aversive response to glutamate agonists using CTA methods. Furthermore, this was the case at concentrations between 0.0001 and 100 mM (*RS*)-α-cyclopropyl-4-phosphonophenylglycine (CPPG), and 1-

aminoindan-1,5-dicarboxylic acid (AIDA), which are antagonists of mGluR. From these results it can be inferred that brain-expressed mGluR4 and mGluR1 may also take part in the umami taste transduction. The assumption that there are multiple receptors for umami is preexisting because of several lines of data showing for example that the differentiation between the taste of umami and sucrose was only suppressed but not fully eliminated in knockout mice. There was also a Ca^{2+}-response to MSG from taste cells in sliced circumvallate papillae of T1R3 knockout mice. Also a very important fact showing that other umami receptors may exist, is the expression, not only of taste-type, but also of brain-type mGluR1 and m-GluR4 in taste buds. The indication that glutamate can trigger a taste at lower concentrations than mentioned formerly is preexisting, because of the results this study is providing. The concentration range was between 0.0001and 30mM. This data also strengthens the hypothesis of brain-mGluR taking part in umami taste transduction. The stimulation of brain-mGluR receptors occurs at low μM concentrations. In addition to that, the repletion is reached below the low mM mark of activation for the taste-mGluRs and the T1R1+T1R3 receptors. In conclusion, the mice responded to glutamate compounds and L-AP4, whereas the conditioned taste aversion (CTA) responses were suppressed by antagonists for mGluR1 and mGluR4. These findings and also the results of other studies so far propose that the brain-type mGluR1 and mGluR4 are maybe involved in perception of umami taste in mice.[38]

T1R1/T1R3 from which the signaling pathway starts, is a heteromeric G-protein. The activation of phospholipase C β2 (PLCβ2) is caused by the subunit Gβ3γ13, so that the Gβγ subunit from a heteromeric G-protein plays an important role in transferring the signal of the umami taste. The activation of the monovalent-selective cation channel TRPM5 (transient receptor potential cation channel subfamily M member 5) is caused by releasing Ca^{2+} from intracellular stores, whereas the whole process starts with phospholipase C β2 (PLCβ2) producing inositol triphosphate (IP_3) and diacylglycerol. IP3 leads to releasing Ca^{2+} by binding to the IP_3 receptor (IP_3R3). The release of ATP (adenosine-5'-triphosphate) provoked by TRMP5 causes a taste cell depolarization onto gustatory afferent fibers.[8,12]

There is a similarity in the mechanism between the knockout of T1R3 and the knockout of TRPM5, IP_3R3, PLCβ2 which leads to a reduced response to umami sensing. Certain substances which act as inhibitors of Ca^{2+} and PLCβ2 sustain the intracellular Ca^{2+}-

stores and thus prohibit the responses of glutamate and nucleotides added to the taste pore. The α-gustducin appears in T1R1/T1R3 in fungiform and palatal taste buds, instead a dissimilar Gα subunit, which is not fully identified, is present in T1R1/T1R3 in circumvallate and foliate taste buds.[8,13] The subunits α-gustducin and α-transducin lower the intracellular cAMP (Adenosine-monophosphate) concentrations by stimulating phosphodiesterases (PDEs), which leads to a different umami taste impression when a knockout of these subunits is conducted. So, an interaction between α-gustducin and α-transducin in the umami taste signaling can be concluded. [8]

"Molecular mechanism for the umami taste synergism" a released paper in 2008, characterized the Venus flytrap domain (VFTD) of T1R1. The VFTD is found in the umami receptor and is an N-terminal domain which is compiled of two globular subdomains.[14] In another study the team tried to find a good umami sensing model, referring to the pig as laboratory animal. By using molecular modeling the researchers matched the Venus flytrap binding domains and the biology of T1R1 sequences, also involving L-amino acid agonists of multiple mammals. Laboratory rodents, humans and pigs were included in these trials. The highest response of umami taste was induced by the L-amino acid L-glutamate and MSG in human and pig models. The umami taste receptor of laboratory rodents like mice and rats reacted to most of the 18 used L-amino acids in comparison to pigs, which only sensed eight different L-amino acids or to humans which only sensed two L-amino acids. So the scientists suggested that there is a higher similarity between the umami sensing model of pigs and humans, than laboratory rodents and humans.[15]

A seven-transmembrane domain (TMD) and small extracellular cysteine-rich domain (CRD) are linked to the Venus flytrap domain (VFTD). A large extracellular Venus flytrap domain (VFTD) is present in different receptors, including the class C G-protein coupled receptors T1R1/T1R3, calcium sensing receptors, T1R2 (T1R2/T1R3) which is the sweet receptor component and also the metabotropic glutamate receptor (mGluRs). The execution of an x-ray crystallography, by means of the extracellular domains of mGluRs have been identified, suggests the existence of two lobes in the VFTD. Between these two lobes, in the hinge region the ligand binding site is located. The mouse T1R1/T1R3 responds mostly to L-amino acids, but much weaker to acidic amino acids than to others, while in comparison the human T1R1/T1R3 responds exclusively to 1-Glu.[39]

Although, five residues have been identified as crucial for binding 1-Glu at the hinge region of hT1R1, the residues are maintained between human and mouse T1R1. This fact indicates that the additional and critical residues for acidic amino and acid recognition should be determined. The mechanism has been evaluated using human-mouse chimeric receptors and point mutants of T1R1/T1R3. This finding has been accounted as the mechanism showing the difference between species in their amino acid recognition.[39]

The modulation of amino acid recognition in mouse- and human-type T1R1/T1R3 was studied with point mutants of T1R1/T1R3 and chimeric human-mouse receptors. Due to this analysis 12 key residues were discovered in the extracellular Venus flytrap domain of T1R1.

The residues crucial for the recognition of acidic amino acid in human-type T1R1/T1R3 are present in the orthosteric ligand binding site, which was unveiled by molecular modeling, whereas the main residues for the mouse-type response were neither at the orthosteric ligand binding site nor the allosteric binding site for the natural umami taste enhancer inosine-5′-monophosphate (IMP), but outside of both regions. For the investigation of the key domains for amino acid recognition a luminescence-based assay was used instead of a fluorescence-based assay, because of the difficulties detecting responses to 1-Asp. [39]

HT1R1/hT1R3 showed the highest response intensities to 1-Glu and 1-Asp out of the 17 appraised amino acids. Additional response to 1-Ala, 1-Ser, 1-Gln, 1-Asn, 1-Arg, and 1-His was detected when exposed to hT1R1/hT1R3, whereas a weaker response was triggered by 1-Ala, 1-Ser, 1-Gln, and 1-Asn at high concentrations. In comparison, the mT1R1/mT1R3 showed much higher response intensities to multiple L-amino acids than to acidic amino acids (Asp, Glu). From these findings it can be deviated that the differences in ligand specificity between mouse and human T1R1/T1R3 are due to the sequence differences in these receptors.[39]

L-Theanine shows a remarkable impact on tea quality and taste. This particular amino acid occurs in green tea. L-Theanine leads to an activation of T1R1 + T1R3-expressing cells, which was showed after analyzing the activity of L-Theanine on the T1R1 + T1R3 umami taste receptor. L-theanine binds to L-amino acid binding site in the Venus flytrap domain of T1R1.[40]

Due to the similarity of L-theanine and Glu, plus the induced umami synergy with 5′-

ribonucleotide it can be predicted that L-theanine activates T1R1+T1R3. The investigators used human T1R1+T1R3 (hT1R1+T1R3)- and mouse T1R1+T1R3 (mT1R1+T1R3)-expressing human embryonic kidney 293T (HEK293T) cells, to determine if the taste of L-theanine is exposed via T1R1+T1R3. For the identification of the L-theanine binding site T1R1 mutants were utilized and also the cellular response to L-theanine in the attendence of IMP was investigated.[40]

T1R3 was at first found in gustatory tissue, but is additionally found in other cell types and tissues like the heart, skeletal muscle, intestine and pancreatic β-cells. Not only the taste recognition is controlled by T1R1/T1R3, the receptor also has a function as an amino acid sensor which controls the mechanism for the secretion of hormones, like insulin, cholecystokinin, and duodenal HCO_3^-. In addition to that, the mammalian rapamycin complex 1 (MTORC1), which inhibits autophagy, is activated by T1R3. A higher rate of autophagy in the skeletal muscle, heart and liver is a result of a T1R3 knockout in mice. [41]

How the expression of T1R3 works precisely is still unknown, though T1R3 is important for a variety of physiological functions and is found in several cell types and tissues. For the characterization of the genomic region upstream of *T1R3* in mammals, the authors used functional and comparative genomics.[41] Two different evolutionary conserved regions (ECRs) with distinct functions were described by the scientists. One of these regions represents the repressor, the other region the promoter of human T1R3 expression. Also the muscle regulatory factors Myogenin and MyoD manage the T1R3 expression. Additionaly the expression level of T1R3 alters and raises with myogenic differentiation of murine myoblasts.

The expression of murine T1R3 is determined due to overexpression of MyoD or myogenin. So it can be inferred that the stimulation of T1R3 during C2C12 cell myogenesis is triggered by myogenin and basal expression levels of T1R3 in C2C12 cells are maintained by MyoD.[41]

The skeletal muscle tissue expresses T1R1, which operates with T1R3 in umami taste sensing. Interestingly in the T1R1/T1R3 complex, T1R3 acts like a nutrient-sensor for amino acids, managing autophagy in muscle tissue. As a result of a T1R3 knockout in mice the autophagy frequency of skeletal muscle cells is much higher. In times of amino acid destitution the skeletal muscle provides stored amino acids, furthermore T1R3 is crucial for exposing nutrient demands. These findings suggest the potential of MyoD

and Myogenin regulating skeletal muscle metabolism and homeostasis with the regulation of *T1R3* promoter activity.[41]

The Ca-sensing receptor (CaSR) and kokumi

The Ca-sensing receptor (CaSR) which is a class C G-protein-coupled receptor is a extracellular amino acid sensor and is found in a variety of tissues, like the central nervous system, brain, the vasculature and gastrointestinal tract.[42] CaSR was also found in the kidney and parathyroid gland and has a important role in the calcium homeostasis. Furthermore, CaSR detects an enhancing blood calcium level, continuously decreasing the parathyroid hormone secretion, inducing urinary calcium excretion and enhancing calcitonin secretion to stabilize the blood calcium and return it to normal levels. Due to a lack of calcium the palatability of calcium increases, suggesting that there is a calcium transduction mechanism in taste cells. Agonists like neomycin, NPS R-568, and several L-amino acids, induced a response in isolated taste cells. [43] This receptor responds to various amino acids, but it was also shown that the receptor responds to peptides like γ-glutamyl peptides and glutathione (kokumi substances). Therefore, CaSR serves as a taste receptor for Ca^{2+} and protein and in addition is responsible for the release of dietary hormones in the intestine via amino acid sensing. The assumption that CaSR serves as a taste receptor first occurred when a 'calcimimetic' substance, a positive allosteric modulator of the CaSR in bullfrogs induced a stimulation of the taste cells resulting in neuronal responses. In rats and mice CaSR were found in the foliate, circumvallate and the fungiform papillae. There exist three different classes of taste cells: Type I (glial-like) cells are responsible for the elimination of neurotransmitters via degradation or absorption, the G-protein-coupled receptors (including T1R and T2R) which sense sweet, bitter and umami compounds are located on type II cells transferring the taste qualities and the G-protein gustducin and lastly the type III cells are presynaptic, forming synaptic contacts with nerve terminals and processing the signals from type II cells. CaSR is expressed in type III cells, but there is also some evidence and possibility that the receptors are expressed in type I cells. The problem was that they were not able to show functional coupling to the PLC-dependent Ca^{2+}-signalling pathways in type I cells. A lot of results have been obtained with rodent taste cells, but there is also evidence that CaSR also plays a role in human taste transduction. Glutathione and γ-glutamyl-valine-glycine are kokumi taste

substances, they just alter salty, sweet and umami tastes without causing any taste. These substances also activate CaSR, furthermore in CaSR-expressing HEK-293 cells, with half maximal effective concentration (EC_{50}) values, it was shown that CaSR agonist activity and kokumi taste intensity correlated. Also the inhibitor of γ-glutamyl-valine-glycine namely NPS 2143, decreases the kokumi effect of CaSR agonists.[42] Some results revealed that water extracts from garlic, which include GSH, lead to a more intense umami taste and so the authors called this inducing character "*kokumi* flavor". From all γ-glutamyl peptides, which are CaSR agonists, γ-glutamyl-valinyl-glycine (γGVG) is the most stimulating kokumi substance and leads to the highest kokumi flavor activity. To measure cellular responses the kokumi stimuli were applied focally on the apical tips of the taste buds. It was determined that *kokumi* substances stimulated a $[Ca^{2+}]_i$ response in taste cells in the posterior tongue, suggesting the detection of kokumi substances by CaSR-expressing taste cells. In the kokumi transduction there is a release of stored Ca^{2+} and no involvement of Ca^{2+} influx. This was shown with the stimulation of the cells by γGVG, which was not affected by Ca^{2+}-free conditions. [43]

The question appeared if γGVG (CaSR ligand) and glutamate stimulate the same mouse type II cells. It was assumed that γGVG induces the same taste as L-glutamate by stimulating the same taste receptors as umami compounds. For this reason scientists applied monopotassium L-glutamate (MPG) + inosine monophosphate (IMP) and γGVG focally on circumvallate taste buds. This test showed that γGVG (100 μM) and MPG (100 mM) + IMP (1 mM) induced a Ca^{2+}-response in different taste cells, suggesting that there are specific receptors for γGVG and MPG + IMP in different cells that produce a Ca^{2+}-response. But there is still the potential that some cells produce a Ca^{2+} response to both agonists and that there is a cell-to-cell signalling. These results show that the CaSR in native taste tissues and the umami taste receptors differentiate from each other, although some studies suggested that CaSR may dimerize with the T1R3 receptor subunit which elicits umami and sweet taste CaSR is specifically for kokumi transduction and is not directly involved in umami and sweet taste signalling. The taste cells are responsive for kokumi substance-sensing. The taste buds responsible for kokumi sensing are non-presynaptic and also presynaptic (Type III), but the precise assignment for the kokumi taste cells need to be clarified. The activation of sensory

afferent fibers in the same taste bud can be induced by activation of kokumi taste cells and by ATP which is released from the receptor. It is also possible that ATP effects the cells in the taste bud, because it acts as a paracrine transmitter.[43]

The umami aftertaste

One temporal feature of taste is the aftertaste which is not separately seen as a taste quality but as an important factor for exhibiting food quality or preference and choice of foods. Dashi which is a broth made from kelp or dried bonito is a common food additive used in traditional Japanese food and induces umami taste. In addition to dashi, soy sauce is added when cooking because it contains glutamic acid and dashi contains besides additional 5′-ribonucleotides. So both combined they induce an enhanced umami taste and intensity, but on the other hand it was shown that the umami aftertaste was decreased. This indicates that some components in the soy sauce decrease the umami aftertaste. In a study [44] the authors looked for the components and the key factors that have an effect on umami aftertaste with methods like sensory analysis, size fractionation, enzymatic treatment and chemical analysis. As a result the components that decrease the umami aftertaste were isolated and a strategy was found for producing soy sauces that enhance umami taste more sufficient. These aftertaste decreasing components are cellulose polysaccharides with a molecular weight of 44900 to 49700, whereas molecules with a weight of less than 1000 have a positive effect on the umami taste. With this result it will be possible to produce food containing less or none polysaccharides with a molecular weight of 44900 to 49700 and increase the palatability of food and enhance the umami aftertaste. [44]

Taste loss in elderly people

Elderly people often show a decreased gustatory function that exert a negative influence on health, because it can affect dietary intake and can lead to changes in eating behaviors. In general, the loss of smell and taste acuity leads to a decreased quality of life, by enhancing the risk of consuming spoiled meat and by inhibiting the enjoyment of eating or the smell of flowers and fresh air. The inability to share such experiences with other people can cause social disturbances, personal isolation and furthermore lead

to depression.[53,60] The detection thresholds for bitter and sour tastes, e.g. show an enhancement in elderly people, but the sensation for sweet, salty and umami is reduced with age. This kind of taste-loss leads to a higher consumption of sweet and salty foods and furthermore to different kind of health issues like obesity, hypertension and cardiovascular diseases. Many various factors can cause a taste-loss in elderly people, including a reduction of olfactory function, less oral health or taste receptor cells dysfunction and the general association with aging like diseases, poor health and a greater consumption of medicament's. The number of taste bud cells in the circumvallate papillae is decreased in elderly people, as was proved microscopically. Furthermore, with histomorphometric analysis it was shown that in the laryngeal surface of the epiglottis the mean density of taste buds was lower compared to younger people. There were not so many studies carried out to examine if umami taste also declines age-related, but three studies tested the detection threshold for MSG and it was shown that the threshold increases with age. It was determined that for elderly people a 2.2-fold higher concentration was needed to detect the umami taste. Also inosine monophosphate and other glutamate salts were tested presenting a higher threshold in older people. Some studies suggest that a higher intake of drugs can also lead to changes in taste sensation, because they can affect the neurons responsible for the taste transduction or can affect the function of taste buds. A lately conducted study showed that elderly people take an average of 4.4 various medicament's, such as anti-hypertensives, antibiotics, NSAIDs and corticosteroids. [53,54]

Metallic taste

As the importance of umami, in relation of nutrition and healthy food, gained more recognition in the last years, researchers tried to find more information about taste and especially more about undiscovered mechanisms and new receptors. One example is the not so well known metallic taste which was identified in various foods, such as acesulfam-K, a sweetener, magnesium and calcium salts. On the other hand electrical stimulation of the chorda tympani and as well of the tongue led to a metallic taste. During pregnancy, in burning mouth syndrome and after taking ferrous sulfate ($FeSO_4$), a taste impairment was discovered, often described as a metallic taste. This kind of perception was hardly classified as a primary or basic taste, but as the occurrence of this

taste began to heap up, the interest for further studies increased, for example encountering potential mechanisms and examining by which circumstances it is triggered. For example, the perception of ferrous sulfate is mostly driven by the retronasal smell and transport. So the nasal occlusion has a big effect on the metallic taste of ferrous sulfate and is greatly reduced. Otherwise the nasal occlusion has no effect on the stimuli of a copper penny. Due to the different stimulation of metallic taste, such as electrical stimulation, stimulation with metals, ferrous sulfate and solutions of divalent salts, a study tried to figure out the differences and resemblances between these stimulation's. The main goal in this study was to find out if a nasal occlusion has an effect on the metallic taste stimulated by electrical stimulation and stimulation with metals, similar to the decrement discovered with ferrous sulfate after nasal closure. This study was conducted to examine if there are other possible mechanisms which trigger a metallic taste. If the perception from electrical and metallic stimulation is not altered by nasal closure this suggests that a different mechanism via gustatory receptors exists. Metallic taste is not a common sensation and is not sensed every day from foods, but can be experienced from lipid oxidation, packaging transfer or when something is directly in contact with metal utensils and foils. The study was divided in two experiments. In the first one the researchers tried to find out if copper and electrical stimuli, generated with a battery, can trigger a metallic sensation in four different areas of the mouth. The second one included a nasal closure to examine if these stimuli are affected by retronasal smell, like the stimulus by ferrous sulfate. It is possible that ferrous sulfate catalyzes a fast lipid oxidation in the mouth and produces 1-octene-3-one, trans-4,5-epoxy-decenal and (*Z*)-1,5-octadien-3-one, which sometimes are also related as metallic-smelling substances. These compounds are often mentioned in gas chromatography analysis and food literature with thresholds under 1 ppb. [45,46] A different study examined the effect of ferrous (Fe^{2+}), cuprous (Cu^{+}), cupric (Cu^{2+}), and ferric (Fe^{3+}) ion solutions on the lipid oxidation in the saliva. In comparison with the control solution (reagent water) the ion solutions led to an increased lipid oxidation, where Fe^{2+} showed a higher lipid oxidation capacity and Cu^{2+}, Cu^{+}, and Fe^{3+} showed a lower lipid oxidation one. No differences in qualitative sensation between those solutions were observed. Thus, this lipid oxidation seems to induce a metallic taste and can be minimized by using antioxidants and chelating agents like Vitamin C, Vitamin E in the group of antioxidants and EDTA, lactoferrin in the group of chelating agents. The intake of Vitamin C or Vitamin E after sipping a ferrous iron solution was not so effective in reducing the metallic taste. Whereas the intake of EDTA or lactoferrin were

more effective, furthermore lactoferrin completely removed the metallic taste. [49]

Due to a diverse description of the taste from salty, sour to metallic, evoked by anodal electrical stimulation, the researchers implied salt-acid mixtures besides the anodal electrical stimulation to verify if there are any differences between these sensations. They used 0.001, 0.003 and 0.01 M citric acid, 0.03, 0.10 and 0.30 M NaCl and a mixture containing both, namely 0.003 M citric acid and 0.10 M NaCl. It was shown that a cooper penny with a zinc core induces a more intense metallic taste than a simple cooper penny. The electrical stimulation was applied with a small battery (1.5 V battery) to various oral regions and was fixed to a plastic handle. The stimulation was induced in four different areas of the tongue, namely the medial tongue, the anterior dorsal tongue, buccal surface and the inside of the upper lip. The subjects closed their eyes before the stimulus was applied, so they were not affected visually and also rinsed between the different stimuli. The only information they received was, where and in which area the stimulus was going to be applied. The area with the greatest sensitivity was the anterior tongue after the stimuli with 1.5 V, then the medial tongue and the lowest sensitivity showed the area of the upper lip and buccal surface. In correlation the electrical stimulation was more potent than the copper/zinc stimulus. Most of the subjects described the sensation as metallic, but some also described it as salty and sour. By using the salt-acid mixtures before the electrical and metal stimuli, the subjects received adequate quality differences and chose a different descriptor besides salty and sour, namely metallic. The area with the highest sensitivity like the anterior tongue also has the highest amount of fungiform papillae, which is considered to be an evidence that the gustatory pathways are activated by an electric current. On top of that, the thresholds rise in the front region of the tongue when the chorda tympani is separated. The second experiment was executed to verify that a nasal closure has no effect on the sensation triggered by electrical stimuli and solid metal. For the metal stimuli metal foils were used instead of copper penny's. In addition a 3.0 V lithium battery was used besides the 1.5 V silver oxide battery to clarify if the level of electrical stimulation increases and Teflon was used as a control stimulus. The liquid stimuli were carefully dabbed on the tongue so that the area of stimulation was approximately the same size as the area touched by the solid stimuli. The results showed that the nasal occlusion had no essential effect on metallic sensation evoked by electrical stimuli. This suggests that a different mechanism must exist for receiving metallic sensations from electrical current, namely a gustatory sensation through gustatory receptors. The fungiform papillae may

also include trigeminal afferents, but with a greater threshold then on the cheek and lip. To identify if the trigeminal afferents play an important role in the sensation of metallic taste or the chorda tympani is the more important part in this transduction, it would be interesting to test an electrical tongue stimulation on patients with an intact chorda tympani but unilateral trigeminal transection.
It must also be considered that the qualitative reports were also affected by a list which was given to the subjects with several descriptions to choose from such terms like alkaline, metallic, astringency, fishy, irritating, spicy. It was harder for the subjects to describe the taste without presenting the list to them, because a lot of persons never experienced a metallic taste. Also a quite similar word to metallic was used by the subjects namely rusty. A metallic taste can for example be experienced from dental procedures, metal wrappers or packaging or different metal objects. But every chemical substance the tongue is exposed to, induces a multitude of sensory effects and there is more than just a monogustatory tastant. The chlorides and sulfates of Zn, Ca, Fe and Cu induce a metallic taste, but also astringent and bitter sensations. But the salts with Zn and Cu showed no reduction in sensation with nasal closure, in comparison with ferrous sulfate were the reduction was considerable. $CuSO_4$ or $ZnSO_4$ also were more astringent and bitter than metallic. [45,46]

Artificial sweeteners induce metallic taste

As mentioned before also acesulfame-K, which is a artificial sweetener, can lead to an metallic taste. Those artificial sweeteners are included in many foods for dietary reasons, because they reduce the caloric intake and are a good supplement for prohibiting dental decay and especially for diabetics these artificial sweeteners do not increase the blood glucose level. Cyclamate on the other hand led to tumors in rats and is not allowed in the United States anymore, but is still used in more than fifty countries. The concentration of the artificial sweeteners plays an important role as it changes the sense from sweet to bitter/metallic when the concentration is increased. At low concentrations G protein-coupled receptors (GPCRs) T1R2/T1R3 are stimulated which are for the sweet taste, whereas in very high concentrations the bitter tastant sensing GPCRs were stimulated. Another mechanism for the aftertaste, evoked by artificial sweeteners, is their diffusion into the taste receptors where they can change signaling pathways. In the taste receptor cells and in sensory neurons in the mouth there are

TRPV1 receptors which are stimulated by thermal stimuli, acidic stimuli, alcohols, lipids, vanilloids and terpenoids. The correlation between the TRPV1 receptors and metallic taste was assumed when it was shown that high concentrations of Mg^{2+} and Ca^{2+} activate these receptors and the sensation was described primarily as bitter but also as metallic. As previously mentioned, an intense metallic taste can be evoked by different metal salts. Some researchers found out that the TRPV1 receptors can be also activated by these metal salts and can contribute to the metallic sensation. [47]

Possible Taste transduction of metallic taste

A lot of compounds and substances are mediated by somatosensory (TRPV1) systems and also gustatory (TRPM5 and T1R3) systems. The recognition of chemical stimuli which are dissolved in saliva is perceived via the gustatory systems, whereas mechanical and thermal stimuli become aware of via the somatosensory systems. The nerve terminals can be activated by capsaicin and also other chemical substances, which can diffuse into the lingual epithelium. Some researchers executed behavioral tests with mice where the TRPM5 receptors, T1R3 receptors or the TRPV1 channels were absent, to obtain insight in the multiple pathways of taste sensations evoked by complex tasting divalent salts. Low concentrations of $FeSO_4$ and $ZnSO_4$ were favored and higher concentrations were aversive. So, $FeSO_4$ and $ZnSO_4$ showed a pleasurable positive phase at low concentrations transmitted via TRPM5 and T1R3, whereas high concentrations led to a negative phase were TRPM5 was not involved and for $FeSO_4$ TRPV1 was involved. TRPV1 leads to an aversive response, whereas knocking it out leads to preferring $FeSO_4$ and $CuSO_4$. $MgSO_4$ and $CuSO_4$ were transmitted via TRPM5 and TRPV1 pathways. Thus, the individual sensations are dependent on the concentration, because complex descriptors trigger different pathways. Another reason for an aversive reaction is that salts of zinc, iron and copper show an emetic effect in higher concentrations and lead to gastrointestinal illness. In fact many subgroups of receptors are responsible for the taste transduction of complex taste divalent salts. T1R3 which is expressed by a subpopulation of taste cells is activated by calcium and magnesium and leads to aversion. Those cells eventually express another receptor which dimerizes with T1T3 to create a responsive receptor for Mg^{2+} and Ca^{2+}. It can be suggested that this second receptor is the calcium sensing receptor (CaSR), because

T1R1 and T1R2 in combination with T1R3 lead to a preference and not to an aversion as determined for magnesium and calcium. Whereas T1R1, T1R2 or other G-protein-coupled receptor could be the partner of T1R3 when responding to $FeSO_4$ and $ZnSO_4$ in low concentrations, because it leads to a preference. A different subpopulation of taste cells that express TRPM5 and not T1R3 are responsible for the preference for Ca^{2+} and Mg^{2+} and eventually for Zn^{2+} and Fe^{2+}. This was determined in T1R3 knockout mice, because T1R3 leads to an aversion as mentioned before and masks the preference induced by TRPM5. On top of that TRPV1 may also contribute to the aversive taste of $FeSO_4$ and $CuSO_4$. A team of researchers expressed hTRPM5 in HEK293 cells to verify if $FeSO_4$, $ZnSO_4$, $CuSO_4$, and $MgSO_4$ can activate TRPM5 channels. According to the researchers the TRPM5 channels were not activated when the salts were inserted to the extracellular surface. These data together with the behavioral data suggest that a calcium release from stores stimulates the TRPM5 after Fe^{2+} or Zn^{2+} affecting T1R3 and activating PLCβ2. The divalent salts of Cu^{2+}, Fe^{2+} and Zn^{2+} can pass through passive and active transmembrane transport pathways into cells. Tight junctions in the taste bud play an important role as barrier of the TRCs and it is possible that these salts can pass through tight junctions which have a specific permeability to divalent salts. The researchers also found out that the complex tasting divalent salts activate heterologously expressed hTRPV1 which leads to aversive reactions. Further studies will be needed to obtain a better insight in the transduction pathway of metallic taste. [48]

Cancer and metallic taste

A metallic taste was also reported by cancer patients treated with chemotherapy. About 78 % of the patients, with different cancers, treatment phases and also chemotherapy reported a metallic sensation. No studies were conducted to determine the effect of metallic taste on body weight, dietary intake or quality of life. Many different mechanisms can be responsible for sensing a metallic taste. The taste was sensed as a phantogeusia which means the occurrence of the taste appears without any stimulus. It was expected that these taste phantoms can be evoked by localized taste damage, which leads to inhibitory mechanisms in the central nervous system. Furthermore, the central nervous system obtains signals from cranial nerves mediating taste. So when one taste nerve is inhibited, the neural input from other taste nerves is increased. But it is not

clearly known if the metallic taste in cancer patients is a taste phantom or other external mechanisms are involved. For example, chemotherapeutic substances, such as cisplatin and carboplatin, which include the metal compound platinum in their structure, can have a direct effect in the mouth and can lead to a metallic taste, because a lot of drugs are solved in saliva and can interact directly with taste-receptors. Another possible reason for cancer patients experiencing metallic taste is a lower detection threshold for metals when medicated with chemotherapeutic substances and thus metallic silverware or utensils are sensed more intense. The consumption of red meat can lead to an aversion in cancer patients because of a metallic taste or sometimes also described as ´blood taste ´, because of the iron compounds it contains. By using plastic utensils the metallic taste can partly be handled, because some patients treated with cyclophosphamide registered that this method made food more palatable. Also cancer patients with pancreatic cancer which were medicated with gemcitabine registered an improvement using plastic utensils and ice water. Another method to mask the metallic taste and make food more palatable for cancer patients, is the fruit *Synsepalum dulcificum* also called ‘miracle fruit’. This fruit includes the protein miraculin and is also used as a sweetener. A pilot study was conducted were eight patients used this ‘miracle fruit’ as a supplement to test the effects on metallic taste. Actually more patients should have been tested to get a more significant result. Nevertheless, five out of eight patients reported a disappearance of the metallic taste using this supplement and also reported a food improvement with a higher food intake. In general, the use of spices, strong herbs, sour and sweet foods were helpful for patients to mask the metallic taste. In fact taste changes can lead to a lower nutrient intake, appetite, energy and a decreased quality of life, but the effects of metallic taste in this fields is unknown and further studies will be needed. [49] Another interesting finding is that head and neck cancer survivors sense an oral pain with metallic taste as side-effect, which can be a hint that a neural damage occurs leading to this sensations. This neural damage can be elicited by cancer-directed treatment and radiotherapy. Also the activation of the chorda tympani taste nerve, anesthesia of the chorda tympani or damaging the chorda tympani for example during a stepedectomy (surgery performed on the middle ear) can lead to a metallic taste. This damage on the chorda tympani should be repaired to restore the gustatory functions. [50] Patients with breast cancer who received a taxane chemotherapy (docetaxel or paclitaxel) also described a metallic taste. Some of them mentioned a long lasting sensation and that the food they ate had not the same taste anymore. Patients even tried to eat onions, garlic or strong flavored meat just to mask this taste. Some of them even gained a lot of weight

because of eating a lot of candy's before meat and consuming sweetened drinks to mask the metallic taste. The taste disturbances are side-effects that are often neglected during a chemotherapy and there is not so much literature about management strategies and effective prevention, although these taste alterations can lead to a serious reduction of life quality for cancer patients. So more studies about managing those taste alterations would be needed to prepare the patients and make them aware of the side-effects, a chemotherapy can generate, to improve their life quality during the treatment. [51] From all these findings and studies about taste disturbances in cancer patientes, it could be proposed that chemotherapeutics can damage the taste buds or neurons which mediate taste signals leading to an irritating and unpleasant taste often described as metallic.

Taste bud damage by chemotherapy

But not only a metallic taste is sensed during chemotherapy or radiotherapy, moreover all five basic tastes (sweet, sour, bitter, salty, umami) are affected. During the third week of radiotherapy the umami taste is decreased and the recovery of this sensation can take a long time. Umami plays an important role in diet and overall in the nutritional intake, because it affects and enhances pleasure while eating. So the loss of umami sensation can cause a great impairment to quality of life. But no studies were conducted for combined therapy or chemotherapeutic agents that can have an effect on the umami sensation. Furthermore, a lot of central nervous system disorders and diseases can lead to taste disturbances, tumors that occur in the central nervous system to cerebrovascular accidents, different central lesions as well as neurodegenerative diseases, such as Parkinson, Alzheimer, Myasthenia Gravis and multiple sclerosis. In addition a lot of permitted medications have taste-related side-effects, like anti-hypertensives, muscle relaxants, antidepressants, antibiotics and of course the earlier mentioned chemotherapeutics. Eszopiclone, a nonbenzodiazepine hypnotic, which is used for insomnia leads to a metallic taste in two thirds of the population. The targeted therapeutics or chemotherapeutics can eventually damage the taste receptors by direct contact or via secretion in saliva were a taste disturbance can last after drug clearance and is often described as metallic or "chemical" taste. Xerostomia, also called dry mouth syndrome, is not a odor or taste disturbance but can lead to changes in taste sensation. This dry mouth syndrome in cancer patients can be provoked by radiation

exposure leading to damaged salivary glands and causing higher viscosity of saliva or lower secretion. How the salivary glands are damaged by radiation is not known, but three possible mechanisms are suggested. One mechanism is that due to lipid oxidation, induced by radiation, the salivary gland cell membrane is affected which leads to autolysis. Another mechanism is a DNA-damage of salivary glands cells induced by oxidative species produced by irradiation. The last hypothesized mechanism is an induced apoptosis via radiation. Patients with xerostomia also report metallic taste or aftertaste. The exact mechanism why xerostomia induces taste alteration is unproved, but it is possible that the lower salivary secretion decreases the transport of molecules to the taste and olfactory receptors. Furthermore, a higher salivary protein concentration is preexisting which can have an impact on retronasal aroma recognition. But more studies will be needed to verify the connection between changes in saliva and taste alteration, because some studies have not discovered any changes in salivary protein concentration after radiation. Xerostomia can be prevented by using specific techniques where two diagonal beams and one lateral beam were used to treat the upper neck, additionally the parotid gland could be divided by specific blocks and was only irradiated lateral. So using these techniques the quantity of taste buds being damaged by irradiation can be reduced and can be useful in maintaining the taste function. The taste cells in healthy persons are replaced constantly, generating new synaptic connections between taste cells and nerve fibers. In general, cytotoxic chemotherapeutics and also radiation therapy lead to apoptosis of cells with high turnover rates. Furthermore, they decrease the number of receptor cells responsible for taste and olfactory sensations and in addition the structure of the taste buds, like the taste pores, can be changed leading to an altered transport of flavor molecules to the receptor cells. A study examined the effect of irradiation on the taste buds in rats, showing that nerve fibers were not affected and that the taste loss is induced by damage to the taste cells alone and the neural transmission is unaffected. Cancer patients regenerate their taste function by renewing the receptor cells all at once within 0.5 to 1 year after the treatment. During the treatment with chemotherapeutics the taste sensations change and the fast renewal of the receptors can lead to a random connection with different nerve fibers. Furthermore, this process can generate the ongoing taste disturbances after the treatment reported by patients, which claim that their taste returned, but is not same as it was before the treatment. In addition, the concentration of cyclic adenosine monophosphate (AMP), which binds to the membranes and functions as a second messenger, was not changed by radiation. Using suprathreshold concentrations of the basic tastes it was shown that the membranes were

just structurally changed but the function of the membranes was consistent, because the concentration curves did not change significantly. This test in rats can help to understand what is happening to the human taste buds during radiotherapy, because some reports claim that the function or structure of the taste buds in mammals is the same and no differences can be observed in umami, sweet and bitter receptors among mammalians. [52,55-57,59]

Also the neuronal activities can be altered leading to an abnormal taste sensation via the chorda tympani nerve, because cancer treatments can affect neuronal cells and change the afferent taste pathways. No substantial difference was found in taste disturbance between patients medicated with mildly neurotoxic or highly neurotoxic drugs. These taste sensations are sensed without any external stimulus. [55]

Loss of umami taste during chemotherapy

There are not so many studies examining the loss of umami taste during chemotherapy or radiotherapy. One study tried to verify the loss of umami taste in patients treated with radiotherapy for head and neck cancer using a whole-mouth method. It was shown that the thresholds for umami taste were higher after 3 weeks of irradiation. Some patients in this study, namely 48%, showed no alteration in umami taste. This can be ascribed to the whole-mouth method that was used, because this kind of method cannot measure the small differences in taste thresholds, like a taste disc method which is available for sweet, bitter, salty and sour but not for umami. Two different studies showed different results referred to thresholds. One of these studies examined that umami taste thresholds were higher after irradiation and that only umami taste showed a significant impairment compared with the other four basic tastes. This hypothesis that the damage is caused by irradiation is different between umami receptors and the other four basic tastes. Whereas in the other study the results showed that the same impairment is preexisting for the other four basic tastes. In fact more studies will be needed to overcome this discrepancy. [58,59]

Treatment of taste and smell dysfunction

So in fact, a lot of patients suffer from taste and smell dysfunctions, which are also associated with chronic diseases. Furthermore, it is important to understand the origin of

these dysfunctions and how they can be evaluated so that a suitable treatment can be executed to correct them. Because taste and smell are chemical senses, alterations in these senses can expose systemic or local changes in the body's function. There exist two possible changes, namely alterations in smell and taste function or loss of sensory acuity. These changes correlate with chronic diseases of many organ systems including neurodegenerative, endocrine, neurological and immunological ones. But the major problem is that reports, of how to evaluate these biochemical and chronic pathological processes, are lacking. One study was conducted in Washington DC in "The Taste and Smell Clinic" over a period of 40 years evaluating 5183 patients. During these 40 years systematic techniques were accomplished to measure and verify smell and taste dysfunctions. Furthermore, methods were developed to examine the biological factors which lead to chronic sensory disorders. Treatment protocols were produced for medicating these chronic sensory disorders. For the effective treatment of chronic taste and smell dysfunctions the use of a tripartite methodology is important which has been developed in the last 40 years. First of all, clinical evaluations have to be carried out, such as examining clinical symptoms, measuring sensory disorders and perform physical and neurological tests on head and neck. Secondly, biochemical evaluations are important, like examination of pathologies found in urine, erythrocytes, blood or saliva. Thirdly integrating and summing these results up so that effective therapies can be developed. It was thought that chronic smell and taste dysfunctions are isolated symptoms, but these dysfunctions are due to chronic disease processes. In fact a group of pathological syndromes are responsible and have a collective set of symptoms. It is well known that local damage in nasal and oral cavities can cause taste and smell disorders but they do not generate chronic alterations of sensory functions without any chronic diseases process. Some biochemical alterations that cause taste and smell loss are inhibition of nasal mucus, saliva, cGMP and cAMP secretion and also a disorder in the zinc metabolism in these fluids. One main analysis for examination of taste and smell disorders is the evaluation of the growth factor concentration in nasal mucus and saliva, because a low concentration can lead to inflammation and cellular apoptosis generating taste and smell disturbances via inhibition of the renewal of olfactory epithelial cells and taste buds. In summary, to correct these smell and taste disturbances a systematic approach is important. In fact the origin of these dysfunctions has to be evaluated and biochemical changes must be understood to use the right medication and treatment. The impulse to find useful treatments for taste and smell dysfunctions should come from the fact that patients are seeking persistent for medical help. One example

for a former medical problem was the treatment of cardiovascular diseases were the blood lipids had to be evaluated first to use the right medication and prevent cardiovascular diseases. So following the tripartite methodology as mentioned before can help to find a successful treatment for chronic taste and smell dysfunction. [60]

References

[1] U.S. Department of Health & Human Services (**2011**). *http://www.surgeongeneral.gov/obesity/calltoaction/fact_adolescents.htm*

[2] Winkel C., de Klerk A., Visser J., de Rijke E., Bakker J., Koenig T., Renes H. (**2008**). New developments in umami (enhancing) molecules. *Chemistry & Biodiversity, **5**, 1195-1203*

[3] Kurihara K. (**2009**). Glutamate: from discovery as a food flavor to role as a basic taste (umami). *Am J Clin Nutr, **90**, 719S-722S*

[4] Beauchamp G. K. (**2009**). Sensory and receptor responses to umami: an overview of pioneering work. *Am J Clin Nutr, **90**, 723S-727S*

[5] Populin T., Moret S., Truant S., Conte L.S. (**2007**). A survey on the presence of free glutamic acid in foodstuffs, with and without added monosodium glutamate, *Food Chem, **104**, 1712-1717*

[6] Jinap S., Hajeb P. (**2010**). Glutamate. Its applications in food and contribution to health, *Appetite, **55**, 1-10*

[7]Yeomans M.R., Gould N.J., Mobini S., Prescott J. (**2008**). Acquired flavor acceptance and intake facilitated by monosodium glutamate in humans. *Physol Behav, **93**, 958-966*

[8] Kinnamon S.C. (**2009**). Umami taste transduction mechanisms. *Am J Clin Nutr, **90**, 753S-755S*

[9] San Gabriel A., Maekawa T., Uneyama H., Torii K. (**2009**). Metabotropic glutamate receptor type 1 in taste tissue, *Am J Clin Nutr, **90**, 743S-746S*

[10] Toyono T., Kataoka S., Seta Y., Shigemoto R., Toyoshima K. (**2007**). Expression of group II metabotropic glutamate receptors in rat gustatory papillae. *Cell Tissue Res, **328**, 57-63*

[11] Nasiripourdori A., Taly V., Grutter T., Taly A. (**2011**). From toxins targeting ligand gated ion channels to therapeutic molecules. *Toxins*, ***3**, 260-293*

[12] Zhang Z., Zhao Z., Margolskee R., Liman E. (**2007**). The transduction channel TRPM5 is gated by intracellular calcium in taste cells, *J Neurosci, **27**, 5777-5786*

[13] Stone L.M., Barrows J., Finger T.E., Kinnamon S.C. (**2007**), Expression of T1Rs and Gustducin in Palatal Taste Buds of Mice, *Chem Senses, **32**, 255-262*

[14] Zhang F., Klebansky B., Fine R.M., Xu H., Pronin A., Liu H., Tachdjian C., Li X. (**2008**), Molecular mechanism for the umami taste synergism, *P Natl Acad Sci USA, **105**, 20930-20934*

[15] Roura E., Humphrey B., Klasing K., Swart M. (**2011**). Is the pig a good umami sensing model for humans? A comparative taste receptor study, *Flavour Frag J, **26**, 282-285*

[16] Backes M., Obst K., Bojahr J., Thorhauer A., Roudnitzky N., Paetz S., Reichelt K.V., Krammer G.E., Meyerhof W., Ley J.P. (**2015**). Rubemamine and Rubescenamine, Two Naturally Occurring N-Cinnamoyl Phenethylamines with Umami-Taste-Modulating Properties. *J Agr Food Chem, **63**,* 8694-8704.

[17] Su G., Cui C., Zheng L., Yang B., Ren J., Zhao M. (**2012**). Isolation and identification of two novel umami and umami-enhancing peptides from peanut hydrolysate by consecutive chromatography and MALDI-TOF/TOF MS. *Food Chem., **135**,* 479-485.

[18] Uneyama H.,Uematsu A., Iwatsuki K., Nakamura E. (**2013**). Perceived Palatability and Appetite for Protein by Umami Taste Derived from Glutamate. *J Nutr & Food Sci, **01**,* 1-7.

[19] Uneyama H. (**2011**). Nutritional and physiological significance of luminal glutamate-sensing in the gastrointestinal functions. *J Pharm Soci Jap, **131**,* 1699-709.

[20] Torii K. (**2012**). Brain activation by the umami taste substance monosodium L-glutamate via gustatory and visceral signaling pathways, and its physiological significance due to homeostasis after a meal. *J Oral Biosci, **54**,* 144-150.

[21] Vasilevskaia LS, Rymshina MV, Shlygin GK. (**1993**), Effect of glutamate and combined with inosine monophosphate on gastric secretion. *Voprosy Pitaniya, **3**,* 29-33.

[22] Kendig D.M., Hurst N.R., Bradley Z.L., Mahavadi S., Kuemmerle J.F., Lyall V., DeSimone J., Murthy K.S., Grider J.R. (**2014**), Activation of the umami taste receptor (T1R1/T1R3) initiates the peristaltic reflex and pellet propulsion in the distal colon. *Am J Physiol Gastrointest Liver Physiol, **307**,* G1100-7.

[23] Wang J., Inoue T., Higashiyama M., Guth P.H., Engel E., Kaunitz J.D., Akiba Y. (**2011**), Umami receptor activation increases duodenal bicarbonate secretion via glucagon-like peptide-2 release in rats. *J Pharmacol Experi Therap, **339**,* 464-473.

[24] Cho Y.M., Kieffer T.J. (**2010**). Chapter Four–K-cells and Glucose-Dependent Insulinotropic Polypeptide in Health and Disease. *Vitam Horm, **84**,* 111-150.

[25] Egan J.M., Margolskee R.F. (**2008**). Taste cells of the gut and gastrointestinal chemosensation. *Mol Interv, **8**,* 78-81.

[26] Paschetta E., Hvalryg M., Musso G. (**2011**). Glucose-dependent insulinotropic polypeptide: from pathophysiology to therapeutic opportunities in obesity-associated

disorders. *Obes revi, **12**,* 813-28.

[27] Miyaki T., Imada T., Shuzhen Hao S., Kimura E. (**2015**). Monosodium L-glutamate in soup reduces subsequent energy intake from high-fat savoury food in overweight and obese women. *Brit J Nutr, **115**,* 176-184.

[28] Shi Z., Yuan B., Wittert G.A., Pan X., Dai Y., Adams R., Taylor A.W. (**2012**). Monosodium Glutamate Intake, Dietary Patterns and Asthma in Chinese Adults. *PLoS ONE, **7**,* 1-6.

[29] Kim M.J., Son H.J., Kim Y., Misaka T., Rhyu M. (**2015**). Umami-bitter interactions: The suppression of bitterness by umami peptides via human bitter taste receptor. *Biochem bioph res co, **456**,* 586-90.

[30] Singh P.B., Hummel T., Gerber J.C., Landis B.N., Iannilli E. (**2015**). Cerebral processing of umami: A pilot study on the effects of familiarity. *Brain Res, **1614**,* 67-74.

[31] Rosa S.G., Quines C.B., Da Rocha J.T., Bortolatto C.F., Duarte T., Nogueira C.W. (**2015**). Antinociceptive action of diphenyl diselenide in the nociception induced by neonatal administration of monosodium glutamate in rats. *Eu J Pharmacol, **758**,* 64-71.

[32] Quines C.B., Rosa S.G., Da Rocha J.T., Gai B.M., Bortolatto C.F., Duarte M.M.M. F., Nogueira C.W. (**2014**). Monosodium glutamate, a food additive, induces depressive-like and anxiogenic-like behaviors in young Rats. *Life Sci, **107**,* 27-31.

[33] Shimada A., Castrillon E., Baad-hansen L., Ghafouri B., Gerdle B., Ernberg M., Cairns B., Svensson P. (**2015**). Muscle pain sensitivity after glutamate injection is not modified by systemic administration of monosodium glutamate. *J Headache & Pain,* **16** (1), 1-9

[34] Baad-Hansen L., Cairns Be., Ernberg M., Svensson P. (**2010**). Effect of systemic monosodium glutamate (MSG) on headache and pericranial muscle sensitivity. *Cephalalgia, **30**,* 68-76.

[35] Kim D., Choi Y., Park S., Han M.J., Kim S. (**2015**). Attenuating effect of Lactobacillus brevis G101 on the MSG symptom complex in a double-blind, placebo-controlled study. *Nutr Res Practice, **9**,* 673-676

[36] Taruno A, Vingtdeux V., Ohmoto M., Ma Z., Dvoryanchikov G., Li A., Adrien L., Zhao H., Leung S., Abernethy M., Koppel J., Davies P., Civan M.M., Chaudhari N., Matsumoto I., Hellekant G., Tordoff M.G., Marambaud P., Foskett J.K. (**2013**). CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes. *Nature (London,U.K), **495**,* 223-226.

[37] Chaudhari N. (**2013**). Sweet umami: the twain shall meet. *J Physiol, **591**,* 1597.

[38] Nakashima K., Eddy M.C., Katsukawa H., Delay E.R., Ninomiya Y. (**2012**). Behavioral responses to glutamate receptor agonists and antagonists implicate the involvement of brain-expressed mGluR4 and mGluR1 in taste transduction for umami in mice. *Physiol Behav, **105**,* 709-719.

[39] Toda Y., Nakagita T., Hayakawa T., Okada S., Narukawa M., Imai H., Ishimaru Y.,

Misaka T. (**2013**). Two distinct determinants of ligand specificity in T1R1/T1R3 (the umami taste receptor). *J Biol Chem, **288**,* 36863-36877.

[40] Narukawa M., Toda Y., Nakagita T., Hayashi Y., Misaka T. (**2014**). L-Theanine elicits umami taste via the T1R1 + T1R3 umami taste receptor. *Amino Acids, **46**,* 1583-1587.

[41] Kokabu S., Lowery J.W., Toyono T., Seta Y., Hitomi S., Sato T., Enoki Y., Okubo M., Fukushima Y., Yoda T. (**2015**). Muscle regulatory factors regulate T1R3 taste receptor expression. *Biochem Bioph Res Com,* **468** (4), 568-573

[42] Brennan S.C., Davies T.S., Schepelmann M., Riccardi D. (**2014**). Emerging roles of the extracellular calcium-sensing receptor in nutrient sensing: control of taste modulation and intestinal hormone secretion. *Brit J Nutr,* 1-7.

[43] Maruyama Y., Yasuda R., Kuroda M., Eto Y. (**2012**). Kokumi substances, enhancers of basic tastes, induce responses in calcium-sensing receptor expressing taste cells. *PLoS One, 7,* 34489.

[44] Imamura M., Matsushima K. (**2013**). Suppression of Umami Aftertaste by Polysaccharides in Soy Sauce. *J Food Sci, **78**,* C1136–C1143.

[45] Lawless H.T., Stevens D.A., Chapman K.W., Kurtz A. (**2005**). Metallic Taste from Electrical and Chemical Stimulation. *Chem Senses, **30***, 185-194

[46] Lawless H.T., Schlake S., Smythe J., Lim J., Yang H., Chapman K., Bolton B. (**2004**). Metallic Taste and Retronasal Smell. *Chem Senses, **29***, 25-33

[47] Riera C.E., Vogel H., Simon S.A., le Coutre J. (**2007**). Artificial sweeteners and salts producing a metallic taste sensation activate TRPV1 receptors. *Am J Physiol, **293***, R626-R634

[48] Riera C.E., Vogel H., Simon S.A., Damak S., Le Coutre J. (**2009**). Sensory Attributes of Complex Tasting Divalent Salts Are Mediated by TRPM5 and TRPV1 Channels. *J Neurosci*, ***29***, 2654-2662

[49] IJpma I., Renken R.J., Ter Horst G.J., Reyners A.K.L. (**2015**). Metallic taste in cancer patients treated with chemotherapy. *Cancer Treat Rev*, ***41***, 179-186

[50] Logan H.L, Bartoshuk L.M., Fillingim R.B., Tomar S.L., Mendenhall W.M. (**2008**). Metallic taste phantom predicts oral pain among 5-year survivors of head and neck

cancer. *Pain*, ***140***, 323-331

[51] Speck R.M., DeMichele A., Farrar J.T., Hennessy S., Mao J.J., Stineman M.G., Barg F.K. (**2013**). Taste alteration in breast cancer patients treated with taxane chemotherapy: experience, effect, and coping strategies. *Support Care Cancer*, ***21***, 549-555

[52] Epstein J.B., Schmutzer G., Doty R.L. (**2016**). Understanding the impact of taste changes in oncology care. *Support Care Cancer*, ***24***, 1917-1931

[53] Sergi G., Bano G., Pizzato S., Veronese N., Manzato E. (**2016**). Taste Loss in the Elderly: Possible Implications for Dietary Habits. *Crit Rev Food Sci*

[54] Methven L., Allen V.J., Withers C.A., Gosney M.A. (**2012**). Ageing and taste. *P Nutr Soc, **71***, 556-565

[55] Hong J.H., Omur-Ozbek P., Stanek B.T., Dietrich A.M., Duncan S.E., Lee Y.W., Lesser G. (**2009**). Taste and Odor Abnormalities in Cancer Patients. *The journal of supportive oncology, **7***, 58-65

[56] Yamashita H., Nakagawa K. Tago M., Nakamura N., Shiraishi K., Eda M., Nakata H., Nagamatsu N., Yokoyama R., Onimura M., Ohtomo K. (**2006**). Taste dysfunction in patients receiving radiotherapy. *Head Neck, **28***, 508-516

[57] Berteretche M.V., Dalix A.M., d'Ornano A.M.C., Bellisle F., Khayat D., Faurion A. (**2004**). Decreased taste sensitivity in cancer patients under chemotherapy. *Support Care Cancer, **12***, 571-576

[58] Yamashita H., Nakagawa K., Hosoi Y., Kurokawa A., Fukuda Y., Matsumoto I., Misaka T., Abe K. (**2009**). Umami taste dysfunction in patients receiving radiotherapy for head and neck cancer. *Oral Oncol, **45***, 19-23

[59] Shi B., MasudaM., Umezaki T., Kuratomi Y., Kumamoto Y., Yamamoto T., Komiyama S. (**2004**). Irradiation impairment of umami taste in patients with head and neck cancer. *Auris, nasus, larynx, **31***, 401-406

[60] Henkin R.I., Levy L.M, Fordyce A. (**2013**). Taste and smell function in chronic disease: a review of clinical and biochemical evaluations of taste and smell dysfunction in over 5000 patients at The Taste and Smell Clinic in Washington, DC. *Am J Otolaryng, **34***, 477-489

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