

Review Article

Sweet-taste receptors, low-energy sweeteners, glucose absorption and insulin release

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(Received 12 February 2010 – Revised 5 May 2010 – Accepted 27 May 2010 – First published online 12 July 2010)

The present review explores the interactions between sweeteners and enteroendocrine cells, and consequences for glucose absorption and insulin release. A combination of *in vitro*, *in situ*, molecular biology and clinical studies has formed the basis of our knowledge about the taste receptor proteins in the glucose-sensing enteroendocrine cells and the secretion of incretins by these cells. Low-energy (intense) sweeteners have been used as tools to define the role of intestinal sweet-taste receptors in glucose absorption. Recent studies using animal and human cell lines and knockout mice have shown that low-energy sweeteners can stimulate intestinal enteroendocrine cells to release glucagon-like peptide-1 and glucose-dependent insulinotropic peptide. These studies have given rise to major speculations that the ingestion of food and beverages containing low-energy sweeteners may act via these intestinal mechanisms to increase obesity and the metabolic syndrome due to a loss of equilibrium between taste receptor activation, nutrient assimilation and appetite. However, data from numerous publications on the effects of low-energy sweeteners on appetite, insulin and glucose levels, food intake and body weight have shown that there is no consistent evidence that low-energy sweeteners increase appetite or subsequent food intake, cause insulin release or affect blood pressure in normal subjects. Thus, the data from extensive *in vivo* studies in human subjects show that low-energy sweeteners do not have any of the adverse effects predicted by *in vitro*, *in situ* or knockout studies in animals.

Sweet-taste receptors: Glucose absorption: Insulin release: Glucose homeostasis: Low-energy sweeteners: Food intake: Appetite

Sweet-taste receptors on the tongue

Sweetness is one of the five tastes – sour, sweet, bitter, salty and umami – that humans experience. Signals from taste receptors generate nerve impulses that are relayed to and processed in the brain to provide information of whether a food is wholesome or spoiled or whether it is bitter and possibly toxic. Taste signals from the tongue influence individual food preferences and the acceptability of different foods. There has been extensive research on taste perception, and the pathways for sweet taste are now well understood.

A major advancement in our understanding of sweetness perception was the recognition that the sweet-taste receptor is similar to many other signalling mechanisms in the body. The sweet-taste receptor is a transmembrane protein present in the cell membrane that is coupled to a G-protein (second messenger) system. Binding of a sweet substrate to the receptor causes a conformation change in the receptor protein that affects its association with the G-protein. The G-protein associated with the sweet-taste receptor is α -gustducin, which like most G-proteins comprises α , β and γ subunits, and is on the cytoplasmic side of the cell membrane. Binding of a sweet

compound to the receptor causes dissociation of α -gustducin from the receptor, which triggers intracellular events such as the opening of ion channels or the generation of other biochemical signals. For sweetness perception, two G-protein-coupled transmembrane receptor proteins, T1R2 and T1R3, dimerise to form the sweet-taste receptor⁽¹⁾. Stimulation of the T1R2 + T1R3 taste receptor activates peripheral gustatory nerves and, in turn, brain gustatory pathways. Sweet-tasting compounds, such as sugars and low-energy sweeteners, can bind to and stimulate the sweet-taste receptor. (Note: the term low-energy (low-calorie) sweetener is used because it covers both non-nutrient compounds (e.g. acesulphame-K, cyclamate, saccharin and sucralose) and intensely sweet compounds that are metabolised with the release of energy (e.g. aspartame and steviol glycosides), but because of their sweetness potency, the intake and the energy released are low compared with an equally sweet amount of sucrose. These sweeteners are between 300 and 800 times sweeter than sucrose on a weight basis; newer sweeteners, such as neotame, are over 1000 times as sweet as sucrose). Studies with α -gustducin knockout mice showed that this receptor mechanism

Abbreviations: GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; SGLT1, Na⁺/glucose co-transporter.

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was responsible for electrophysiological changes in the brain, which resulted in preferred ingestion of water sweetened with either saccharin or acesulphame-K compared with unsweetened water in two-bottle taste preference tests⁽²⁾. Thus, the mechanism for sweet-taste perception on the tongue has been well defined.

The absorption of glucose from the intestine

Glucose is a highly polar molecule that cannot diffuse across the lipid bilayer of cell membranes of the enterocytes that line the gut. It crosses cell membranes via special transporters, which control uptake from the gut lumen into the hepatic portal vein and also transfer glucose from the blood into tissues, including the brain. The control of absorption across the intestinal wall involves two transporters: Na⁺/glucose co-transporter (SGLT1), which is an active transporter on the apical (luminal) membrane and the glucose facilitative transporter GLUT2, which is present on the basolateral and apical membranes⁽³⁾.

Na⁺/glucose co-transporter

The SGLT1 actively transports glucose from the gut lumen into the enterocytes. The expression of the SGLT1 protein is proportional to the amount of glucose in the gut lumen at low concentrations. Therefore, the capacity for glucose absorption is related to the amount of glucose available in the gut lumen. The transporter is important at low luminal glucose concentrations since it has an apparent K_m of 8–23 mM, but it becomes saturated at approximately 30–50 mM glucose⁽³⁾. The expression of the SGLT1 protein is regulated by a glucose sensor⁽⁴⁾ on the luminal membrane of gut cells, which initiates a signalling pathway, involving a G-protein-coupled receptor, but this sensor was not identified as a sweet-taste receptor until recently⁽⁴⁾.

GLUT2

GLUT2 is a passive transmembrane GLUT that is present in many tissues, including the pancreas. In the intestine, it is normally expressed on the basolateral membrane of enterocytes, and transfers intracellular glucose into the general circulation⁽⁵⁾. GLUT2 has higher capacity but lower affinity than SGLT1 for glucose, and it is not saturated even at concentrations over 100 mM. The contribution of GLUT2 to glucose absorption exceeds that of the SGLT1 at 30–50 mM-glucose and continues to increase at higher glucose concentrations, to become approximately 2- to 3-fold greater than the active component⁽³⁾. Increased concentrations of glucose in the gut lumen result in increased synthesis of GLUT2 and its expression in the luminal (apical) membrane; apical insertion of GLUT2 was not induced at 20 mM-glucose, but it was detected at 30 mM- and increased at 100 mM-glucose⁽³⁾. Apical insertion of GLUT2 at high luminal glucose levels provides a mechanism by which absorptive capacity is rapidly and precisely matched to dietary intake.

These two transporters provide adaptive and interactive mechanisms of glucose absorption. Apical GLUT2 insertion is prevented if SGLT1 activity is blocked, possibly because intracellular Ca²⁺ is essential for GLUT2 insertion and

depolarisation of the apical membrane by transport of glucose through SGLT1 stimulates Ca²⁺ entry via the L-type channel⁽⁵⁾. Thus, SGLT1 and apical GLUT2 work in concert to cover the necessary physiological concentration range from low to high dietary glucose; moreover, SGLT1 activity exerts a regulatory effect over apical GLUT2 insertion⁽⁶⁾.

Sweet-taste receptors in the small intestine and the absorption of glucose

The first indication of the presence of sweet-taste receptors in the small intestine was the detection of α -gustducin in the brush borders of intestinal cells⁽⁷⁾. α -Gustducin and taste-signalling receptors are expressed on the enteroendocrine cells rather than on the enterocytes, which are the cells responsible for glucose absorption. Margolskee *et al.*⁽⁸⁾ showed that the sweet-taste receptor subunit, T1R3, and the G-protein, α -gustducin, are expressed in the enteroendocrine cells and are responsible for intestinal glucose sensing⁽⁴⁾ and for the regulation of the SGLT1 mRNA and protein in the enterocytes. Dietary sugar and low-energy sweeteners increased SGLT1 mRNA and protein expression, and glucose absorptive capacity. Because of the inter-relationship between the activity of SGLT1 and the insertion of GLUT2 into the apical membrane (see above), stimulation of the T1R3 also increased GLUT2 insertion⁽⁸⁾.

The enteroendocrine cells communicate with the enterocytes via the production of signals that are detected by the enterocytes and cause them to increase their expression of the SGLT1. These signals are known as incretins (a name proposed in 1932 for a hormone extracted from the upper gut mucosa, which caused hypoglycaemia – see Egan & Margolskee⁽⁹⁾), and comprise glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). These are released from the basal surface of stimulated enteroendocrine cells and diffuse locally, to act on the enterocytes and to activate afferent neurones in the gut villi, and also enter the blood to act as systemic hormones that have the potential to augment the release of insulin from the pancreas. The released incretins are rapidly inactivated by hydrolysis.

GLP-1 has a wide range of effects on glucose metabolism, including stimulation of insulin release, inhibition of glucagon secretion, reduction of gastric emptying and augmentation of satiety⁽¹⁰⁾.

In vitro studies using the human intestinal cell line (NCI-H716) have shown that stimulation of sweet-taste receptors and α -gustducin in the enteroendocrine cells of the intestine by glucose or the low-energy sweetener sucralose causes the release of GLP-1⁽¹¹⁾. *In vivo* studies in knockout mice⁽¹¹⁾ showed that the sweet-taste receptor mechanism was involved in insulin release in response to a gavage dose of glucose: the rapid early increase in insulin secretion in response to glucose displayed by wild-type mice was absent in the knockout mice, although they eventually achieved peak insulin concentrations higher than those of the wild-type mice. Glucose homeostasis was also altered in the knockout mice with higher plasma glucose concentrations after gavage administration of glucose or after consumption of laboratory chow⁽¹¹⁾.

The physiological consequences of intestinal incretin release on insulin release from the pancreas and glucose homeostasis are discussed below.

The use of low-energy sweeteners in studies on mechanisms of glucose absorption

Low-energy sweeteners have been used in the above-mentioned experiments as tools to define the role of intestinal sweet-taste receptors in glucose absorption. The data are related to *in vitro* studies and short-term *in vivo* investigations in animals, and caution is necessary in the extrapolation of *in vitro* effects to the *in vivo* situation and the extrapolation of data from studies in animals to human subjects. In some cases, the authors speculate outside the available data about the possible consequences of stimulation of intestinal sweet-taste receptors on appetite, glucose homeostasis and diabetes.

Mace *et al.*⁽⁵⁾ used acesulphame-K, saccharin and sucralose as probes for their studies on the impact of stimulation of intestinal sweet-taste receptors on the apical insertion of GLUT2. They used *in situ* intestinal perfusion in rats and studied glucose absorption by the decrease in concentration in the perfusate over time. They also undertook histological examination to investigate the tissue expression of GLUT2. The basal rate of glucose absorption, measured following perfusion with 20 mM-glucose, was doubled by the addition of 1 mM-sucralose, and this was shown to be due to a 3.2-fold increase in GLUT2 by studies using phloretin (which inhibits GLUT2 but not SGLT1). Studies using an inhibitor of phospholipase-C, which is a key part of α -gustducin signalling, demonstrated the involvement of the G-protein sweet-taste receptor in the sucralose-stimulated increase in glucose absorption. Measurement of the apical expression of GLUT2 when perfused with 20 mM-glucose showed that acesulphame-K increased the apical GLUT2 expression to the same extent as 1 mM-sucralose, whereas saccharin increased it by only one-fifth (the concentrations of acesulphame-K and saccharin seem not to be given in the paper, but they are probably the same as those used by Li *et al.*⁽¹²⁾). Li *et al.*⁽¹²⁾ had used the *in vitro* increase in Ca^{2+} in cell constructs containing human T1R2 and T1R3 as a measure of the binding of sweet molecules including many low-energy sweeteners, sucrose and a number of amino acids including tryptophan. The concentrations used were 0.1 mM (neotame), 1 mM (saccharin and sucralose), 2.5 mM (acesulphame-K and aspartame) and 5 mM (cyclamate), and each of these concentrations resulted in an increase in intracellular Ca^{2+} concentration.

Margolskee *et al.*⁽⁸⁾ performed *in vivo* studies on intestinal SGLT1 expression in wild-type and α -gustducin knockout mice and *in vitro* studies on the effects of low-energy sweeteners on sweet-taste receptors expressed on a mouse enteroendocrine GLUTag cell line. Wild-type mice fed a diet containing 70 % sucrose for 2 weeks had 1.6-fold higher intestinal SGLT1 mRNA levels and 1.8-fold higher SGLT1 protein levels than the mice fed 2 % sucrose; no such differences were found in knockout mice. Data for wild-type, but not for knockout, mice fed a low-carbohydrate diet and water containing sucralose showed a 2.2-fold increase in SGLT1 mRNA and a 1.9-fold increase in SGLT1 protein. Data for wild-type mice given low-energy sweeteners in their drinking-water showed increases in SGLT1 mRNA expression of 1.9-fold with acesulphame-K (10 mM) and 1.8-fold with saccharin (20 mM), but no increase with aspartame (1 mM). *In vitro* studies showed that cultured GLUTag cells release the incretins, GLP-1 and GIP into the culture medium, and that the

addition of sucralose (50 mM) to the culture medium increased the release of GLP-1 (about 1.4-fold) and GIP (about 3.8-fold), and also increased intracellular Ca (based on supporting information available via a web link provided in the publication of Margolskee *et al.*⁽⁸⁾). The authors concluded that sweetener-dependent release of GLP-1 and GIP from GLUTag cells depends on the stimulation of sweet-taste receptors.

Jang *et al.*⁽¹¹⁾ investigated the role of sweet-taste receptors and α -gustducin in the intestinal secretion of GLP-1 using a range of *in vitro* and *in vivo* methods. Studies with human duodenal biopsy sections confirmed the organisation of sweet-taste receptors, α -gustducin, and of GLP-1- and GIP-secreting cells outlined in the general description earlier. *In vivo* studies in knockout mice confirmed the importance of sweet-taste receptors and α -gustducin in GLP-1 secretion. The studies using low-energy sweeteners used NCI-H716 cells, a human enteroendocrine L cell line that releases GLP-1 in response to sucrose or glucose in the medium, which correlates with the phosphorylation of extracellular signal-regulated kinase. Addition of sucralose to the medium caused a dose-related increase in GLP-1 release and phosphorylation of extracellular signal-regulated kinase, with the maximum effect being found at 5 mM.

In contrast to these studies showing clear effects of low-energy sweeteners, Fujita *et al.*⁽¹³⁾ showed in rats that doses of acesulphame-K, saccharin, stevia or sucralose (at 1 g/kg body weight) given by gavage did not increase blood concentrations of either GIP or GLP-1, or affect blood glucose levels during an intraperitoneal glucose tolerance test; gavage doses (1 g/kg body weight) of either sucralose or stevia did not alter normal blood glucose levels.

The major speculations about the nutritional impact of these findings on human health and the actions of low-energy sweeteners were published by Egan & Margolskee⁽⁹⁾, who suggested that ingestion of soft-drinks containing non-energy sweeteners may act via these intestinal mechanisms to increase obesity and the metabolic syndrome, due to a disequilibrium between taste receptor activation, nutrient assimilation and appetite. The same article also implied links with increased risk of diabetes and hypertension. In contrast, Mace *et al.*⁽⁵⁾, Margolskee *et al.*⁽⁸⁾ and Jang *et al.*⁽¹¹⁾ speculated that modulation of GLUT2 or SGLT1 expression might be useful in developing strategies for the prevention and/or treatment of malabsorption syndromes and diet-related disorders, including diabetes and obesity.

The relationship between sweet-taste receptors, insulin secretion and glucose absorption in human subjects *in vivo*

Although *in vitro* research and most of the *in vivo* animal studies have indicated actions of low-energy sweeteners on mechanisms involving intestinal sweet-taste receptors and the release of incretins, it is less clear whether these play a significant role *in vivo* in the release of insulin or in glucose homeostasis following ingestion of a low-energy sweetener.

The hypothesis raised by Egan & Margolskee⁽⁹⁾ is that:

- (i) Low-energy sweeteners stimulate the sweet-taste receptors on the enteroendocrine cells in the intestine.
- (ii) This causes an increased expression of the uptake transporters (SGLT1 and GLUT2) on the enterocytes to enhance glucose uptake.

- (iii) The stimulus for this increase is mediated by the release of incretins (GLP-1 and GIP).
- (iv) Released incretins enter the general circulation and increase insulin secretion, which lowers the blood sugar.
- (v) The increased insulin secretion affects glucose homeostasis and the risk of diabetes.
- (vi) The lowered blood sugar increases appetite.
- (vii) The increase in appetite leads to increased weight gain.

This is not logical because if a low-energy sweetener were to increase GLP-1, it would tend to decrease appetite, not increase it; however, all the evidence indicates that low-energy sweeteners provide a sweet taste without significant energy and without any effect on appetite (see below). In addition, if ingestion of a food or beverage sweetened by a low-energy sweetener were to increase GLUT and the rate of glucose absorption from the intestinal lumen, it would do so in the context of a low luminal concentration of glucose, so that there could be only a limited greater glucose absorption and uptake of energy. However, if the same food or beverage contained an equi-sweet amount of sucrose, then this would cause a similar increase in GLUT, but it would be doing so in the context of high luminal concentrations of glucose. In consequence, despite any effect of the low-energy sweetener on glucose uptake, the sucrose-sweetened product must result in greater glucose absorption.

Many aspects of this hypothesis have been considered previously in relation to the stimulation of sweet-taste receptors on the tongue and the so-called 'paradoxical effect' of aspartame on hunger⁽¹⁴⁾ and the hypothetical cephalic phase insulin release⁽¹⁵⁾. The data from numerous publications on low-energy (intense) sweeteners and appetite, blood insulin and glucose levels, food intake and body weight were reviewed in 1994⁽¹⁶⁾, and they indicated no consistent significant effects.

It has been hypothesised that stimulation of sweet-taste receptors on the tongue can act as a signal for insulin release as a mechanism by which the body is prepared for the absorption of dietary glucose, known as a cephalic phase insulin release. This was investigated in a number of studies in the 1990s⁽¹⁶⁾, which showed no evidence of an increase in insulin after tasting various low-energy sweeteners. This issue has been raised again in a recent study⁽¹⁷⁾, in which blood insulin levels were measured in volunteers who tasted different solutions for 45 s. The authors reported that both sucrose and saccharin increased insulin levels during the first 10 min after tasting, but that other solutions (including salt, citric acid, monosodium glutamate, quinine, starch or water) did not. Although twenty subjects were studied for the two sweet substances, only five subjects tasted the other test compounds; the increases in insulin for starch and water were larger than those for the sweeteners, raising questions about the study design and the statistical power of the comparisons. In contrast to these data, no increase in plasma insulin was found in fifteen of sixteen subjects who tasted a solution sweetened with aspartame, saccharin or sucrose⁽¹⁸⁾. Perhaps, the most comprehensive investigation of this phenomenon was by Abdallah *et al.*⁽¹⁹⁾ who found no early changes in insulin, glucose or glucagon levels in twelve subjects who sucked a lozenge sweetened with aspartame, sucrose or polydextrose (placebo) for 5 min in a study that incorporated continuous blood sampling and monitoring in 1 min fractions

over a period of 45 min before and 25 min after tasting. Although there were statistically significant decreases in plasma glucose and insulin after all three stimuli (including the placebo), only the consumption of the sucrose tablet was followed by a post-absorptive increase in plasma glucose and insulin concentrations, which started at about 18 min after the beginning of sucking. The authors concluded that oral stimulation provided by sweet non-flavoured tablets did not induce cephalic phase insulin release.

Härtel *et al.*⁽²⁰⁾ investigated the influence of drinking solutions containing acesulphame-K (165 mg), aspartame (165 mg), cyclamate (800 mg) or saccharin (75 mg) on blood insulin and glucose levels in human volunteers over the following 2 h in comparison with sucrose (30 g) or water controls. Only sucrose caused an increase in blood glucose and insulin levels, which occurred within 5 min and peaked at 30 min after ingestion. In comparison with the water control, and in contrast to the dose of sucrose, none of the low-energy sweeteners showed significant changes in glucose or insulin indicative of a cephalic phase or later insulin release. Because of the duration of observations (120 min), these comprehensive *in vivo* human data are equally applicable to the newer hypothesis of an intestinal incretin-induced insulin effect.

In addition to these data on the older sweeteners, further information can be obtained from clinical research on the newer sweeteners, sucralose and stevioside/rebaudioside-A.

A high single oral dose of sucralose (1000 mg) given to thirteen subjects with insulin-dependent diabetes mellitus and thirteen subjects with non-insulin-dependent diabetes mellitus did not affect the area under the concentration time curve of either plasma glucose or serum C-peptide, indicating no acute effect of sucralose on glucose homeostasis⁽²¹⁾. A single oral dose of sucralose (10 mg/kg) did not affect the glucose-induced changes in blood glucose levels when given with a large test dose of sucrose (100 g)⁽²²⁾. A randomised, double-blind, placebo-controlled study in which 128 subjects with type 2 diabetes were given either sucralose (667 mg/d for 13 weeks) or placebo showed no effect on fasting plasma glucose, HbA1c (glycated Hb) or fasting serum C-peptide, demonstrating no effect on glucose homeostasis⁽²³⁾.

The most convincing evidence on the consequences of stimulation of the intestinal sweet-taste receptors in human subjects comes from recent studies that have measured circulating levels of incretins, insulin and glucose in human volunteers. Ma *et al.*⁽²⁴⁾ measured circulating levels of GLP-1, GIP, insulin and glucose in seven human volunteers after intragastric infusions of doses of sucrose (50 g), sucralose (80 or 800 mg) or saline control given in 500 ml of iso-osmotic solutions. Increases in GLP-1, GIP, insulin and glucose were found only after the administration of the sucrose test dose; the data for sucralose did not differ from the saline control. Delayed gastric emptying, a response that is important to the release of and responses to incretins⁽²⁵⁾, was found only after the sucrose dose. Since sucralose is 600 times sweeter than sucrose, and it is not absorbed significantly, the sweetness delivered to the intestinal taste receptors must have been over ten times greater with the high sucralose test dose than with sucrose. Despite this, there was no indication of an effect on circulating incretins, insulin or glucose in human subjects.

In contrast, a greater increase in GLP-1 has been reported when a glucose-tolerance test using 75 g of glucose was

performed in volunteers 10 min after the consumption of 250 ml of diet soda (sweetened with sucralose and acesulphame-K; concentrations not stated) than after the consumption of 250 ml of a non-sweetened carbonated drink⁽²⁶⁾. The increases in blood glucose and insulin from the glucose tolerance test were not altered by the nature of the prior beverage. Because exogenous GLP-1 results in lower postprandial blood glucose levels and decreased insulin secretion, an effect primarily due to delayed gastric emptying⁽²⁵⁾, the authors proposed that prior stimulation of intestinal sweet-taste receptors with the low-energy sweetener drink may have produced two effects that balance each other, an increase in GLP-1 that would tend to lower blood glucose and an increase in intestinal GLUT2 that could increase blood glucose. However, there was no effect on the time course of the increases in GLP-1, glucose or insulin, suggesting that there was no difference in gastric emptying. The absorption of glucose is affected by a number of variables including the rate of delivery to the small intestine⁽²⁷⁾ and the length of intestine exposed to glucose⁽²⁸⁾, but the biological basis of the different findings for GLP-1 in these two recent studies is unclear. It is difficult to understand how prior exposure of the intestinal taste receptors to a low-energy sweetener that does not itself cause an increase in blood GLP-1, even at very high intake levels, would increase the GLP-1 rise after a subsequent large intake of glucose. It is interesting that the studies used different methods of administration (intragastric in the study of Ma *et al.*⁽²⁴⁾ and oral in the study of Brown *et al.*⁽²⁶⁾), suggesting that the lingual taste receptors might have influenced the subsequent rise in GLP-1, a possibility that warrants further study. Whatever the reason, the physiologically most important observation was that there was no change in blood glucose or insulin level when the low-energy sweetener was given either alone or before glucose administration.

The sixty-ninth meeting of the WHO/FAO Joint Expert Committee on Food Additives in 2008⁽²⁹⁾ considered data from the clinical trials on the effects of steviol glycosides on blood pressure in healthy volunteers with normal or low-normal blood pressure and on glucose homeostasis in men and women with type 2 diabetes mellitus. The Committee concluded that 1000 mg of rebaudioside-A/d given for 16 weeks did not have adverse effects on diabetic control or on blood pressure in patients with type 2 diabetes. No clinically significant changes in blood pressure parameters were observed in normotensive individuals or in a subset of individuals with blood pressure below the median who took rebaudioside-A at a dose of 1000 mg/d for 4 weeks.

Thus, the extensive *in vivo* human database (see above and Blackburn⁽³⁰⁾, de la Hunty *et al.*⁽³¹⁾, Bellisle & Drewnowski⁽³²⁾ and Anton *et al.*⁽³³⁾) shows that low-energy sweeteners do not have any unwanted effects on appetite or subsequent food intake, insulin or blood glucose levels, glucose homeostasis or blood pressure.

It seems that the authors of papers who reported elegant research on the molecular biology or glucose signalling in the intestine and who speculated about a possible loss of equilibrium between taste receptor activation, nutrient assimilation and appetite were unaware of the extensive database demonstrating the safety and efficacy of low-energy sweeteners. Much of the data used to support a possible intestinal incretin effect on insulin levels, glucose absorption, appetite and body weight were from animal tissues or *in vivo* animal studies, and

would have been detected in the extensive preclinical animal toxicity studies required before approval. Any physiological perturbations, such as changes in glucose homeostasis, produced at the levels of intake by human subjects would have resulted in serious adverse sequelae at the 100-fold higher dose levels used in animal safety studies. Regulatory safety studies include numerous pre-defined endpoints including blood biochemistry measurements, which would have revealed any significant effect on glucose homeostasis. It should not be forgotten that the Acceptable Daily Intake (ADI) for low-energy sweeteners are 100-fold lower than the daily intakes that did not produce any adverse effects in animals.

Overall, the available data show that there is no consistent evidence that intense sweeteners cause insulin release or lower blood sugar in normal subjects.

Acknowledgements

A. G. R. acts as a scientific consultant to the International Sweeteners Association, Brussels. S. V. M. acts as a scientific consultant to Tate & Lyle, Inc., Decatur, IL, USA.

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