TRPM5 as a target in the battle against diabetes and obesity.

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TRPM5 is a nonselective monovalent cation channel activated by increases in intracellular Ca^{2+} . It has a distinct expression pattern throughout the body where expression occurs in chemosensitive tissues from solitary chemosensory cells to the taste receptor cells and in pancreatic β -cells. The role of TRPM5 has been investigated with the use of knockout mouse models. *Trpm5*^{-/-} mice have a lack of type II taste perception and show reduced glucose-induced insulin secretion. Expression levels of TRPM5 are reduced in obesity and mutations in TRPM5 have been associated with type II diabetes and metabolic syndrome. In this review we aim to give an overview of the activation, selectivity, modulation and physiological roles of TRPM5.

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26 Introduction

27 Transient receptor potential channels (TRP) are a superfamily of cation channels, expressed in
28 a large variety of species from yeasts over invertebrates to vertebrates, including mammals. 28
29 mammalian members of the TRP channel family have been described.¹ Based on amino acid
30 homology, TRPs are divided into seven subfamilies: TRPN (NOMPC-like), TRPC (Canonical),
31 TRPV (Vanilloid), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucolipin) and TRPA
32 (Ankyrin). Besides TRPN, every subfamily is represented in mammals. 27 human TRP
33 channels have been identified. Every TRP protein is composed of 6 transmembrane domains
34 (TM S1-S6) with cytosolic N-and C-termini of variable length. TRPs form tetrameric
35 quaternary structures.² Each subunit contributes to a part of the centrally located selectivity
36 filter and ion-conducting pore. This topology has recently been confirmed with the elucidation
37 of the protein structure from TRPV1, TRPV2, TRPV6, TRPP2 and TRPA1.^{3–10} TRP channel
38 activity can be modulated by a plethora of stimuli.² It is shown that both physical (like
39 temperature) and chemical stimuli can activate TRP channels by shifting their voltage
40 sensitivity from a physiologically irrelevant voltage range to more relevant values.¹¹ Several
41 TRP channels show a temperature sensitive activation. For example, TRPM8 is activated by
42 cold temperatures and TRPV1 by hot temperatures.¹¹

43 All TRP channels are cation channels with a huge diversity in their ion selectivity e.g. TRPV5
44 and TRPV6 are highly permeable to Ca^{2+} , while TRPM6 and TRPM7 show high permeability
45 to Mg^{2+} and TRPM4 and TRPM5 are not permeable to divalent cations. TRPV1, TRPML1 and
46 TRPP3 are also permeable to H^+ .¹² Because of their sensitivity to multiple stimuli and their

cation selectivity, TRP channels can be regarded as signal integrators capable of influencing Ca^{2+} signaling and/or membrane potential.² Over the recent years it has become more and more apparent that these channels play an important role in human pathophysiology. While hereditary channelopathies have only been attributed to a few TRP channels, genetic analysis data indicates that mutations in most TRP genes are potential disease risk factors (reviewed by Nilius *et al.*^{12,13}). Expanding our knowledge about these channels may elucidate disease mechanisms and can lead to new therapies targeting TRP channels. Several TRP channels have been studied regarding their function in metabolic diseases. Almost a dozen different TRP channels are expressed in the pancreatic β -cells where they are mainly involved in cellular depolarization and calcium influx. The role of different TRP channels in β -cell function and excitability or metabolic diseases like obesity has been in detail described before.^{14,15} In this review the aim is to present the current knowledge about TRPM5 in metabolic diseases as diabetes and obesity.

1. TRPM5

TRPM5 is a membrane protein with six predicted transmembrane domains, like all TRP channels. Currently, there are four splice variants of the human TRPM5 are described,^{16,17} sequence homology between the human and mouse orthologues is ~80%.^{18,19} TRPM5 shares several features with TRPM4; they are both gated by raises in intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$), show voltage-dependency, are impermeable to Ca^{2+} ions, show high permeability to monovalent cations and have a single channel conductance of 20-25 pS.¹⁸⁻²⁰ These characteristics suggest TRPM4 and/or TRPM5 play a key role in membrane excitability.¹⁸ Ca^{2+} -activated non-selective cation channels (CANs) have been described in many cell types, like sensory cells, myocytes, neurons in the central nervous system and pancreatic β -cells. The most likely molecular candidates for these currents are TRPM4 or TRPM5, which makes it interesting to further investigate their properties, expression profile and physiological relevance.²¹

Ion substitution experiments have shown TRPM5 is equally permeable to K^+ , Na^+ , Cs^+ and Li^+ , but impermeable to divalent cations. TRPM5 is not blocked by divalent cations, as removal of external Ca^{2+} , Mg^{2+} or both did not change the rectification properties and did not increase the inward current.^{22,23}

Determination of the Ca^{2+} sensitivity varies depending on the experimental conditions. Measurements of the EC_{50} using inside-out patch clamp led to half activation values of 20-30 μM .^{18,23} On the other hand, the whole-cell patch clamp technique gives EC_{50} values that are approximately 10–30 times lower, thus indicating a higher Ca^{2+} sensitivity.¹⁸ However, others have found an EC_{50} value of 30 μM using the whole-cell technique, resembling the EC_{50} in inside-out conditions.²¹ Further research will elucidate the unknown cellular factors that contribute to this apparent difference, which could be changes in phosphorylation state that modulate the Ca^{2+} sensitivity.¹⁸ In general, the recordings suggest that the TRPM5 channel can be activated by global Ca^{2+} elevations resulting from either receptor-mediated release from intracellular stored Ca^{2+} or influx of extracellular Ca^{2+} .

1.1. TRPM5 Expression

Expression of TRPM5 was initially investigated through northern blotting and revealed high expression levels in murine liver and lower expression in the heart, brain, kidney, spleen, lung and testes.¹⁶ RT-PCR experiments confirmed this and showed expression in the stomach, intestine, prostate, pancreas and pituitary.²⁴ In the human protein atlas, antibody reactivity for TRPM5 confirmed the aforementioned expression and shows additional TRPM5 expression in endocrine tissues, skeletal muscle, pancreatic islets of Langerhans, throughout the GI tract and in both male and female reproductive organs with a medium to low prevalence.²⁵ The human proteome map, which uses mass spectrometry to identify peptide sequences, shows TRPM5 protein in the frontal cortex, spinal cord and liver.²⁶ Further research showed TRPM5 expression in murine taste tissue, the stomach and small intestine.²⁷ Immunohistochemical

detection of TRPM5 revealed expression is limited to a very discrete subset of cells. A genetic Cre knock-in TRPM5 mouse was generated, and confirmed the expression of TRPM5 in taste bud cells and olfactory epithelium.²⁸ A general observation is that TRPM5 is coexpressed with one or more signalling components of the canonical bitter, sweet and umami taste transduction cascade (PLC β_2 , α -gustducin).

Type II Taste Receptor cells - Type II Taste Receptor cells (TRC) in the taste buds express TRPM5 where it acts as a signal integrator of bitter, sweet and umami taste.²⁹ The TRPM5 channel shows co-expression with TAS1R, TAS2R, PLC β_2 , and α -gustducin.^{29,30} TRPM5 expression is mainly enriched in the basolateral region of the taste receptor cells.^{23,30} This localization suggests TRPM5 does not act as a chemosensor, but as a mediator of the signal transduction between primary sensor and the neurotransmitter release.³¹

Pancreatic β -cells - It is shown that TRPM5 has a functional role in pancreatic β -cells where it is involved in regulation of glucose-induced insulin secretion in mice.³² Analysis of the TRPM5-GFP reporter mouse²⁸ shows fluorescence, and hence TRPM5 expression in the pancreatic islets.³³ It is however unclear if the TRPM5 expression is limited to the β -cell or also occurs in other celltypes. Recent efforts are made to investigate the role of TRPM5 in human tissue. The use of TRPM5-antibodies yields variable results with *in situ* stainings²⁵. In human islets and isolated β -cells a low level of TRPM5 mRNA has been detected.³⁴ Experiments to show a functional role for TRPM5 in human β -cells is currently lacking. The low mRNA levels suggest a low protein level and thereby a small TRPM5-dependent conductance. This is in line with the observations in murine β -cells where TRPM5 is active during phases of high membrane resistance, when small currents can induce a substantial shift of the membrane potential. The availability of high quality human islets will help to provide valuable insight in the TRPM5 function in the pancreatic β -cell.³⁵

Diffuse chemosensory system - Besides its role in canonical taste signaling, the expression profile of TRPM5 suggests a broader functional role in chemosensation. TRPM5 together with other elements of the taste signal transduction cascade are expressed in at least a subset of solitary chemosensory cells (SCCs) throughout much of the olfactory, respiratory and digestive epithelia of rat and mice. SCCs form the so-called diffuse chemosensory system. This system may be participating in the recognition and processing of diverse environmental stimuli.^{31,36,37} TRPM5, Tas1R2, Tas1R3, Gβ3, Gγ13, α-gustducin, PLCβ₂ and IP₃R are expressed in NCI-H716 cells, a human enteroendocrine L-cell line, and in human duodenal tissue. α-gustducin expression is also found in murine L-cells, responsible for the secretion of the incretin hormone glucagon-like peptide-1 (GLP-1).³⁸

In line with chemosensory role of solitary TRPM5 expressing cells in the respiratory and intestinal tract, many epithelial tissues express TRPM5 in a discrete subset of their luminal cells, cholinergic brush cells. There is TRPM5 expression in the murine conjunctival cholinergic chemosensory cells in the eye, murine and rat cholinergic urethral brush cells, murine biliary brush cells in the gall bladder, murine medullary epithelial cells in the thymus and murine chemosensory cells in the auditory tube.³⁹⁻⁴³ These studies used a transgenic mouse (or rat) expressing GFP driven by the promoter of choline acetyltransferase (hence cholinergic cells). TRPM5 expression was shown using immunostaining. The functional role of TRPM5 and consequences of activation of these cells still needs to be determined. It is however postulated they initiate protective reflexes in reaction to hazardous chemicals, like bacterial compounds.⁴⁴

Next to sensing stimuli external to the body, taste signaling molecules also play a role in sensing internal stimuli. Taste signaling molecules (incl. TRPM5) were found to be expressed in the choroid plexus and other areas in the brainstem of rat. It is suggested these proteins play a role in sensing the chemical composition of brain extracellular fluids.^{45,46} TRPM5 might play a role

in the pathogen sensing in type II immunity recruiting tuft cells in response to intestinal parasites.⁴⁷

The brain – In the murine prefrontal cortex, the layer 5 pyramidal neurons display a characteristic electrical pattern in the response to muscarinic acetylcholine receptor agonists (e.g. carbachol): a burst of action potentials activating a slow afterdepolarization, which can eventually lead to the generation of intrinsic persistent firing. CANs (Ca²⁺-activated nonselective cation channels) are thought to underlie these slow afterdepolarizations, with TRPM5 being a possible candidate. Using immunofluorescence, TRPM5 expression is shown in these cells in the murine prefrontal cortex. Indeed, knockout of *Trpm5* in prefrontal cortex neurons of mice decreases the carbachol-induced induction of slow afterdepolarization.⁴⁸ TRPM5 functions as a signal integrator detecting the activation of GPCR and Ca²⁺ increases, much in the same way as in the type II taste receptor cells.

1.2. Modulation of TRPM5

Knowledge about the modulation of TRPM5 helps to elucidate its physiological role. Chemical TRPM5 modulators are of great economical interest, as they can help optimizing a desired taste palate in industrial prepared food products. In addition, TRPM5 is implicated in glucose-dependent insulin secretion. Therefore, TRPM5 potentiation could lead to increased postprandial insulin secretion and reduced glycaemia. In this way, TRPM5 modulators can be used as lead compound for the development of a new class of anti-diabetic medication, stimulating the physiological way of insulin secretion.

1.2.1. Physiological modulation of TRPM5

Elevation of [Ca²⁺]_i activates TRPM5. This activation is voltage-dependent, characterized by a strong outward rectification.^{18,21–23,32,49} This voltage dependency is a physiologically relevant feature of many TRP channels.

It was shown that TRPM5 channel activity is modulated by temperature changes in the 14-38°C range, which is the result of a temperature-dependent shift of the activation curve to more negative V_m .⁵⁰ This temperature modulation is secondary to Ca^{2+} induced activation. TRPM5 can thus act as coincidence detector of warm temperatures and stimuli increasing $[Ca^{2+}]_i$. This is illustrated by experiments that show that increasing the temperature from 15°C to 35°C was able to increase sweet stimulation of chorda tympani nerves in electrophysiological recordings, an effect that was lost in *Trpm5*^{-/-} mice.^{51,52}

TRPM5 was found to be blocked by extracellular acidification. The amino acid residues responsible for the blocking by lower pH were identified and are conserved between human and mouse TRPM5, indicating an evolutionary conserved regulating role.⁵³

TRPM5 gating appears to be modulated by lipids. TRPM5 activity subsides after prolonged exposure to Ca^{2+} in both whole-cell experiments and in patch excision experiments.^{22,23} PIP_2 lowers the Ca^{2+} threshold for activation after desensitization of the TRPM5 channel, suggesting that PIP_2 hydrolysis is the reason for current rundown in TRPM5.²³ It is hypothesized that the process of desensitization involves loss of bound PIP_2 through a Ca^{2+} -dependent mechanism. In this way, PIP_2 could play an important role in TRPM5-mediated taste modulation.²³ Generation of arachidonic acid in rat taste cells was shown to activate TRPM5 and modulate taste signaling.⁵⁴ It is likely that additional stimuli are involved in the physiological modulation of TRPM5 gating, the discovery of which could help to clarify the role and functioning of the channel.

1.2.2. Pharmacological modulation of TPRM5

There is both an unmet scientific need and economic value in high affinity modulation of TRPM5. The available pharmacology of the TRPM5 channels is limited. Many of the early compounds that have TRPM5-modulatory activity have either not been tested on selectivity and

specificity or are not selective, work only at high concentrations difficult to reach pharmacologically or work via TRPM5-independent pathways in the body making them inappropriate to use as drugs. Limited evidence is available, but increasing, of small molecules tested in *in vivo* preparations (Table 1).

Blocking agents of TRPM5 can be used to discriminate TRPM5 currents in native cells and tissue. For this purpose, selectivity between TRPM5 and other channels, like TRPM4 is required. Early work identified some compounds with TRPM5-inhibiting activity but show poor selectivity. Using inside-out patches it was shown that TRPM4 shows a 10-fold higher sensitivity to flufenamic acid than TRPM5 ($IC_{50} = 2.5 \mu M \pm 6.1 \mu M$ and $24.5 \mu M \pm 2.2 \mu M$, respectively) and spermine is able to inhibit TRPM4 and TRPM5 within similar concentration range ($IC_{50} = 35 \mu M \pm 11 \mu M$ and $37 \mu M \pm 12 \mu M$, respectively).¹⁸

Triphenylphosphine oxide (TPPO) is the most interesting described TRPM5 inhibitor to date.⁵⁵ It was identified in a screening approach using a membrane potential assay. TPPO is a TRPM5 blocker with IC_{50} values of $\sim 12 \mu M$ and $\sim 30 \mu M$ for murine and human TRPM5, respectively. TRPA1, TRPV1 and TRPM4 are not inhibited by TPPO, proving selectivity. TPPO has been used as a pharmacological tool to study TRPM5-dependent insulin secretion from rat islets and taste perception in mice.^{56,57} However, TPPO has limited solubility in water ($\sim 100 \mu M$) and is needed in high concentrations to inhibit taste responses, which may limit its use in consumption products.

Recently, rutamarin, a phytochemical isolated from the *Ruta graveolens* plant was identified as a TRPM5 potentiator.⁵⁸ Based on the distinct taste of this plant, bioactive compounds were tested on taste-mediating proteins. 10 different phytochemicals were isolated and their activity on Tas2R receptors (bitter taste receptors) and certain TRP channels, involved in sensory and

taste signalling, was analysed. Rutamarin has poor selectivity for TRPM5 as it has potentiating activity on TRPV1 and inhibits TRPM8 activity.

Talavera *et al.*⁵⁹ investigated the effect of quinine and quinidine on TRPM5 in order to elucidate the role of TRPM5 in bitter-sweet taste interactions. Using the whole-cell patch clamp technique, an IC₅₀ value of ~50 μ M and ~42 μ M at -50 mV was obtained, upon quinine and quinidine application, respectively. Murine gustatory nerve recordings show *Trpm5*^{-/-} nerves are non-responsive to these compounds. Nicotine was identified as a TRPM5 inhibitor using whole-cell patch clamp (IC₅₀ = 1.3 μ M at -50 mV), but lacks TRPM5-specificity as it interacts with TRPA1 and the acetylcholine receptor.^{60,61}

Steviol glycosides are sweet tasting compounds isolated from the leaves of *Stevia rebaudiana*. We recently identified steviol glycosides as stevioside and rebaudioside A as compounds potentiating the Ca²⁺-induced TRPM5 activity.⁴⁹ Patch clamp recordings revealed the TRPM5-potentiating effect of steviol glycosides in overexpression studies and selectivity was tested vs. TRPM3, TRPM4, TRPM8, TRPV1 and TRPA1. In a two bottle taste preference test to assess the taste sensation, steviol potentiates the preference for sweet, bitter and umami tastants while being taste neutral on its own. This taste enhancing effect was also present in sweet receptor knock-out mice, but completely abolished in *Trpm5*^{-/-} mice. *In vivo* studies on mice show that stevioside administration to mice, in physiological concentrations, leads to increased glucose-induced insulin secretion and delays the development of high-fat diet induced diabetes. This effect was completely absent in *Trpm5*^{-/-} mice. These experiments show that the targeting of TRPM5 is a valid approach to increase the postprandial insulin secretion and prevent hyperglycemia.

Stevioside has long been used in Japan and South America as a non-caloric sweetener and so far no negative effects of the use of physiological dosage of stevioside or steviol glycosides

have been described either in healthy subjects or in a clinical trial setting.^{62,63} Currently described studies that use steviol glycosides to treat type 2 diabetic subjects are either suffering from low numbers of participants and consequently insufficient statistical power,⁶⁴ or included patients that are on some form of insulin therapy, that could interfere with the anticipated effect of stevioside.⁶⁵ Ultimately a clinical trial could confirm the previously obtained results with mice, when care is taken to the statistical power and inclusion criteria for healthy volunteers and type 2 diabetic patients. The development of stevioside-based drugs to treat diabetes is expected to be far less expensive than de novo drug development. Targeting TRPM5 with stevioside can form the basis for new, inexpensive antidiabetic medication.

2. Taste

The sensory system is essential to become aware of the outside world regarding its chemical (taste, olfaction) and physical parameters (temperature, vision, hearing and mechanical). The perception of taste consists of five modalities: sweet, umami, salt, bitter and sour.^{30,66} Recent work suggests a separate sixth primary taste modality elicited by the digestive products of fat (fatty acids).^{67–70} Another candidate as primary taste modality is glucose oligomers or starches that elucidate a sensation independent from the sweet receptor.⁷¹ However further evidence is required to fully classify fatty acid perception and starch perception as primary taste. Further statements in this review are mainly limited to the established five primary taste modalities. The recognition of sweet and umami allows the selection of food rich in carbohydrates and proteins, respectively. Salt taste is indicative for nutrients important for maintaining electrolyte balance. Excessive bitterness or sourness are aversive and warn us against the intake of noxious or poisonous chemicals, like bitter plant alkaloids present in spoiled food and sour, unripe fruit.^{30,66}

TRCs are responsible for taste perception. These cells of epithelial origin are organized as taste buds together with support and precursor cells. The taste buds are located in papillae of the tongue and palate epithelium. Three types of papillae exist: circumvallate, foliate and fungiform. Each receptor cell responds to a single taste modality. TRCs are innervated by afferent nerve endings transmitting information via synapses in the brain stem and thalamus to the taste centers of the cortex. There are currently two models on how taste information is coded. In the labeled-line model (Figure 1) each neuron carries taste information of a single modality via segregated pathways. In the across fibre pattern, taste modality and intensity are encoded by ensembles of broadly tuned neurons.^{29,66,72–74} The taste of sweet, bitter and umami is detected by the type II TRCs. They carry a distinct combination of taste-receptors on their apical surface. The taste receptors determine which taste is detected by an individual cell. There are two subfamilies of taste receptors. The TAS1R subfamily is responsible for umami and sweet taste detection, while the TAS2R subfamily is responsible for bitter taste detection. The TAS1R subfamily consists of three GPCRs: TAS1R1, TAS1R2 and TAS1R3. These subtypes form heterodimers TAS1R2+3 and TAS1R1+3 with selectivity for sweet and umami, respectively. The sweet receptor is activated by a range of structurally different molecules elucidating a sweet taste. These agonists include mono- and disaccharides, sweet D-amino acids, sweet proteins, natural and synthetic low/non-caloric sweeteners as saponins, saccharines and cyclamate.^{72,75,76} Taste sensation is an evolutionary important trait, and sequence variations of the taste receptors allows species to be optimally adapted in their habitat. For example, humans can only sense umami as monosodium glutamate and aspartate, while most mammals taste a broad range of L-amino acids.⁶⁶

While birds in general lack the Tas1R2 receptor and thereby lost sweet sensation, the hummingbird has a functional adapted umami receptor Tas1R1+3 that is activated by the sweet taste of nectar.⁷⁷ Bitter taste is detected by a family of about 30 highly divergent GPCRs:

TAS2R. One TRC expresses multiple TAS2R subtypes enabling it to respond to a wide variety of bitter compounds.⁶⁶

2.1. The role of TRPM5 in taste

Type II TRCs are reacting to sweet, umami and bitter tasting compounds, due to the different receptors expressed at their apical membrane in the taste pore. The complex intracellular signal transduction cascade is the same. Altogether, binding of the sweet, umami or bitter receptor complex activates the heterotrimeric G protein gustducin, composed of the α -gustducin, G β 3, and G γ 13 subunits. The subsequent release of the subunit G $\beta\gamma$ results in PLC β ₂ activation. The latter enzyme breaks down PIP₂ into DAG and IP₃. IP₃ causes intracellular Ca²⁺ release from the ER via activation of the IP₃R. The rise in [Ca²⁺]_i activates TRPM5 which leads to depolarization and subsequent release of the neurotransmitter ATP through the voltage sensitive channel CALHM1 (calcium homeostasis modulator 1).^{23,66,72,78–80}

TRPM5 is specifically expressed in the Type II TRCs in the taste buds, and has an essential function in the signal transduction downstream of the receptor.^{28,30} In both nerve recordings and behavioral assays, *Trpm5*^{-/-} mice show a significant reduction or total abolishment of the responses to sweet, bitter and umami, while retaining responses to sour and salt.^{29,49,81} In intact tastes cells, elevation of intracellular IP₃ or Ca²⁺ induces TRPM5-dependent currents. In inside-out patches, elevations of Ca²⁺ activate the TRPM5 current but not IP₃.⁸² IP₃R^{-/-} mice have impaired responses to sweet, bitter and umami tastants.⁷⁸ The IP₃R is the link in the signal transduction between PLC and TRPM5. Reduction of TRPM5 function by replacing extracellular Na⁺ by NMDG⁺ (which is not permeated by TRPM5) or knockout of *Trpm5* were able to completely eliminate ATP secretion by the type II TRCs.⁷⁹ Ca²⁺ is the physiological activator of TRPM5, as chelating intracellular Ca²⁺ (using BAPTA), inducing store depletion

(using IP₃) or adding IP₃R inhibitor (heparin) were all able to abolish the TRPM5-dependent currents in type II taste cells.^{21–23} In this way IP₃R-mediated Ca²⁺ release and subsequent activation of TRPM5 is essential for taste perception in type II TRCs.

As TRPM5 is an essential component of taste signalling, it can be expected that modulation of TRPM5 influences taste perception. Indeed, as discussed in previous paragraphs, TRPM5 modulation by moderate heat and by quinine alter taste perception in mice.^{50,59} Warming the tongue was shown to enhance TRPM5 sweet-taste responses in mouse gustatory nerves, much in the same way as potentiation of TRPM5 with steviol and stevioside, a feature lost in *Trpm5*^{-/-} mice. This temperature sensitivity of TRPM5 might underlie certain effects of taste, such as enhanced sweetness perception at high temperatures and thermal taste (a phenomenon whereby heating or cooling of the tongue evokes sensations of taste in the absence of tastants) and a reduced taste perception of colder food.⁵⁰ Quinine was shown to dose-dependently inhibit TRPM5 currents and abolishes the gustatory response of sweet-sensitive gustatory nerves in WT but not in *Trpm5*^{-/-} mice. This inhibition of TRPM5 by bitter compounds constitutes a molecular basis of bitter-sweet interaction, whereby bitter tastants directly inhibit the sweet transduction pathway.⁶⁰

TRPM5 has been shown to play a role in taste sensation of fat,^{83,84} which is proposed to involve type II TRCs.⁶⁷ As TRPM5 is an essential signaling molecule in type II TRCs it is unsurprising that it has a prominent role in the sensation of fat. TRPM5 was shown to be involved in taste perception of linoleic acid (LA) in both *ex vivo* experiments in murine taste cells and *in vivo* experiments.⁸³ The inward current and rise in [Ca²⁺]_i upon LA were significantly reduced when the activity of G-proteins or PLC were inhibited. Addition of TPPO or knockout of *Trpm5* resulted in significantly reduced inward current and rise in [Ca²⁺]_i upon LA, indicating TRPM5 is involved. *Trpm5*^{-/-} mice showed reduced sensitivity to and no preference for LA. Others have

shown *Trpm5*^{-/-} mice lost preference for soybean oil emulsions.⁸⁴ The mechanism of fat taste is thus at least in part similar to the perception of sweet, bitter and umami.

3. The role of TRPM5 in the diffuse chemosensory system

TRPM5 expression is enriched in the vomeronasal organ and the olfactory epithelium, which is consistent with a proposed role in for TRPM5 in the olfaction of odorants and pheromones. TRPM5 expression is present in mature olfactory sensory neurons (OSNs) of mice in specific regions of the olfactory epithelium (ventral and lateral).⁸⁵ Here, TRPM5 was shown to be implicated in sensing of pheromones.^{85,86} A subset of glomeruli targeted by TRPM5-expressing OSNs was shown to be activated by several chemical signals, like mouse urine.⁸⁵ In addition, the number OSNs expressing TRPM5 increased by unilateral naris occlusion and elektroolfactogram recordings showed TRPM5 plays a role in maintaining pheromone detection after naris occlusion.⁸⁶ SCCs of the murine nasal cavity that express TRPM5 are suggested to play a role in the detection of odorous irritants.⁸⁷ High levels of various odorants were able to induce local event-related potentials and changed intracellular Ca²⁺ levels of isolated TRPM5-expressing SCCs. These SCCs are likely to receive trigeminal innervation, the activation of which leads to protective reflexes. TRPM5 expression is not only found in the nasal cavity, but also in SCCs scattered in the rest of the respiratory tract, from the main olfactory epithelium to the bronchioles of mice and rats.³⁶ These cells are suggested to play several roles in order to prevent damage caused by pathogens and irritants.

Additional TRPM5 expression has been shown in the intestinal tract.^{31,88} Most of the TRPM5-positive cells were identified as SCCs, more specifically brush cells or tuft cells. Again, these cells co-express most genes implicated in the canonical taste signalling (e.g. *Gai1*, *Gai2*, *Gβ13*, *α-gustducin*, *PLCβ2*, *PLCγ2*,...).³⁷ The brush cells play an important role in parasite detection and type 2 immunity in the gut. Recently it has been shown that the taste signalling pathway is

360 implicated.⁴⁷ *Gustducin*^{-/-} and *Trpm5*^{-/-} mice have impaired expansion of brush cells, goblet
361 cells, eosinophils and type 2 innate lymphoid cells upon parasite colonisation. This shows that
362 TRPM5-mediated chemosensation by the brush cells is important for an effective type 2
363 immune response to parasites colonizing the intestines.

364 TRPM5 was shown to be expressed in a subset of SCCs in mouse duodenum that co-express β -
365 endorphin and Met-enkephalin. Secretion of β -endorphin into the lumen of *ex-vivo* gut
366 preparation was stimulated by a hypertonic stimulus via a TRPM5-dependent process. The
367 physiological role of TRPM5 in this context remains to be determined.⁸⁸

368 Enteroendocrine cells are another subset of SCCs. The L-cells release glucagon-like peptide-1
369 (GLP-1) and the K-cells release gastric inhibitory polypeptide (GIP). GLP-1 and GIP are
370 incretin hormones and stimulate postprandial insulin secretion by acting on cell-surface
371 receptors expressed on the pancreatic β -cell. GLP-1 slows down gastric emptying and thus
372 influences macronutrient absorption and increases satiety by working in on the brain's hunger
373 centre. In general, GLP-1 is implicated in metabolic function through regulation of
374 macronutrient processing and glucose homeostasis.^{89,90} Notably, GLP-1 receptor (GLP-1R)
375 agonists are used as antidiabetics based on their ability to stimulate insulin secretion. Knockout
376 of α -*Gustducin* in mice decreased GLP-1 release both *in vivo* and *ex vivo* in response to
377 glucose.³⁸ The reduced GLP-1 release was reflected in a delayed rise in plasma insulin after
378 glucose gavage in mice. In NCI-H716 cells, a human enteroendocrine L-cell line, sucralose-
379 mediated GLP-1 secretion was inhibited by the Tas1R3 inhibitor, Lactisole. Glucose-mediated
380 GLP-1 release was blocked by the phospholipase C inhibitor, U73122. So Tas1R3, α -gustducin
381 and PLC β_2 seem to be implicated in GLP-1 release. On the other hand, a direct role for TRPM5
382 in the secretory process of GLP-1 has not been identified.

4. Insulin secretion

The pancreatic islets of Langerhans contain β -cells, the functional units responsible for insulin production and secretion. Insulin is the only blood glucose lowering hormone regulating glucose homeostasis. Insulin secretion is a strictly regulated process controlled by a wide array of metabolic (e.g. glucose), neural, hormonal and possible pharmacological factors. Insulin promotes glucose uptake and nutrient storage in the liver, fat and muscle tissue. Dysregulation of insulin secretion or altered insulin sensitivity can lead to hypoglycemia when secreted excessively. Insufficient insulin secretion results in hyperglycemia and is related to the development of type 2 diabetes.^{89,91}

In the consensus model of glucose-stimulated insulin release, glucose enters the β -cell by facilitated diffusion via the GLUT-2 transporter. Glucose is metabolized resulting in a rise of ATP/ADP ratio. The rise in intracellular ATP levels ($[ATP]_i$) causes closure of ATP-sensitive K^+ channels (K_{ATP} channels). A depolarizing conductance then drives the V_m away from the equilibrium potential for K^+ . Next, Ca^{2+} enters the cell through VDCCs, followed by Ca^{2+} -induced Ca^{2+} release. The increase in $[Ca^{2+}]_i$ allows activation of the exocytotic machinery and eventually insulin secretion.^{89,91,92}

This classical view, however, cannot explain the pulsatile nature of glucose-stimulated insulin secretion. Indeed, oscillations in insulin secretion are observed containing a fast component with a period of tens of seconds and a slow component with a period of 4–6 min. These oscillations reflect a complex interaction between metabolic (glycolytic oscillations) and electrical mechanisms ($[Ca^{2+}]_i$ feedback onto ion channels). These interactions were implemented in a mathematical model called the glycolytic- Ca^{2+} or ‘dual oscillator model’.⁹³ Fast, slow and compound $[Ca^{2+}]_i$ oscillations can be observed, in parallel with oscillatory changes in V_m . The compound oscillations show fast bursts superimposed on the slow plateaus,

separated by electrically silent or interburst intervals. The slow component can be reflected by an oscillating glycolytic rate. The fast component can be attributed to Ca^{2+} feedback onto ion channels, during high glycolytic rate when glycolysis is non-oscillatory. First, concerning the Ca^{2+} feedback, Ca^{2+} activates at least one type of K^{+} ion channels. K^{+} efflux through these channels forms a part of the hyperpolarizing current necessary for the oscillatory behaviour. Ca^{2+} influences cellular metabolism as active Ca^{2+} extrusion consumes ATP. In addition, the Ca^{2+} transport through mitochondrial inner membrane uniporters depolarizes the mitochondrial inner membrane, resulting in decreased ATP production. Second, it is hypothesized that the occurrence of oscillations is dependent on glucokinase and aldolase activity and the glycolytic enzyme phosphofructokinase is responsible for the frequency of the glycolytic oscillatory mechanism resulting from positive feedback of its own product fructose 1,6-bisphosphate on its activity followed by depletion of its substrate fructose 6-phosphate.^{32,93,94} The exact mechanism resulting in these complex oscillatory patterns upon glucose stimulation has not yet been elucidated. It can be concluded that stimulus-secretion coupling in β -cells is highly complex and unlikely to be explained by one simple model.

4.1. The role of TRPM5 in insulin secretion

The resting potential of the β -cell is slightly more positive than K^{+} equilibrium potential. Furthermore, closure of the K_{ATP} channel upon glucose stimulation on its own is not enough to bring membrane potential away from the K^{+} equilibrium potential. This indicates the presence of an inward background current. The contribution of this background current to total membrane permeability increases upon glucose stimulation as closure of K_{ATP} channels increases the electrical resistance of the membrane. The background current can be attributed to a Cl^{-} efflux and/or Ca^{2+} and/or Na^{+} influx.^{89,92,95} More specifically, the presence of CANs (Ca^{2+} -activated nonselective cation channels) has been described in human β -cells and related cell lines. These channels display a conductance of 20-25 pS. Two TRP channels are candidate

for these currents: TRPM4 and TRPM5.^{95,96} TRPM4 deletion in mice, however, does not alter results for glucose tolerance test and glucose-induced insulin release from the islets remains unchanged.⁹⁷ Recent studies have provided more insight into the role of TRPM5 in insulin release by the β -cells, indicating that TRPM5 provides positive feedback between increases in $[Ca^{2+}]_i$ and membrane depolarization (Figure 2).

Colsooul *et al.*³² demonstrated co-localization of TRPM5 with insulin in murine β -cells. Whole-cell currents in single pancreatic islet cells, obtained by raising the $[Ca^{2+}]_i$ either via dialyses with pipette solution or via flash-uncaging of Ca^{2+} , were significantly reduced in *Trpm5*^{-/-} compared to WT β -cells. These changes are reflected in an altered phenotype, giving evidence for a role of TRPM5 in insulin secretion. Indeed, *Trpm5*^{-/-} isolated islets showed significant reduction of glucose-induced calcium activity and insulin release. Moreover, *Trpm5*^{-/-} mice showed reduced insulin secretion and glucose clearance in both intraperitoneal (IPGTT) and oral (OGTT) glucose tolerance test. $[Ca^{2+}]_i$ oscillations upon glucose stimulation, measured by Fura-2 Ca^{2+} imaging, differed between WT and *Trpm5*^{-/-} in that *Trpm5*^{-/-} islet lacked fast type oscillations. These Ca^{2+} oscillations were shown to be strictly coupled to V_m changes. TRPM5 contributes to the depolarizing current during interburst interval driving V_m toward threshold potential resulting in new bursts.⁹⁸ Why TRPM5 seems only to be important in the fast oscillating subpopulation can be explained by the double oscillatory model. The TRPM5-dependent depolarization is coupled to the glycolytic rate of the cell. During high glycolytic rate, when K_{ATP} channels are closed, TRPM5 current is able to bring the membrane potential away from the K^+ equilibrium potential in the interburst interval. During oscillating glycolytic rate, however the relative small TRPM5-dependent current is not able to counteract the K^+ influx through K_{ATP} channels.

Brixel *et al.*⁹⁹ confirm a role for TRPM5 in membrane depolarization upon glucose stimulation. Besides glucose-induced insulin secretion, L-arginine-induced insulin secretion (in the

presence of low glucose levels) is also shown to be impaired in the *Trpm5*^{-/-} mice. The authors postulate this is indicative for an additional role for TRPM5 in the insulin secretion process, independent from K_{ATP} channel inhibition. However, no further experimental evidence is provided to illustrate *in vivo* relevance of this mechanism and one could argue this hypothesis. Indeed, the positively charged amino acid L-arginine passively enters the cell which leads to a depolarization of the cell membrane. This induces Ca²⁺ release via VDCC. This rise in [Ca²⁺]_i activates TRPM5 which conduct a depolarizing current. TRPM5 simply potentiates the L-arginine-induced depolarization.

Inhibition of TRPM5 with TPPO was able to reduce glucose-induced or L-arginine induced insulin secretion from rat islets and [Ca²⁺]_i increase in rat β-cells.⁵⁶ Furthermore, inhibition of TRPM5 was shown to decrease the K_{ATP} channel-independent glucose-mediated insulin release and [Ca²⁺]_i increase. The K_{ATP} channel independent insulin secretion occurs when a high extracellular K⁺ solution of diazoxide is used to depolarize the β-cells and clamp the intracellular Ca²⁺ thereby circumventing the K_{ATP} channel-mediated pathways. Potentiation of TRPM5 with stevioside leads to increased depolarization in murine β-cells reflected by faster Ca²⁺ oscillation and increased glucose-induced insulin secretion.⁴⁹

In addition to its role in glucose-induced insulin release, blocking TRPM5 by TPPO was also able to inhibit the GLP-1 potentiated glucose-induced insulin release. A recent report has further investigated the mechanism by which physiological ranges of GLP-1 mediate insulin secretion in both murine and human β-cells and islets. A role for TRPM5 in GLP-1-mediated insulin release is suggested. It was found that low physiological GLP-1 concentrations in 6 mM glucose conditions depolarize the β-cells, trigger action potential firing and induce insulin secretion via both cAMP/PKA-and PLC/PKC-dependent mechanisms. On application of isradipine, an L-type Ca²⁺ channel blocker, GLP-1 was still capable of depolarizing the β-cell. However, no action potentials were induced, indicating the important role of L-type Ca²⁺ channels. Most

interestingly, the effect of GLP-1 on electrical activity in presence of isradipine was abolished by lowering extracellular Na^+ . Na^+ influx thus seems to be important in GLP-1 induced electrical modulations and is mediated by TRPM5 and TRPM4. β -cells of *Trpm5*^{-/-} and *Trpm4*^{-/-} mice show a significant reduction of the GLP-1 mediated potentiation of glucose-induced insulin secretion. This effect is mirrored in an equal reduction of action potential firing of *Trpm5*^{-/-} and *Trpm4*^{-/-} β -cells. Activation of Na^+ -permeable TRPM5 and TRPM4 channels is shown to be mediated largely by mobilization of intracellular Ca^{2+} from thapsigargin-sensitive Ca^{2+} stores. Activation of TRPM5 and TRPM4 via a PKC-dependent mechanism is a subject for further investigation.¹⁰⁰

Kyriazis *et al.*¹⁰¹ suggest TRPM5 plays a role in sweet receptor mediated fructose-induced potentiation of glucose-stimulated insulin secretion. *Trpm5*^{-/-} murine β -cells failed to show intracellular calcium mobilization upon a fructose stimulus, impairing insulin release. On the contrary, Krishnan *et al.*⁵⁶ did not find that inhibition of TRPM5 by TPPO affected insulin secretion of rat islets and $[\text{Ca}^{2+}]_i$ increase in rat β -cells in response to fructose. This difference is suggested to be due to a difference in experimental setup and the use of a different animal model.

Finally, in accordance with above mentioned results in *Trpm5*^{-/-} mice, stimulation of TRPM5 with steviol glycosides increases action potential firing in the β -cells and stimulates glucose-induced insulin secretion.⁴⁹ Taken together, these results show that TRPM5 is an important link in the insulin secretion pathway of the pancreatic β -cells, and might be a useful drug target to increase insulin secretion.

5. The role of TRPM5 in the metabolic syndrome

The metabolic syndrome (MetS) is characterized by a combination of at least three of several metabolic medical conditions: visceral obesity, elevated blood pressure or hypertension, insulin

resistance and impaired insulin tolerance, resulting in elevated plasma glycemia, high serum triglycerides and low HDL levels. MetS is associated with the increased risk for developing cardiovascular disease and type II diabetes and an increased mortality.¹⁰²

TRPM5 plays a role in carbohydrate metabolism on different levels: taste perception and insulin secretion. It is indirectly implicated in the release of incretins and other factors from the gut. TRPM5 is therefore involved in the complex process of energy intake and glucose homeostasis. In this way, TRPM5 could constitute a new target in the search for pharmaceuticals for the treatment or prevention of MetS.

5.1. Obesity

Since 1980, the worldwide prevalence of overweight (body mass index (BMI) ≥ 25) and obesity (BMI ≥ 30) has more than doubled.¹⁰³ Obesity is caused by an interaction between genetic and environmental factors resulting in an energy imbalance between calories consumed and calories expended. It cannot be denied that the increased prevalence of this condition can be ascribed to a decrease in physical activity and/or an increased consumption of energy-dense foods that are high in fat and added, refined, sugars.¹⁰⁴ This largely preventable condition has many health consequences. BMI is a risk factor for noncommunicable diseases such as cardiovascular diseases, musculoskeletal disorders, cancer and diabetes. Consequently, overweight and obesity are a great burden of disease to our society.¹⁰⁴ The treatment of obesity and its related comorbidities is one of the big challenges for the medical industry. Treatment currently consists first of all of life style changes. For morbidly obese patient (BMI > 45) bariatric surgery may be the only effective treatment. The development of pharmacological treatment has shown to be more difficult. Drugs that target the central nervous system, like rimonabant (cannabinoid-1 receptor agonist), have had minimal success due to side-effects. Discovery of peripheral drug targets, involved in food intake and metabolism, would improve the effectiveness of pharmacological intervention because targeting the periphery limits unwanted side effects.⁹⁰

5.1.1. TRPM5 and caloric intake

TRPM5 is proposed to be such a peripheral target.¹⁰⁵ Supplementation of quinine, a bitter tasting TRPM5 inhibitor to the diet of WT mice leads to less weight gain compared to control diet.⁵⁹ This effect however was not significantly different in *Trpm5*^{-/-} mice, indicating at least a partial TRPM5-independent mechanism.¹⁰⁶ Other studies systematically tested the effect of high-calorie diets on WT and *Trpm5*^{-/-} mice. The effect of *Trpm5* KO on diet-induced obesity upon sucrose and polycose supplementation to the diet was investigated. *Trpm5*^{-/-} mice show a reduced caloric intake which is associated with a reduced weight gain compared to WT mice on both the sweet tasting sucrose and polycose diet. This is in line with the taste deficiency of *Trpm5*^{-/-} mice that lack short-term preferences for sucrose and polycose. Another recent study corroborated these results.¹⁰⁷ An independently generated *Trpm5*^{-/-} mouse consumed less calories, gained less weight and had an improved glucose tolerance compared to WT mice when fed a high fat diet (HFD) or high sugar and fat cafeteria diet. Interestingly, both glycemic values and circulating insulin levels were lower in *Trpm5*^{-/-} mice. WT mice significantly increased their caloric intake when fed a HFD supplemented with a palatable chocolate ball compared to HFD alone, due to increased consumption of the chocolate ball. *Trpm5*^{-/-} mice had the same caloric intake when fed the combination compared to HFD alone, indicating that TRPM5-dependent sweet taste induced overeating as the cause for the increased energy intake and glucose intolerance development seen for WT mice on cafeteria diet and HFD.¹⁰⁷ We recently reported that when WT and *Trpm5*^{-/-} mice on a HFD consume the same amount of calories, their weight gain is not different.⁴⁹ This indicates the taste dependent reduction of caloric intake as the factor towards the apparent obesity-resistant phenotype of *Trpm5*^{-/-} mice. Forced feeding studies with strict control of caloric intake in WT and *Trpm5*^{-/-} animals would be able to confirm this, but have not been performed. Furthermore, reduced insulin levels in the *Trpm5* KO mice might lead to impaired nutrient uptake in the tissues, extended elevated blood glucose and

reduced hunger feeling. Currently, one can only speculate about the exact mechanism behind the apparent protective effect of the loss of TRPM5 against obesity.

5.2. Type 2 diabetes mellitus

Type 2 Diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by disturbed carbohydrate, protein and fat metabolism resulting from (a combination of) impaired insulin secretion and peripheral insulin resistance.¹⁰⁸ In 2014, the global prevalence of diabetes was estimated to be 9% among adults. T2DM constitutes 90% of all diabetes cases. DM is associated with macro-and microvascular complications, thus constituting a great burden of disease to our society. The World Health Organisation estimates that by 2030 diabetes will be the seventh leading cause of death.¹⁰⁹ T2DM results from a combination of lifestyle, environmental and genetic factors. It is a polygenic disease and mutations in genes encoding ion channels or regulating ion channel function, membrane targeting or expression in the β -cell have been implicated in the pathophysiology of T2DM. Small changes in electrical excitability of the β -cell will be mirrored by changes in insulin secretory capacity.¹⁰⁸

T2DM is initially treated with lifestyle modifications and oral medication, including sulfonylureas (SU), metformin and incretin mimetics. SUs increase β -cell insulin secretion via K_{ATP} channel inhibition. Inhibition of the K_{ATP} channel results in depolarization, activation of VDCC, Ca^{2+} influx and subsequent insulin release. SUs increase insulin levels independently of blood glucose levels, risking hypoglycaemia. These drugs are associated with weight gain, an unwanted side effect. GLP-1 receptor agonists or inhibitors of dipeptidylpeptidase-4 (DDP4), which inactivates GLP-1, modulate the incretin signalling. These drugs increase insulin secretion and suppress glucagon secretion both by a glucose-dependent mechanism. GLP-1 receptor agonists delay gastric emptying, increasing satiety. In addition, they work on the brain's hunger centres influencing eating behaviour. In some cases, this results in modest weight loss. However, these drugs are expensive, require subcutaneous administration and are

associated with unwanted side effects, like nausea and vomiting.¹¹⁰ Therefore, it is interesting to keep pursuing improvement of current therapies and to keep searching for new targets and therapies that are safe and have limited adverse effects.

5.2.1. TRPM5 and type 2 diabetes

Blood glucose concentrations from diabetic patients is negatively correlated with TRPM5 expression in the small intestine. Also, acute perfusion of glucose *in vivo* in mice led to a significant decrease in Tas1R2 expression. So, the expression of intestinal taste molecules must be under dynamic control by metabolic and luminal factors.¹¹¹ Colsoul *et al.*¹¹² found that TRPM5 expression in islets of obese Ob/Ob and Db/Db mice was significantly decreased. These leptin-deficient mice display a prediabetic phenotype. They are deficient in sensing satiety and therefore suffer from hyperphagia-induced obesity. This leads to elevated plasma glucose levels and insulin resistance resulting in severely impaired glucose tolerance. It was shown TRPM5 was downregulated due to elevated insulin levels. This reduced expression is reflected by irregular Ca^{2+} oscillations and lack of fast oscillations, similar as seen in *Trpm5*^{-/-} mice.³² Steadily elevated insulin levels in the leptin-deficient (Db/Db) mice prevent its own release by downregulation of TRPM5, a positive regulator of insulin secretion. This could be linked to impairment of pulsatile insulin release which is an early marker for β -cell dysfunction in T2DM.¹¹² Ketterer *et al.*¹¹³ investigated the impact of nine common SNPs, three of which were found to be related to increased glucose levels during OGTT. One of these SNPs in TRPM5 was also shown to be significantly associated with insulin secretion. These results are in line with previous animal studies showing that *Trpm5* KO results in reduced insulin secretion, impaired glucose clearance and reduced glucose tolerance.^{32,99} All three TRPM5 SNP's were nominally associated with reduced OGTT-derived insulin sensitivity in humans. This contrasts with results showing a normal insulin tolerance test and hyperinsulinemic euglycemic clamp in WT and *Trpm5*^{-/-} mice.^{19,32} Although the functional impact of these variants is not clear yet,

606 these data indicate a link exists between T2DM and TRPM5. A case-control study investigated
607 TRP channel gene expressions and TRPM5 gene polymorphisms in the obesity-related MetS
608 in a Turkish population.¹¹⁴ The A allele of the rs4929982 (Arg578Gln) polymorphism was
609 positively associated with MetS. Also, TRPM5 gene expression was shown to be significantly
610 reduced in MetS patients. In genetic and diet-induced obese animals, TRPM5 expression was
611 downregulated compared to the lean WT controls in the brainstem and the duodenum, showing
612 that obesity modulates TRPM5 expression.¹¹⁵ Thus, TRPM5 could play a role in the complex
613 etiology of MetS and T2DM which is characterised by glucose intolerance. Wolfram syndrome
614 is a rare autosomal recessive neurodegenerative disease caused by a mutation in the wolframin
615 gene (WFS1). One of the symptoms from the disease is juvenile onset T2DM. Recently RNA-
616 seq from pancreatic islets from WT and *Wfs1*^{-/-} animals revealed a downregulation of TRPM5
617 in the islets of diabetes-prone *Wfs1*^{-/-} mice, indicating a negative association of TRPM5
618 expression levels with the development of T2DM.¹¹⁶

619 As discussed in previous paragraphs, TRPM5 is expressed in taste buds and pancreatic β -cells.
620 TRPM5 is implicated in frequency of Ca^{2+} oscillations and regulation of insulin release by β -
621 cells. *Trpm5* KO results in decreased glucose tolerance due to impaired glucose-induced insulin
622 release.^{32,99} In this way, TRPM5 potentiating compounds as stevioside could constitute a new
623 class of insulin secretagogues.⁴⁹ The TRPM5 mediated current in the β -cell is only significant
624 when glucose plasma levels are high and K_{ATP} channels are closed.⁵⁶ So, TRPM5 potentiation
625 would result in increased glucose-dependent insulin secretion. This reduces the risk of
626 hypoglycaemia like is observed for the sulfonylureas, that work independent of glucose plasma
627 levels.

6. Modulation of TRPM5 to prevent/treat obesity and type 2 diabetes

The prevalence of obesity and type 2 diabetes is steadily increasing. The leading cause is access and excessive intake of cheap and high-caloric food/beverages combined with a sedentary lifestyle and reduced physical activity. Controlling caloric intake is, therefore, a first target in treatment and prevention of these multi-factorial conditions. Taste is one of the factors influencing prandial satiety and caloric intake.¹¹⁷ TRPM5 is required for taste perception, so it can be regarded as a target to modulate caloric intake. Both potentiation and inhibition of TRPM5 can have distinct functional roles in taste perception. It has been shown in mice that *Trpm5* knockout can reduce energy intake, preventing body weight gain.^{105,107} Blockage of the TRPM5 channel on the tongue could be used as a strategy to reduce caloric intake and achieve weight reduction by inhibiting the gustatory rewards.⁹⁰ Indeed, impairment of taste perception in humans (ageusia), due to stroke, drug therapy, radiation treatment or aging, is associated with decreases in caloric intake and body weight.¹¹⁸

On the other hand, there is great economical interest in reducing caloric content while maintaining taste of food and beverages. In this context, potentiating TRPM5 at the tongue can be considered. By combining a TRPM5 potentiating compound as stevioside with a caloric sweet receptor agonist, the concentration of the latter can be reduced while taste sensation is maintained. This strategy is conceptually similar to the effect of positive allosteric modulators of the sweet taste receptor, which do not induce a taste sensation on their own, but enhance the taste of sweet receptor agonists.^{72,119}

The use of artificial low-caloric sweeteners (LCS) as sucralose, aspartame and saccharin for body weight control is however controversial. Populations of people with high LCS consumption partially overlap with obesity and people with a general unhealthy diet. This

652 makes it challenging to study the effects of the LCS independent of interfering morbidities.
653 These LCS interfere with glycemic control and energy homeostasis mechanisms. Several
654 studies indicate that LCS interfere with the gut microbiome, and in this way contribute to the
655 development of glucose intolerance.¹²⁰ Furthermore, interaction between LCS and sweet-taste
656 receptors in the digestive system interferes with glucose absorption and triggers insulin
657 secretion.¹²¹ High consumption of LCS changes the taste sensitivity and metabolic response on
658 a glucose load.¹²² Overall, the effect of replacing caloric sugars with LCS on body weight and
659 composition is moderately positive. A meta-analysis of randomized controlled trials found a
660 significant reduction in body weight, fat mass and BMI with the use of LCS.¹²³ Stevia extract,
661 containing mainly rebaudioside A and stevioside, is a natural non-caloric sweetener which is
662 increasingly being used in commercial beverages and food products. Stevioside does not
663 interfere with the gut microbiome composition or alters the insulin sensitivity in mice.¹²⁴ On
664 the contrary stevioside increases postprandial insulin secretion, preventing the onset of high-fat
665 diet induced diabetes.⁴⁹ No negative effects in humans have been described but rigorous
666 randomised controlled trails are currently lacking.

667 TRPM5 potentiating compounds would enable a reduction of the sugar and fat content in food
668 or without limiting the taste stimulus. This would result in a reduction of energy intake and
669 reduced obesity and associated complications as type 2 diabetes. TRPM5 has also been
670 identified as an important factor in the regulation of glucose-induced insulin secretion in the
671 pancreatic β -cell. In this way a potentiation of TRPM5 can stimulate the β -cells to secrete more
672 insulin, preventing the onset of T2DM. There is effectively a bimodal advantage (taste and
673 insulin release) of TRPM5 potentiation in the prevention/treatment of type 2 diabetes. The
674 identification of steviol glycosides as novel TRPM5 potentiating compounds and future efforts
675 for new compounds either inhibiting or potentiating TRPM5 enables us to investigate the
676 impact of TRPM5 modulation in detail. More importantly, it will allow us to translate this

677 research to human, which is lacking at the moment. Identification of functional TRPM5 in the
678 human β -cell will increase the evidence that TRPM5 is a target for prevention and treatment of
679 type 2 diabetes.

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685 REFERENCES

- 686 1. Nilius, B. & Owsianik, G. The transient receptor potential family of ion channels. *Genome*
687 *Biol.* **12**, 218 (2011).
- 688 2. Ramsey, I. S., Delling, M. & Clapham, D. E. An introduction to TRP Channels. *Annu. Rev. Physiol.* **68**, 619–647 (2006). doi:10.1146/annurev.physiol.68.040204.100431
689
- 690 3. Liao, M., Cao, E., Julius, D. & Cheng, Y. Structure of the TRPV1 ion channel determined by
691 electron cryo-microscopy. *Nature* **504**, 107–112 (2013).
- 692 4. Paulsen, C. E., Armache, J., Gao, Y., Cheng, Y. & Julius, D. Structure of the TRPA1 ion
693 channel suggests regulatory mechanisms. *Nature* **520**, 511–517 (2015).
- 694 5. Saotome, K., Singh, A. K., Yelshanskaya, M. V. & Sobolevsky, A. I. Crystal structure of the
695 epithelial calcium channel TRPV6. *Nature* **534**, 506–511 (2016).
- 696 6. Zubcevic, L. *et al.* Cryo-electron microscopy structure of the TRPV2 ion channel. *Nat. Struct. Mol. Biol.* **23**, 1–9 (2016).
697
- 698 7. Gao, Y., Cao, E., Julius, D. & Cheng, Y. TRPV1 structures in nanodiscs reveal mechanisms of
699 ligand and lipid action. *Nature* **534**, 347–351 (2016).
- 700 8. Barad, B. A. *et al.* EMRinger: side chain-directed model and map validation for 3D cryo-
701 electron microscopy. *Nat. Methods* **12**, 943–946 (2015).
- 702 9. Huynh, K. W. *et al.* Structure of the full-length TRPV2 channel by cryo-EM. *Nat. Commun.* **7**,
703 11130 (2016).
- 704 10. Wilkes, M. *et al.* Molecular insights into lipid-assisted Ca²⁺ regulation of the TRP channel
705 Polycystin-2. *Nat. Struct. Mol. Biol.* **24**, 123–130 (2017).
- 706 11. Nilius, B. *et al.* Gating of TRP channels: a voltage connection? in *The Journal of physiology*
707 **567**, 35–44 (2005).
- 708 12. Nilius, B. TRP channels in disease. *Biochim. Biophys. Acta* **1772**, 805–812 (2007).
- 709 13. Nilius, B. & Owsianik, G. Transient receptor potential channelopathies. *Pflugers Arch.* **460**,

710 437–50 (2010).

711 14. Philippaert, K. & Vennekens, R. in *TRP Channels as Therapeutic Targets* (ed. Szallasi, A.)
712 343–363 (978-0-12-420024-1, 2015).

713 15. Zsombok, A. & Derbenev, A. TRP Channels as Therapeutic Targets in Diabetes and Obesity.
714 *Pharmaceuticals* **9**, 50 (2016).

715 16. Enklaar, T. *et al.* Mtr1, a novel biallelically expressed gene in the center of the mouse distal
716 chromosome 7 imprinting cluster, is a member of the Trp gene family. *Genomics* **67**, 179–187
717 (2000).

718 17. Oztuzcu, S. *et al.* Association of TRPM Channel Gene Polymorphisms with Systemic
719 Sclerosis. **770**, 763–770 (2015).

720 18. Ullrich, N. D. *et al.* Comparison of functional properties of the Ca²⁺-activated cation channels
721 TRPM4 and TRPM5 from mice. *Cell Calcium* **37**, 267–78 (2005).

722 19. Colsoul, B., Kecskes, M., Philippaert, K., Menigoz, A. & Vennekens, R. in (eds. Szallasi, A. &
723 Bíró, T.) 103–125 (Humana Press, 2012). doi:10.1007/978-1-62703-077-9_6

724 20. Guinamard, R., Sallé, L. & Simard, C. The non-selective monovalent cationic channels
725 TRPM4 and TRPM5. *Adv. Exp. Med. Biol.* **704**, 147–71 (2011).

726 21. Hofmann, T., Chubanov, V., Gudermann, T. & Montell, C. TRPM5 is a voltage-modulated and
727 Ca²⁺-activated monovalent selective cation channel. *Curr. Biol.* **13**, 1153–1158 (2003).

728 22. Prawitt, D. *et al.* TRPM5 is a transient Ca²⁺-activated cation channel responding to rapid
729 changes in [Ca²⁺]_i. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 15166–71 (2003).

730 23. Liu, D. & Liman, E. R. Intracellular Ca²⁺ and the phospholipid PIP₂ regulate the taste
731 transduction ion channel TRPM5. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 15160–5 (2003).

732 24. Fonfria, E. *et al.* Tissue distribution profiles of the human TRPM cation channel family. *J.*
733 *Recept. Signal Transduct. Res.* **26**, 159–78 (2006).

734 25. Uhlén, M. *et al.* Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419

735 (2015).

736 26. Kim, M.-S. *et al.* A draft map of the human proteome. *Nature* **509**, 575–81 (2014).

737 27. Pérez, C. a *et al.* A transient receptor potential channel expressed in taste receptor cells. *Nat.*
738 *Neurosci.* **5**, 1169–76 (2002).

739 28. Kusumakshi, S. *et al.* A Binary Genetic Approach to Characterize TRPM5 Cells in Mice.
740 *Chem. Senses* **40**, 413–425 (2015).

741 29. Zhang, Y. *et al.* Coding of sweet, bitter, and umami tastes: different receptor cells sharing
742 similar signaling pathways. *Cell* **112**, 293–301 (2003).

743 30. Pérez, C. a., Margolskee, R. F., Kinnamon, S. C. & Ogura, T. Making sense with TRP
744 channels: store-operated calcium entry and the ion channel Trpm5 in taste receptor cells. *Cell*
745 *Calcium* **33**, 541–549 (2003).

746 31. Kaske, S. *et al.* TRPM5, a taste-signaling transient receptor potential ion-channel, is a
747 ubiquitous signaling component in chemosensory cells. *BMC Neurosci.* **8**, 49 (2007).

748 32. Colsoul, B. *et al.* Loss of high-frequency glucose-induced Ca²⁺ oscillations in pancreatic islets
749 correlates with impaired glucose tolerance in Trpm5^{-/-} mice. *Proc. Natl. Acad. Sci. U. S. A.*
750 **107**, 5208–13 (2010).

751 33. Wyatt, A. *et al.* Genetic strategies to analyze primary TRP channel-expressing cells in mice.
752 *Cell Calcium* 1–14 (2017). doi:10.1016/j.ceca.2017.05.009

753 34. Marabita, F. & Islam, M. S. Expression of Transient Receptor Potential Channels in the
754 Purified Human Pancreatic β -Cells. *Pancreas* **0**, 1 (2016).

755 35. Lyon, J. *et al.* Research-Focused Isolation of Human Islets From Donors With and Without
756 Diabetes at the Alberta Diabetes Institute IsletCore. *Endocrinology* **157**, 560–9 (2016).

757 36. Tizzano, M., Cristofolletti, M., Sbarbati, A. & Finger, T. E. Expression of taste receptors in
758 solitary chemosensory cells of rodent airways. *BMC Pulm. Med.* **11**, 3 (2011).

759 37. Bezençon, A. *et al.* Murine Intestinal Cells Expressing Trpm5 Are Mostly Brush Cells and

Express Markers of Neuronal and inflammatory cells. *J. Comp. Neurol.* **509**, 514–525 (2008).

38. Jang, H.-J. *et al.* Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 15069–74 (2007).

39. Wiederhold, S. *et al.* A novel cholinergic epithelial cell with chemosensory traits in the murine conjunctiva. *Int. Immunopharmacol.* 6–11 (2015). doi:10.1016/j.intimp.2015.06.027

40. Deckmann, K. *et al.* Bitter triggers acetylcholine release from polymodal urethral chemosensory cells and bladder reflexes. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 8287–92 (2014).

41. Schütz, B. *et al.* Chemical coding and chemosensory properties of cholinergic brush cells in the mouse gastrointestinal and biliary tract. *Front. Physiol.* **6**, 1–14 (2015).

42. Panneck, A. R. *et al.* Cholinergic epithelial cell with chemosensory traits in murine thymic medulla. *Cell Tissue Res.* **358**, 737–748 (2014).

43. Krasteva, G. *et al.* Cholinergic chemosensory cells in the auditory tube. *Histochem. Cell Biol.* **137**, 483–497 (2012).

44. Howitt, M.R., Lavoie, S., Michaud, M., Blum, A.M., Tran, S.V., Weinstock, J.V., Gallini, C.A., Redding, K., Margolskee, R.F., Osborne, L.C. and Artis, D. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science (80-.)*. **351**, 1329–1333. (2016).

45. Dehkordi, O. *et al.* Neuronal expression of bitter taste receptors and downstream signaling molecules in the rat brainstem. *Brain Res.* **1475**, 1–10 (2012).

46. Tomas, J., Santos, C., Quintela, T. & Gonçalves, I. Tasting of the cerebrospinal fluid: Another function of the choroid plexus. *Neuroscience* **320**, 160–171 (2016).

47. Howitt, M. R. *et al.* Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* **351**, 1329–33 (2016).

48. Lei, Y. *et al.* Differential contribution of TRPM4 and TRPM5 nonselective cation channels to the slow afterdepolarization in mouse prefrontal cortex neurons. *Front. Cell. Neurosci.* **8**, 1–14 (2014).

- 786 49. Philippaert, K. *et al.* Steviol glycosides enhance pancreatic beta-cell function and taste
787 sensation by potentiation of TRPM5 channel activity. *Nat. Commun.* **8**, 14733 (2017).
- 788 50. Talavera, K. *et al.* Heat activation of TRPM5 underlies thermal sensitivity of sweet taste.
789 *Nature* **438**, 1022–5 (2005).
- 790 51. Lu, B., Breza, J. M. & Contreras, R. J. Temperature Influences Chorda Tympani Nerve
791 Responses to Sweet, Salty, Sour, Umami, and Bitter Stimuli in Mice. *Chem. Senses* **0**, bjw082
792 (2016).
- 793 52. Talavera, K., Ninomiya, Y., Winkel, C., Voets, T. & Nilius, B. Influence of temperature on
794 taste perception. *Cell. Mol. Life Sci.* **64**, 377–381 (2007).
- 795 53. Liu, D., Zhang, Z. & Liman, E. R. Extracellular acid block and acid-enhanced inactivation of
796 the Ca²⁺-activated cation channel TRPM5 involve residues in the S3-S4 and S5-S6
797 extracellular domains. *J. Biol. Chem.* **280**, 20691–20699 (2005).
- 798 54. Oike, H., Wakamori, M., Mori, Y., Nakanishi, H. & Taguchi, R. Arachidonic acid can function
799 as a signaling modulator by activating the TRPM5 cation channel in taste receptor cells.
800 *Biochim. Biophys. Acta* **1761**, 1078–1084 (2006).
- 801 55. Palmer, R. K. *et al.* Triphenylphosphine oxide is a potent and selective inhibitor of the transient
802 receptor potential melastatin-5 ion channel. *Assay Drug Dev. Technol.* **8**, 703–713 (2010).
- 803 56. Krishnan, K. *et al.* Role of transient receptor potential melastatin-like subtype 5 channel in
804 insulin secretion from rat β -cells. *Pancreas* **43**, 597–604 (2014).
- 805 57. Ren, Z. *et al.* TRPM5-dependent amiloride- and benzamil-insensitive NaCl chorda tympani
806 taste nerve response. *Am. J. Physiol. Gastrointest. Liver Physiol.* **305**, G106-17 (2013).
- 807 58. Mancuso, G., Borgonovo, G., Scaglioni, L. & Bassoli, A. Phytochemicals from Ruta
808 graveolens Activate TAS2R Bitter Taste Receptors and TRP Channels Involved in Gustation
809 and Nociception. *Molecules* **20**, 18907–18922 (2015).
- 810 59. Talavera, K. *et al.* The taste transduction channel TRPM5 is a locus for bitter-sweet taste
811 interactions. *FASEB J.* **22**, 1343–55 (2008).

- 812 60. Gees, M. *et al.* Differential effects of bitter compounds on the taste transduction channels
813 TRPM5 and IP3 receptor type 3. *Chem. Senses* **39**, 295–311 (2014).
- 814 61. Talavera, K. *et al.* Nicotine activates the chemosensory cation channel TRPA1. *Nat. Neurosci.*
815 **12**, 1293–1299 (2009).
- 816 62. Geuns, J. M. C., Buyse, J., Vankeirsbilck, A. & Temme, E. H. M. Metabolism of stevioside by
817 healthy subjects. *Exp. Biol. Med. (Maywood)*. **232**, 164–173 (2007).
- 818 63. Chan, P. *et al.* A double-blind placebo-controlled study of the effectiveness and tolerability of
819 oral stevioside in human hypertension. *Br. J. Clin. Pharmacol.* **50**, 215–220 (2000).
- 820 64. Gregersen, S., Jeppesen, P. B., Holst, J. J. & Hermansen, K. Antihyperglycemic effects of
821 stevioside in type 2 diabetic subjects. *Metabolism* **53**, 73–76 (2004).
- 822 65. Maki, K. C. *et al.* Chronic consumption of rebaudioside A, a steviol glycoside, in men and
823 women with type 2 diabetes mellitus. *Food Chem. Toxicol.* **46**, (2008).
- 824 66. Chandrashekar, J., Hoon, M. a, Ryba, N. J. P. & Zuker, C. S. The receptors and cells for
825 mammalian taste. *Nature* **444**, 288–94 (2006).
- 826 67. Keast, R. S. & Costanzo, A. Is fat the sixth taste primary? Evidence and implications. *Flavour*
827 **4**, 5 (2015).
- 828 68. Running, C. a., Craig, B. a. & Mattes, R. D. Oleogustus: The Unique Taste of Fat. *Chem.*
829 *Senses* **40**, 507–516 (2015).
- 830 69. Chaudhari, N. & Roper, S. D. The cell biology of taste. *J. Cell Biol.* **190**, 285–96 (2010).
- 831 70. Laugerette, F., Gaillard, D., Passilly-Degrace, P., Niot, I. & Besnard, P. Do we taste fat?
832 *Biochimie* **89**, 265–269 (2007).
- 833 71. Lapis, T. J., Penner, M. H. & Lim, J. Humans can taste glucose oligomers independent of the
834 hT1R2/hT1R3 sweet taste receptor. *Chem. Senses* **41**, 755–762 (2016).
- 835 72. Dubois, G. E. Physiology & Behavior Molecular mechanism of sweetness sensation. *Physiol.*
836 *Behav.* **164**, 453–463 (2016).

- 837 73. Liman, E. R., Zhang, Y. V. & Montell, C. Peripheral coding of taste. *Neuron* **81**, 984–1000
838 (2014).
- 839 74. Carleton, A., Accolla, R. & Simon, S. A. Coding in the mammalian gustatory system. *Trends*
840 *Neurosci.* **33**, 326–334 (2010).
- 841 75. Hellfritsch, C., Brockhoff, A., Stähler, F., Meyerhof, W. & Hofmann, T. Human psychometric
842 and taste receptor responses to steviol glycosides. *J. Agric. Food Chem.* **60**, 6782–6793 (2012).
- 843 76. Ahmed, J. *et al.* SuperSweet-A resource on natural and artificial sweetening agents. *Nucleic*
844 *Acids Res.* **39**, 1–6 (2011).
- 845 77. Baldwin, M. W. *et al.* Sensory biology. Evolution of sweet taste perception in hummingbirds
846 by transformation of the ancestral umami receptor. *Science* **345**, 929–33 (2014).
- 847 78. Hisatsune, C. *et al.* Abnormal taste perception in mice lacking the type 3 inositol 1,4,5-
848 trisphosphate receptor. *J. Biol. Chem.* **282**, 37225–37231 (2007).
- 849 79. Huang, Y. A. & Roper, S. D. Intracellular Ca(2+) and TRPM5-mediated membrane
850 depolarization produce ATP secretion from taste receptor cells. *J. Physiol.* **588**, 2343–50
851 (2010).
- 852 80. Taruno, A. *et al.* CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter
853 and umami tastes. *Nature* **495**, 223–6 (2013).
- 854 81. Damak, S. *et al.* Trpm5 null mice respond to bitter, sweet, and umami compounds. *Chem.*
855 *Senses* **31**, 253–64 (2006).
- 856 82. Zhang, Z., Zhao, Z., Margolskee, R. & Liman, E. The transduction channel TRPM5 is gated by
857 intracellular calcium in taste cells. *J. Neurosci.* **27**, 5777–86 (2007).
- 858 83. Liu, P., Shah, B. P., Croasdell, S. & Gilbertson, T. a. Transient receptor potential channel type
859 M5 is essential for fat taste. *J. Neurosci.* **31**, 8634–8642 (2011).
- 860 84. Sclafani, A., Zukerman, S., Glendinning, J. I. & Margolskee, R. F. Fat and Carbohydrate
861 Preferences in Mice: the contribution of alfa-gusducin and Trpm5 taste signaling proteins. *Am.*
862 *J. Regul. Integr. Comp. Physiol.* **293**, 1–21 (2008).

- 863 85. Lin, W., Margolskee, R., Donnert, G., Hell, S. W. & Restrepo, D. Olfactory neurons expressing
864 transient receptor potential channel M5 (TRPM5) are involved in sensing semiochemicals.
865 *Proc. Natl. Acad. Sci. U. S. A.* **104**, 2471–2476 (2007).
- 866 86. Oshimoto, A. *et al.* Potential Role of Transient Receptor Potential Channel M5 in Sensing
867 Putative Pheromones in Mouse Olfactory Sensory Neurons. *PLoS One* **8**, (2013).
- 868 87. Lin, W., Ogura, T., Margolskee, R. F., Finger, T. E. & Restrepo, D. TRPM5-Expressing
869 Solitary Chemosensory Cells Respond to Odorous Irritants. *J. Neurophysiol.* **99**, 1451–1460
870 (2008).
- 871 88. Kokrashvili, Z. *et al.* Release of endogenous opioids from duodenal enteroendocrine cells
872 requires Trpm5. *Gastroenterology* **137**, 598–606, 606–2 (2009).
- 873 89. Colsoul, B., Vennekens, R. & Nilius, B. Transient receptor potential cation channels in
874 pancreatic β cells. *Rev. Physiol. Biochem. Pharmacol.* **161**, 87–110 (2011).
- 875 90. Palmer, R. K. & Lunn, C. A. TRP channels as targets for therapeutic intervention in obesity:
876 focus on TRPV1 and TRPM5. *Curr. Top. Med. Chem.* **13**, 247–57 (2013).
- 877 91. Henquin, J. Triggering and Amplifying Pathways of Regulation of Insulin Secretion by
878 Glucose. *Diabetes* **49**, 1751–1760 (2000).
- 879 92. Henquin, J. C., Nenquin, M., Ravier, M. A. & Szollosi, A. Shortcomings of current models of
880 glucose-induced insulin secretion. *Diabetes. Obes. Metab.* **11 Suppl 4**, 168–179 (2009).
- 881 93. Bertram, R., Sherman, A. & Satin, L. S. Metabolic and electrical oscillations: partners in
882 controlling pulsatile insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* **293**, E890-900
883 (2007).
- 884 94. Westermark, O. & Lansner, A. A Model of Phosphofructokinase and Glycolytic Oscillations in
885 the Pancreatic β -cell. **85**, 126–139 (2003).
- 886 95. Ashcroft, F. M. & Rorsman, P. Electrophysiology of the pancreatic beta-cell. *Prog. Biophys.*
887 *Mol. Biol.* **54**, 87–143 (1989).
- 888 96. Liman, E. R. A TRP channel contributes to insulin secretion by pancreatic β -cells. *Islets* **2**,

889 331–3

890 97. Vennekens, R. *et al.* Increased IgE-dependent mast cell activation and anaphylactic responses
891 in mice lacking the calcium-activated nonselective cation channel TRPM4. *Nat. Immunol.* **8**,
892 312–20 (2007).

893 98. Gees, M., Colsoul, B. & Nilius, B. The role of transient receptor potential cation channels in
894 Ca²⁺ signaling. *Cold Spring Harb. Perspect. Biol.* **2**, a003962 (2010).

895 99. Brixel, L. R. *et al.* TRPM5 regulates glucose-stimulated insulin secretion. *Pflugers Arch.* **460**,
896 69–76 (2010).

897 100. Shigeto, M. *et al.* GLP-1 stimulates insulin secretion by PKC-dependent TRPM4 and TRPM5
898 activation. *J. Clin. Invest.* **125**, 4714–28 (2015).

899 101. Kyriazis, G. a, Soundarapandian, M. M. & Tyrberg, B. Sweet taste receptor signaling in beta
900 cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. *Proc. Natl.*
901 *Acad. Sci. U. S. A.* **109**, E524–32 (2012).

902 102. Grundy, S. M. *et al.* Definition of metabolic syndrome: Report of the National Heart, Lung,
903 and Blood Institute/American Heart Association conference on scientific issues related to
904 definition. *Circulation* **109**, 433–8 (2004).

905 103. World Health Organization. Global action plan for the prevention and control of
906 noncommunicable diseases 2013–2020. *World Heal. Organ.* 102 (2013). doi:978 92 4 1506236

907 104. World Health organisation. Obesity and overweight: Fact sheet N°311. (2015).

908 105. Glendinning, J. I., Gillman, J., Zamer, H., Margolskee, R. F. & Sclafani, A. The role of T1r3
909 and Trpm5 in carbohydrate-induced obesity in mice. *Physiol. Behav.* **107**, 50–8 (2012).

910 106. Cettour-Rose, P., Bezençon, C., Darimont, C., le Coutre, J. & Damak, S. Quinine controls body
911 weight gain without affecting food intake in male C57BL6 mice. *BMC Physiol.* **13**, 5 (2013).

912 107. Larsson, M. H., Håkansson, P., Jansen, F. P., Magnell, K. & Brodin, P. Ablation of TRPM5 in
913 mice results in reduced body weight gain and improved glucose tolerance and protects from
914 excessive consumption of sweet palatable food when fed high caloric diets. *PLoS One* **10**, 1–19

915 (2015).

916 108. Smani, T., Shapovalov, G., Skryma, R., Prevarskaya, N. & Rosado, J. A. Biochimica et
917 Biophysica Acta Functional and physiopathological implications of TRP channels. *Biochim.*
918 *Biophys. Acta* **1853**, 1772–1782 (2015).

919 109. World Health organisation. Diabetes: Fact sheet number 312. (2015).

920 110. Lebovitz, H. E. Type 2 diabetes mellitus--current therapies and the emergence of surgical
921 options. *Nat. Rev. Endocrinol.* **7**, 408–19 (2011).

922 111. Young, R. L. *et al.* Expression of taste molecules in the upper gastrointestinal tract in humans
923 with and without type 2 diabetes. *Gut* **58**, 337–46 (2009).

924 112. Colsoul, B. *et al.* Insulin downregulates the expression of the Ca(2+)-activated nonselective
925 cation channel TRPM5 in pancreatic islets from leptin-deficient mouse models. *Pflugers Arch.*
926 **466**, 611–621 (2013).

927 113. Ketterer, C. *et al.* Genetic variation within the TRPM5 locus associates with prediabetic
928 phenotypes in subjects at increased risk for type 2 diabetes. *Metabolism.* **60**, 1325–1333
929 (2011).

930 114. Tabur, S. *et al.* Role of the transient receptor potential (TRP) channel gene expressions and
931 TRP melastatin (TRPM) channel gene polymorphisms in obesity-related metabolic syndrome.
932 *Eur. Rev. Med. Pharmacol. Sci.* **19**, 1388–1397 (2015).

933 115. Herrera Moro Chao, D. *et al.* Impact of obesity on taste receptor expression in extra-oral
934 tissues: emphasis on hypothalamus and brainstem. *Sci. Rep.* **6**, 29094 (2016).

935 116. Ivask, M., Hugill, A. & Köks, S. RNA-sequencing of WFS1-deficient pancreatic islets.
936 *Physiol. Rep.* **4**, e12750 (2016).

937 117. Oliveira-Maia, A. J., Roberts, C. D., Simon, S. A. & Nicolelis, M. A. L. Gustatory and reward
938 brain circuits in the control of food intake. *Adv. Tech. Stand. Neurosurg.* **36**, 31–59 (2011).

939 118. Maheswaran, T. *et al.* Gustatory dysfunction. *J. Pharm. Bioallied Sci.* **6**, 30 (2014).

- 940 119. Servant, G. *et al.* Positive allosteric modulators of the human sweet taste receptor enhance
941 sweet taste. *Proc. Natl. Acad. Sci.* **107**, 4746–4751 (2010).
- 942 120. Nettleton, J. E., Reimer, R. A. & Shearer, J. Physiology & Behavior Reshaping the gut
943 microbiota: Impact of low calorie sweeteners and the link to insulin resistance? *Physiol. Behav.*
944 **164**, 488–493 (2016).
- 945 121. Bryant, C. & McLaughlin, J. Low calorie sweeteners: Evidence remains lacking for effects on
946 human gut function. *Physiol. Behav.* **164**, 482–485 (2016).
- 947 122. Pepino, M. Y. Metabolic effects of non-nutritive sweeteners. *Physiol. Behav.* **152**, 450–455
948 (2015).
- 949 123. Miller, P. E. & Perez, V. Low-calorie sweeteners and body weight and composition : a meta-
950 analysis of randomized controlled trials and prospective. *Am. J. Clin. Nutr.* 1–13 (2014).
951 doi:10.3945/ajcn.113.082826.
- 952 124. Gardana, C., Simonetti, P., Canzi, E., Zanchi, R. & Pietta, P. Metabolism of Stevioside and
953 Rebaudioside A from Stevia rebaudiana Extracts by Human Microflora. *J. Agric. Food Chem.*
954 **51**, 6618–6622 (2003).

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958 Figure 1: Schematic representation of a taste bud, according to the labelled-line model, with the
 959 different cell types indicated. For the type II receptor cells, the expression of TRPM5 and the specific
 960 taste receptors is indicated.

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963 Figure 2: The role of TRPM5 in the pancreatic β -cells and type 2 taste receptor cells (figure adapted
 964 from³⁹).

Compound	Activity	Selectivity	Model	Ref.
Flufenamic acid	IC ₅₀ 24.5±2.2µM	TRPM4 IC ₅₀ 2.8±6.1µM	I/O patch overexpression	18
Clotrimazole	IC ₅₀ 1±0.4µM	TRPM4 IC ₅₀ 3.9±0.6µM	I/O patch overexpression	18
Spermine	IC ₅₀ 37±12µM	TRPM4 35±11µM	I/O patch overexpression	18
Quinine	IC ₅₀ 50±5µM (-50mV) IC ₅₀ 1.6±0.2mM	TRPM4 IC ₅₀ 450±50µM (-50mV) <i>Trpm5</i> ^{-/-} unresponsive	Whole cell patch clamp Murine gustatory nerve recording	59
Quinidine	IC ₅₀ 42±3µM 1.22±0.14mM	<i>n.d.</i> <i>Trpm5</i> ^{-/-} unresponsive	Whole cell patch clamp Murine gustatory nerve recording	59
TriPhenyl Phosphine Oxide (TPPO)	IC ₅₀ 12µM	Vs. TRPM4b, TRPV1 and TRPA1	Whole cell patch overexpression, membrane potential dye	55
Nicotine	IC ₅₀ 1.34±0.1mM (-50mV)	Vs. <i>TRPA1</i> EC ₅₀ 17µM	Whole cell patch clamp	60,61
Rutamarin	EC ₅₀ ±26.7 µM	Vs. TRPA1, Tas2R(0), TRPM8 (-), TRPV1(+)	Membrane potential dye	58
Stevioside Rebaudioside A Steviol	EC ₅₀ 690 nM 25 mg.kg ⁻¹ .day ⁻¹	Vs. TRPM3, TRPM4, TRPM8, TRPA1, TRPV1	Patch Clamp, Calcium activity in pancreatic islets. Murine model on taste and diabetes	49

Table 1: Pharmacological compounds modulating TRPM5 activity with the tested model and selectivity. *n.d.*: not determined