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Journal Club

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Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1

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Abstract

Problem/Motivation: Why does glucose, given intravenously, produce less pancreatic insulin secretion than when given orally?

It is known that GLP-1, which is released from gut endocrine cells, stimulates pancreatic insulin secretion, and that with I.V glucose administration there is no GLP-1 release.

However, the pathway by which luminal carbohydrate causes the release of GLP-1 from the gut endocrine cells is not known.

The purpose of this study is to determine that pathway.

Hypothesis: elements of taste signalling previously found in the gut, such as gustducin, may be involved in this process.

Abstract (Contd)

Methods: Enteroendocrine cells from both humans and mice were examined for the presence of taste receptors and taste transduction elements.

Glucose was administered to both wild type mice, positive for gustducin, and gustducin negative knockout mice.

Blood levels of GLP-1, insulin, and glucose were measured and compared.

An enteroendocrine cell line in humans was treated with sucrose, glucose, sucralose, 2-deoxy-glucose, and lactisole.

Their effects on GLP-1 release was measured.

Abstract (Contd)

Results: A large percentage of human and mice cells which secrete GLP-1 also express gustducin.

GLP-1 is secreted in response to sucrose, glucose, and sucralose in wild type mice and human cells, but not in the gustducin null mice or human cells silenced for the gustducin-gene.

Insulin and glucose levels in the mice also were affected by gustducin expression.

Conclusion: Enteroendocrine cells use the same mechanisms to sense sweet as taste cells of the tongue.

These sweet receptors trigger a cascade of events to increase insulin response in preparation for incoming glucose.

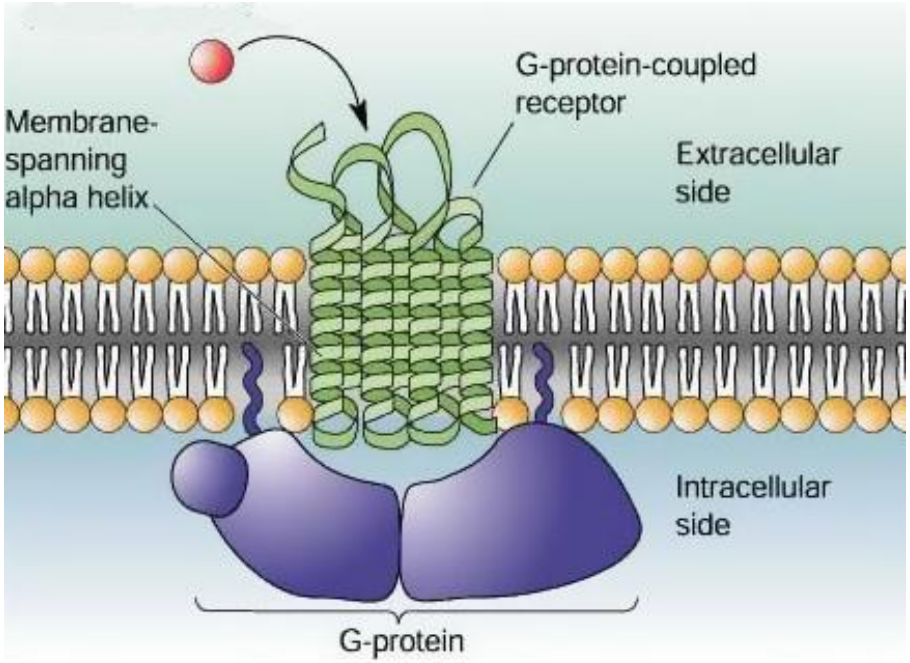
Introduction:
Background and definitions

Gustducin: A g–protein which is involved in the transduction of the sweet taste. It is activated by a g-protein coupled receptor.

It is found in the taste receptor cells of the lingual epithelium.

More recently it was found in the brush cells of the stomach, duodenum, pancreatic ducts, and colon.

G-protein coupled receptor:
A transmembrane receptor which binds signalling molecules on the outside of the cell, causing a cascade of signals and changes within the cell, via the g-protein to which it is coupled.



Introduction:
Background and definitions

Incretin: A gastrointestinal hormone which stimulates the beta cells of the pancreas to secrete insulin.

The two known incretins are glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) also known as Gastric inhibitory peptide.

- The latter name for GIP referring to the ability of incretins to slow gastric emptying; increasing satiety and slowing absorption.

L cell: A cell found in the gastrointestinal tract which secretes the peptide hormone, or incretin, **GLP-1**; thus making it an enteroendocrine cell

K cell: An enteroendocrine cell, similar to the L cell. Differentiated by the fact that it secretes **GIP** rather than GLP-1.

Review of Literature

Author's summarized previously established data including:

- The taste receptors already found present on lingual epithelium
- How these taste receptors work
- Research which has identified these receptors in other areas of the body

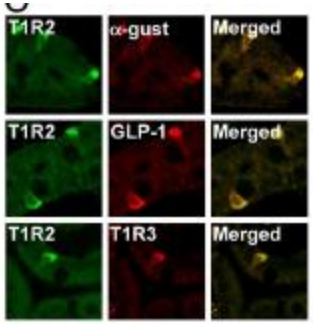
A review of the literature available showed no discrepancies, bias, or inappropriate conclusions made by these authors in presenting the current research and evidence to support their theory.

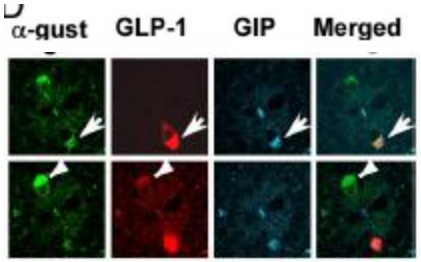
Methods/ results

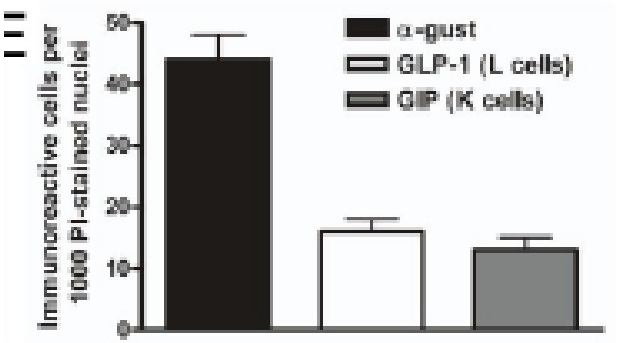
Detecting the presence of Taste signaling elements

Using immunoflouresencent imaging on **human** duodenal enteroendocrine cells:

- >90% of L cells express gustducin
- <50% of K cells express gustducin
- 5-10% of incretin cells express both GIP and GLP-1 (K/L cell)
- The number of gustducin containing cells > than all K+ L cells combined
  - May play a role for non –endocrine cells







Methods/ results

Detecting the presence of Taste signaling elements

Using IF imaging on **Mouse** duodenal enteroendocrine cells:

- L cells of the mouse GI tract frequently express gustducin
- Unlike K cells in humans, very rarely express gustducin
  - (~ 50% of human K cells)

Methods/ results

Testing the role of gut-expressed gustducin in **mice**

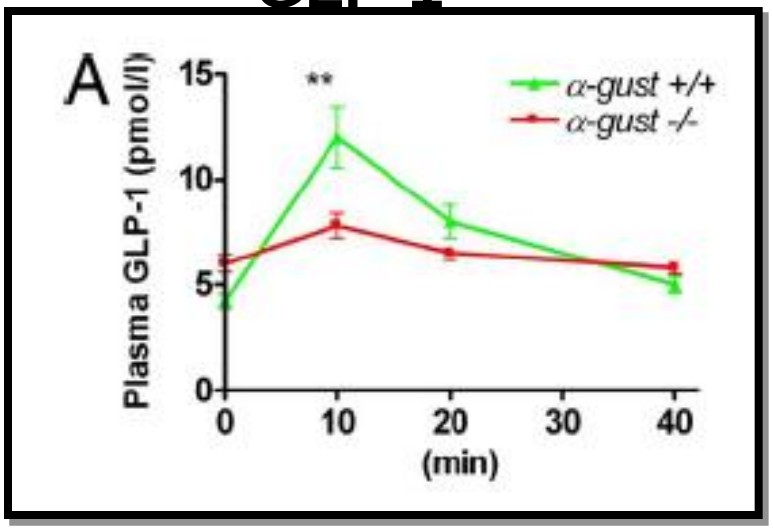
Gavage administration of glucose

- Directly into the stomach, using a feeding needle to both:
  - Wild type mice (α-gust +/+)
  - Knockout mice (α-gust -/-)

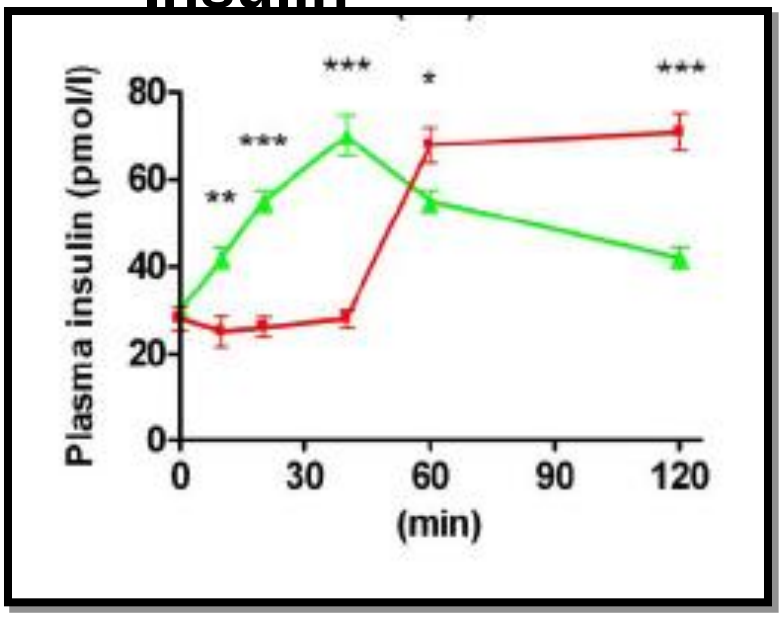
Results:

- Knockout mice
  - no rise in plasma GLP
  - Delayed rise in insulin
  - Higher peak in glucose, remained higher than wild mice for 2 hours
- Wild mice
  - Rapid rise in GLP
  - Rapid insulin secretion
  - Lower peak in glucose

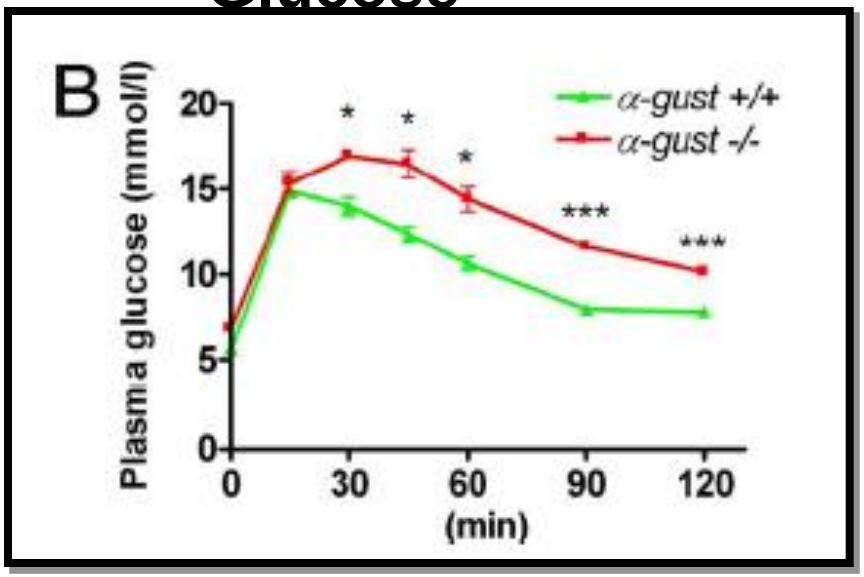
GLP-1



Insulin



Glucose



Methods/ results

In vitro testing of GLP-1 secretion of **mice** duodena

Proximal duodena of both wild type and knockout mice were harvested, minced and placed in a culture medium.

Unstimulated (no glucose):

- Knockout mice baseline of172 mMol GLP-1 per gram of tissue
- Wild type mice baseline of 127 mMol GLP-1 per gram of tissue

•In 10% glucose

- •Knockout mice had a 2 fold increase from baseline to 322 mMol/g of tissue
- •Wild type mice had a 3.5 fold increase from baseline to 445mMol/g of tissue

In mice:

- •A great deal of GLP-1 secretion is dependant on gustducin.

- •Other gustducin independent mechanisms do contribute to a glucose stimulated GLP-1 release

Methods

In vitro testing of GLP-1 secretion of **Human** cell line NCI-H716

To ensure that only L cells which contain gustducin were tested, Just cells from the NCI-H716 cell line were isolated and cultured

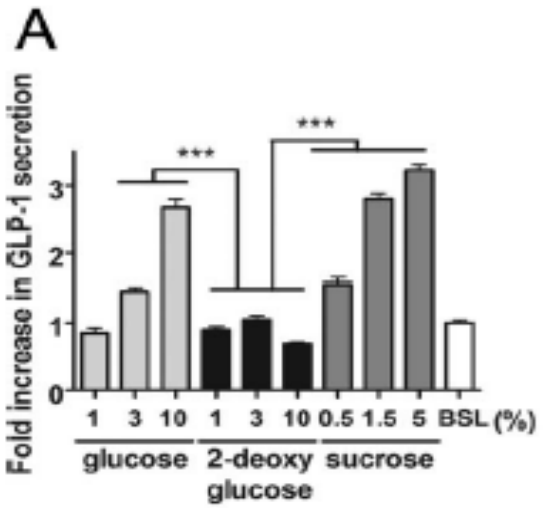
- In these cells expression of GLP-1 and gustducin was confirmed by IF microscopy

- The following were applied to the medium as variables:
  - **Sucrose**
  - **Glucose**
  - **Sucralose**
  - **Lactisole** (antagonizing inhibitor of the taste receptor coupled to gustducin)
  - **2-deoxy-glucose** (non-metabolizable, non-sweet sugar)
    - Used to rule out osmotic effects as the mechanism for stimulation

Results

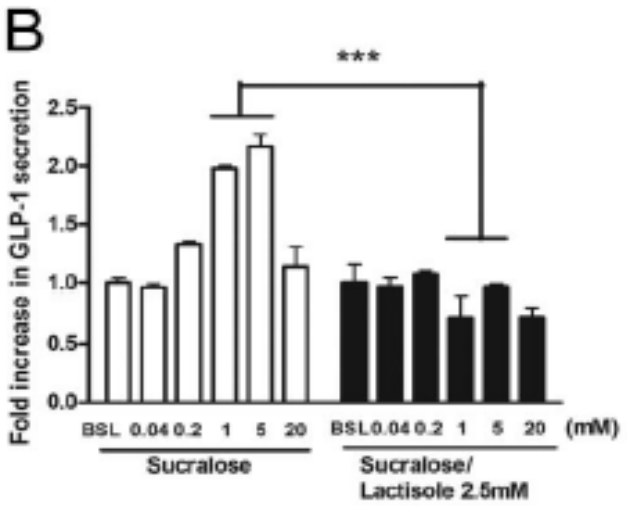
•Glucose and Sucrose led to a concentration dependent release of GLP-1

•2-deoxy-glucose did not increase GLP-1



•Sucralose (like glucose and sucrose) led to a concentration dependent release of GLP-1

• When lactisole is applied prior to sucralose, it inhibits this sucralose responsive GLP-1 release.



Methods

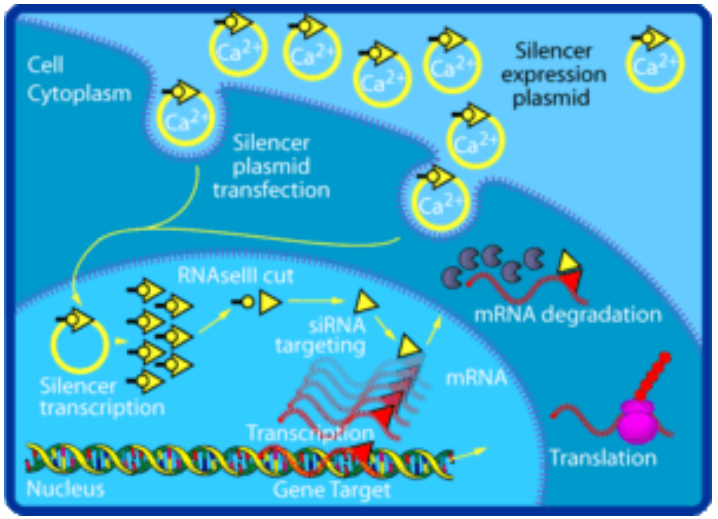
Silencing RNA was used to decrease the expression of the gene for gustducin in NCI-H716 cells

These cells were cultured and glucose was applied to the medium

Results

Basal GLP-1 secretion was not affected

Glucose mediated GLP-1 release was decreased

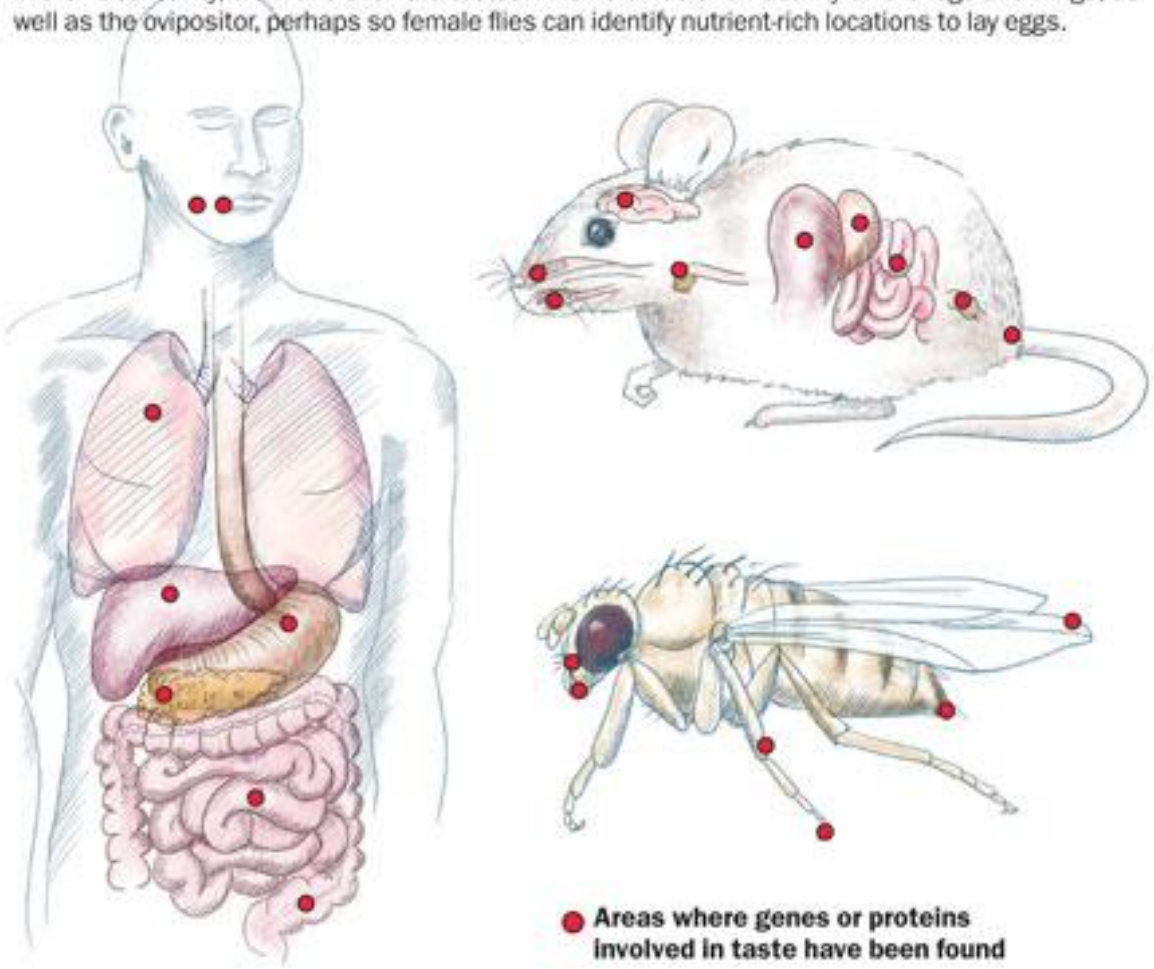


Conclusion

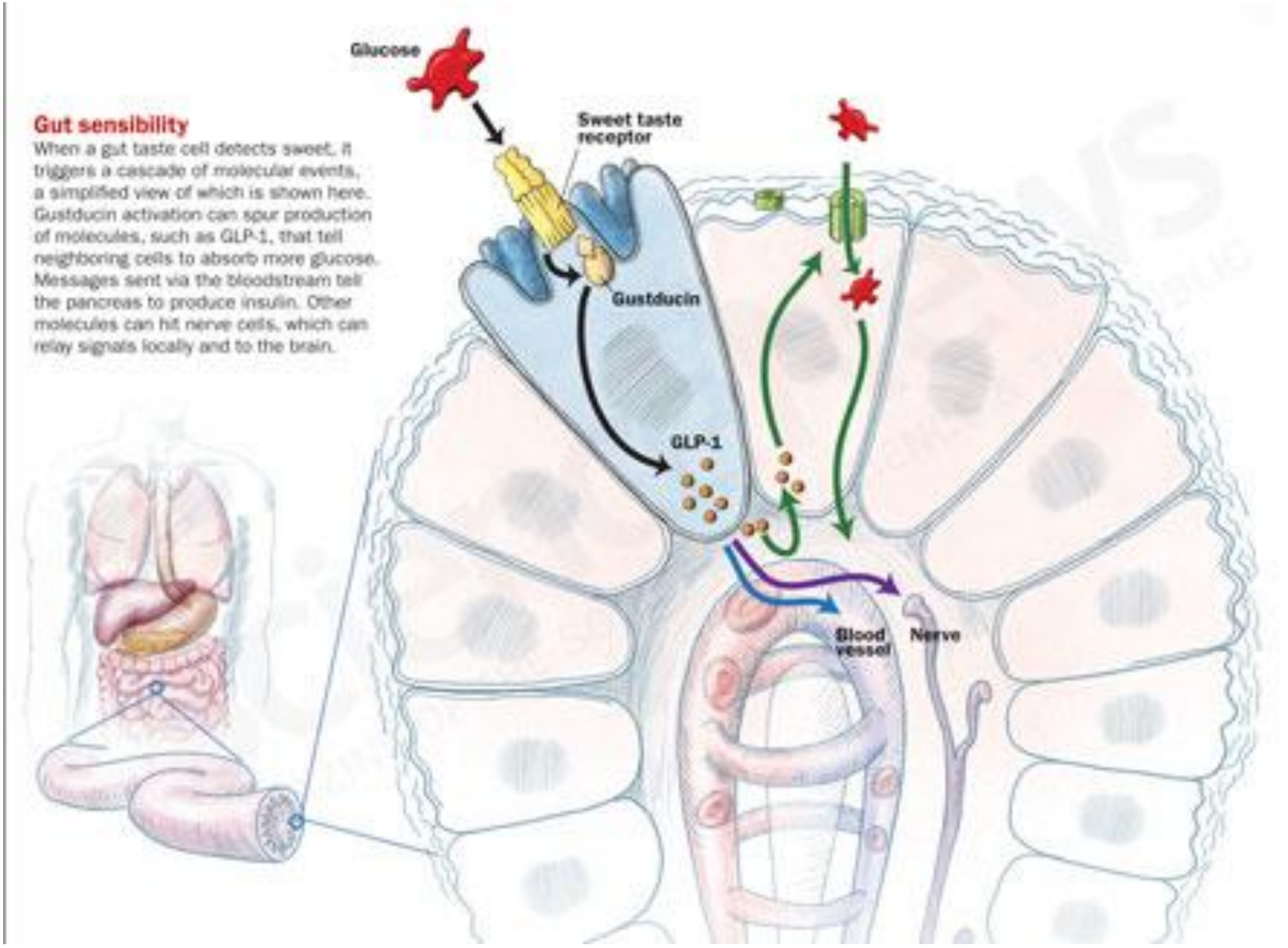
The data collected in this study indicate that the same taste-receptors and G-protein which are found on the tongue and involved in our perception of sweet are part of a signalling pathway which regulates intestinal hormone secretion.

These hormones, known as incretins, play an important role in regulating blood glucose levels.

Inner taste Activity in taste-related genes and proteins has been found throughout the body, not just in the mouth. For humans, the tongue and cheeks are the usual suspects, but activity has also been identified in the liver, lungs and gut. Mouse taste-related genes and proteins are active in the nasal cavity, brain and even the testes. And fruit flies show activity on the legs and wings, as well as the ovipositor, perhaps so female flies can identify nutrient-rich locations to lay eggs.



● Areas where genes or proteins involved in taste have been found


Gut sensibility
When a gut taste cell detects sweet, it triggers a cascade of molecular events, a simplified view of which is shown here. Gustducin activation can spur production of molecules, such as GLP-1, that tell neighboring cells to absorb more glucose. Messages sent via the bloodstream tell the pancreas to produce insulin. Other molecules can hit nerve cells, which can relay signals locally and to the brain.
Glucose
Sweet taste receptor
Gustducin
GLP-1
Blood vessel
Nerve

Application

•There is an 83% to 86% cure rate of diabetes with Roux-en-Y gastric bypass and a >95% cure rate with bilopancreatic diversion within days of surgery.

•Researchers have struggled to determine the reasoning behind this.

•It was previously known that:

- peak levels of GLP-1 In Roux-en-Y gastric bypass patients are:
  - •higher than non-bypass patients
  - •achieved sooner than with non-bypass patients
- •small intestine L- cell concentrations are greater distally than in the duodenum
  - •Supported by the time course of GLP-1 levels obtained from this study

Application: conclusion

By confirming the presence, and identifying the role of a-gustducin, this study indicates that:

•The diabetic remission rate in bariatric surgery patients is directly related to the increased stimulation of the gustducin pathway.

•This increased stimulation is made possible by the bypass of the duodenum into areas of larger L- cell concentrations.

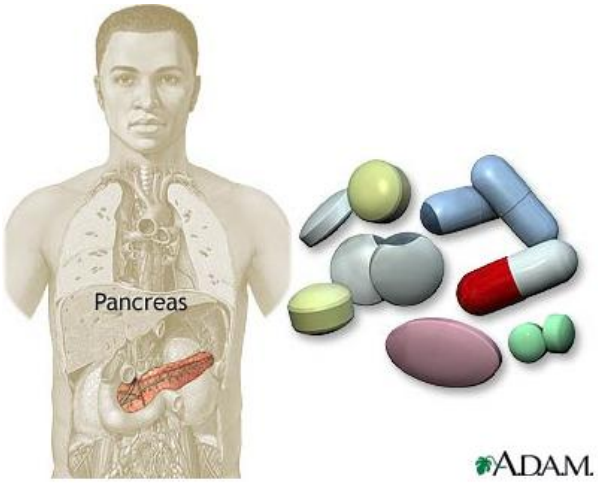
Now that the mechanisms behind diabetes remission in weight loss surgery is understood…

How can we apply this information for patients in which weight loss surgery is not an option?

One option: Drug therapy…

Byetta (exanitide):

- •GLP-1 agonist
- •Lasts longer than human GLP-1, which has short half life
- •Taken by injection 2X daily
- • Increase mass and preserve function of Beta cells



Group Discussion Question

Would there be an increased benefit, over a GLP-1 agonist, to developing a drug which uses the sweet taste receptor pathway to increase endogenous levels of GLP-1?

Group Discussion Question

Knowing that Sucralose elicits the GLP-1 response, without increasing blood glucose itself, would you recommend diabetic patients take it with their meals?

Why or why not?

Would you recommend it to pre-diabetic patients, or patients with a strong genetic pre-disposition for diabetes?

Do you think there could be any negative effects to consuming artificially sweetened beverages separate from mealtime?

Could this increased insulin release lead to hypoglycemia? Hunger? Sugar cravings?

Could the increased stimulation of taste receptors or from diet beverage consumption "wear out" these receptors more quickly?

A recent study found a 67% greater relative risk of incident type 2 diabetes, in those with at least daily consumption of diet soda, compared with non-consumers.

Would other Non-nutritive sweeteners induce the same effect as Sucralose?

Others studies testing Non-Nutritive Sweetener's effect on GLP-1 secretion have only been performed in rats. These studies show that neither sucralose, acesulfame, stevia, or saccharin increase GLP-1 response.

This is likely due to a difference in species:

- Studies have shown that mice don't taste aspartame as sweet
- (whereas humans do).

- Even differences exist between closely related animals like the primate family
  - gorillas taste aspartame as sweet, but marmosets do not.

Clearly more testing needs to be done on the wide variety of Non-nutritive sweeteners and GLP-1 release in humans

References

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