Replacing Sugars with the Alternative Sweeteners Erythritol and D-allulose

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Abbreviations

3-OMG	3-O-Methyl-D-glucose
ADI	acceptable daily intake
AgRP	agouti-related peptide
ARC	arcuate nucleus
AUC	area under the curve
BfR	German Federal Institute for Risk Assessment
BMI	body mass index
BOLD	Blood-Oxygen-Level Dependent
CART	cocaine- and amphetamine-regulated transcript
CASR	calcium-sensing receptor
CCK	cholecystokinin
CIOMS	Council for International Organizations of Medical Sciences
ClinO	Clinical Trial Ordinance
CNS	central nervous system
CVD	cardiovascular disease
DPP-IV	dipeptidyl peptidase-IV
ECLIA	electrochemiluminescence assay
EDTA	ethylenediaminetetraacetic acid
EEC	enteroendocrine cell
EFSA	European Food Safety Authority
EKNZ	Ethics Committee Northwestern- and central Switzerland
ELISA	enzyme-linked immunosorbent assay
EU	European Union
FDA	US Food and Drug Administration
GCP	good clinical practice
GDP	gross domestic product

GHIS	Global Hedonic Intensity Scale
GHSR1A	growth hormone-secretagogue receptor 1A
GI	gastrointestinal
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide 1
GLUT2	glucose transporter 2
GLUT5	glucose transporter 5
GOAT	O-acyltransferase
GRAS	Generally Recognized As Safe
GSIS	Global Sensory Intensity Scale
HbA1c	glycated hemoglobin
HCl	hydrochloric acid
HDL	high-density lipoprotein
HFCS	high-fructose corn syrup
hsCRP	high-sensitive C-reactive protein
ICH	International Council on Harmonisation
icv	intracerebroventricular
IgG	immunoglobulin G
LCS	low-caloric sweetener
LDL	low-density lipoprotein
MRI	Magnetic Resonance Imaging
NAFLD	non-alcoholic fatty liver disease
NCD	non-communicable disease
NPY	neuropeptide Y
NTS	nucleus of the solitary tract
OGTT	oral glucose tolerance test
OLETF	Otsuka Long-Evans Tokushima fatty

POMC	pro-opiomelanocortin
PPP	pentose phosphate pathway
PYY	peptide tyrosine tyrosine
RCT	randomized-controlled trial
RIA	radioimmunoassay
SGLT-1	sodium-glucose transporter 1
SSB	sugar-sweetened beverage
T2DM	type 2 diabetes mellitus
UNESCO	United Nations Educational, Scientific and Cultural Organization
US	United States
VAS	visual analogue scale
WHO	World Health Organization

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Summary

Obesity is a worldwide growing public health concern. The causes of obesity are complex and the health consequences significant. They not only include an increased risk of non-communicable diseases (NCDs), such as type two diabetes mellitus (T2DM) or cardiovascular disease (CVD), but also a number of psychosocial issues, such as low self-esteem, depression, and discrimination. The availability and affordability of high-caloric foods, especially sugar-sweetened beverages (SSBs) and their overconsumption, contribute to its development. Currently, pharmacotherapy or surgery are the main management tools applied to treat obesity and associated NCDs. These interventions have, however, a significant impact on quality of life, and are associated with a financial burden. In 2015, the World Health Organisation (WHO) published a guideline to limit sugar intake worldwide as a simple, effective, and low-cost strategy that can be used both preventively and therapeutically. A possible way to implement this guideline is to substitute sugar with artificial low-caloric sweeteners (LCS) to meet the desire for sweet taste with minimal or no caloric intake. However, the potential health impacts of artificial LCS ingestion remain a topic of ongoing debate, with no definitive consensus regarding their overall benefits or harms. Therefore, two other alternative sweetener classes, low-caloric bulk sweeteners and rare sugars, are emerging. Representatives include the non-caloric bulk sweetener erythritol and the rare sugar D-allulose.

The focus of this thesis was to study the effects of substituting sugar with erythritol and D-allulose in healthy humans. These aims were pursued in two clinical studies.

In part I of the first study, we investigated the involvement of the gut sweet taste receptor T1R2/T1R3 in the release of gastrointestinal (GI) satiation hormones, such as cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide tyrosine tyrosine (PYY) in response to erythritol and D-allulose. Participants received an intragastric administration of erythritol, D-allulose, or tap water, with or without lactisole (a T1R2/T1R3 receptor antagonist), respectively, in six sessions. Erythritol and D-allulose induced a significant release of CCK, GLP-1 ,and PYY compared to tap water. Lactisole did not affect the erythritol- and D-allulose-induced release of these GI satiation hormones. The lack of effect of lactisole suggests that

erythritol- and D-allulose-induced GI satiation hormone release is not mediated via T1R2/T1R3 in the gut and that other receptor/transporters are responsible.

In part II of the first study, we assessed different metabolic parameters and safety aspects of acute intragastric administration of erythritol and D-allulose. We found that both alternative sweeteners show beneficial physiological effects regarding glycemic control and the release of ghrelin, and have no effects on blood lipids, uric acid and high-sensitive C-reactive protein (hsCRP). This indicates that erythritol and D-allulose are, at least in the short-term, effective as sugar alternatives and have a positive safety profile.

In the second study, we compared the effect of oral administration of erythritol to sucrose, sucralose, or tap water on energy intake during a subsequent *ad libitum* test meal and examined the release of CCK in response to these substances. We found that (total) energy intake was significantly lower after erythritol compared to sucrose, sucralose, or tap water. Before the start of the *ad libitum* test meal, erythritol led to a significant increase in CCK compared to the other substances. These findings document once more the potential role for erythritol as a useful sugar alternative. Moreover, our results challenge the existing paradigm that only nutrients with calories are able to induce a satiating effect and decrease subsequent energy intake.

In summary, the results of both studies demonstrate that alternative sweeteners have unique physiological effects and should be evaluated independently and not as a homogenous group. Additionally, the acute findings of erythritol but also D-allulose indicate that they are simple and effective sugar alternatives that can be used by healthy individuals, but also by people with obesity and other NCDs, as a preventative or therapeutic approach. Whether these acute effects sustain in more long-term studies needs to be investigated.

Chapter 1

General Introduction

1 General Introduction

The wiring of the human brain throughout evolution has led it to recognize sweet as a naturally rewarding and reinforcing taste due to the caloric content of carbohydrates. This innate preference for sweet taste has evolved by natural selection to provide the necessary motivation to recognize and acquire adequate sources of energy in general, and sugar in particular [1]. This psychobiological mechanism, although adaptive in ancestral times of scarcity, may now be our worst enemy when it comes to resisting the abundance of sweet, energy-dense foods that are readily and cheaply available.

The sharp rise in sugar consumption, mainly through sucrose (i.e. table sugar) and glucose-fructose syrups from sugar-sweetened beverages (SSBs), is a key contributor to the dramatic global rise of non-communicable diseases (NCDs) including obesity, type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), gout, cardiovascular disease (CVD), and cancer [2-8]. These NCDs have become some of the greatest global health burdens of the 21st century in terms of prevalence, morbidity, and mortality [9-11], thereby placing a growing demand on healthcare, social, and economic resources. Indeed, the economic burden of obesity was estimated to be around 2 trillion US dollars or 2.8% of the global gross domestic product (GDP) in 2014 [12]. A more recent study indicates that the GDP will rise globally to 3.29% until 2060 if the current trends continue [13]. The mechanisms underlying the harmful effects of high sugar consumption are complex. For instance, obesity is a multifactorial condition with a complex etiology and a risk factor for many other NCDs. In the World Health Organisation (WHO) European Region, overweight and obesity have reached epidemic proportions, and it is estimated that around 60% of adults have either overweight or obesity [14]. A factor that contributes to overweight and obesity is the regular consumption of sugar-sweetened foods throughout the day, especially in the form of SSBs leading to constant hyperglycaemia with subsequent insulin release [3, 14]. SSBs are the largest source of added sugar in the diet; a typical 330 mL serving of soda delivers around 35-37 g of sugar and 140-150 calories. In Switzerland, almost 40% of the added sugars come from SSBs [15]. As a consequence, glucose concentrations rise, which in turn induces the secretion of insulin. The latter decreases blood glucose while inhibiting both glycogenolysis and fat oxidation [16, 17]. In other words, these

stored energy reserves are only available when insulin concentrations decrease again. Constantly elevated insulin concentrations, as is the case with permanent sugar consumption, encourage the development of adipose tissue [3]. In addition, if high doses of sugar are consumed within a short period of time, it can lead to excessive insulin responses and consequently to hypoglycemia and further episodes of food cravings [3, 18].

Other metabolic effects of high sugar intake depend on the type of sugar. Glucose, for example, leads to an increase in blood glucose concentrations with subsequent insulin secretion, whereas fructose is metabolized insulin-independent. Fructose passes from the small intestine via the portal vein into the liver where it is converted into fatty acids [19, 20]. It has been observed that high doses of acute fructose consumption affect the blood lipid profile negatively [21, 22]. Indeed, regular intake of high amounts of fructose is associated with NAFLD, which in turn promotes the development of insulin resistance [3, 5, 23]. In addition, elevated concentrations of triglycerides and cholesterol in response to repeated daily fructose consumption are associated with an increased risk of CVD as well as the promotion of hyperuricemia, which eventually leads to gout [24-26].

The two monosaccharides, glucose and fructose, also differ in their ability to stimulate gastrointestinal (GI) satiation hormones. Glucose ingestion induces the release of GI satiation hormones from enteroendocrine cells (EECs) located in the small intestine. These GI satiation hormones are then released into the bloodstream and act in an endocrine manner as hormones on peripheral organs or into the extracellular fluid to act in a paracrine manner on nearby cells such as vagal afferents [27]. In contrast, the consumption of fructose stimulates GI satiation hormones less effectively and does not attenuate ghrelin concentrations, a hunger-stimulating hormone [28-31].

Many of the NCDs described above can be alleviated and partially cured with pharmacological and surgical interventions. However, the impact on quality of life and the costs associated with these interventions are substantial. In contrast, the reduction of sugar intake is a simple, effective, and low-cost approach that can be applied both preventively and therapeutically. In 2015, the WHO published a guideline to limit sugar intake to 10% of the total energy intake, preferably to less than 5% [32]. As a comparison, in Switzerland, only 8% of the adult population have a free sugar intake below 5% of the total energy intake and more than half of

the population is over the recommended 10% [33]. In other terms, around 39 kg of refined sugar per head of the population were consumed in Switzerland in 2017. This is equivalent to more than 100 g per day, which exceeds the recommended amount of 25 g by the WHO more than four times [32, 34].

One possible way to achieve the WHO recommendations is to replace sugar intake with alternative sweeteners such as artificial low-caloric sweeteners (LCS) as a possibility to satisfy the desire for sweet taste while providing few or no calories. However, the health consequences of artificial LCS intake are still highly debated. Overall, the existing literature shows mixed results on the effects of artificial LCS on appetite, body weight, or glucose metabolism, with no clear consensus on whether they are beneficial or harmful [35]. As a consequence, two other groups, natural low-caloric bulk sweeteners, and rare sugars are attracting attention.

This thesis deals with the non-caloric bulk sweetener erythritol and the rare sugar D-allulose as sugar alternatives. The overall aim was to study the effects of replacing sugar with erythritol and D-allulose in healthy humans. The introduction provides an overview of the current state of knowledge concerning the gastrointestinal phase of eating control and introduces sucrose and its potential alternatives.

1.1 Gastrointestinal Phase of Eating Control

Dietary energy intake is highly regulated in animals and humans via the gut-brain axis to maintain energy homeostasis in the body. The GI tract is the place of origin for a wide range of signals contributing to the regulation of appetite and energy intake. The process of nutrient metabolism is a multifaceted phenomenon that encompasses the interactions of various nutrients with distinct GI targets in the short-term, as well as the ability to adapt to fluctuations in energy input and output in the long-term [36-38]. This thesis focuses on the short-term effects of the gastrointestinal phase of eating control.

The regulation of energy intake relies on a balance between hunger, satiation and satiety. “Hunger” describes the drive to eat inferred from objective conditions or the urge to eat due to physical sensations in our body (light-headedness, emptiness in stomach). “Satiation” describes the state of meal termination during eating (intra-meal satiety) influenced by satisfaction and stimulation of the brain by expanding the volume of the stomach. “Satiety”, on the other hand,

refers to the feeling of fullness due to nutritional and physiological signals (between-meal satiety), preventing further eating i.e. lack of appetite or hunger. [39-41] These appetite-related sensations are the result of complex interactions between the central nervous system (CNS) and peripheral feedback, which mainly originate from the GI tract and other active tissues and energy depots, such as fat mass or hepatic glycogen [37, 38].

The response of the GI tract to nutrient ingestion is traditionally divided into cephalic, gastric, and intestinal phases. The cephalic phase (primarily pre-and peri-ingestive influences of visual, and oro-nasal sensory stimulation) will not be addressed in this thesis [27].

1.1.1 Gastric Phase

The gastric phase of energy intake is a highly complex, multicompartmental process. Physical digestion of solid foods already begins in the mouth but is primarily a gastric function [42]. The main function of the stomach is to act as a reservoir of ingested nutrients and to perform mechanical and chemical breakdown of the contents into chyme that is pushed to the small intestine at a controlled rate. A relationship between gastric parameters, such as gastric distension, gastric emptying, and gastric hormones (e.g. the hunger hormone ghrelin) and appetite has been shown several times and will be addressed below. Other gastric parameters, such as motility, pH, or pyloric function have been studied to a lesser extent in relation to appetite, particularly energy intake. [27]

1.1.1.1 Gastric Distension

Gastric satiation is conveyed by distension in the stomach. The stomach wall contains specific neural mechanoreceptors that detect tension, stretch, and changes in volume [43]. These receptors send signals to the brain via vagal and spinal sensory nerves, using a variety of neurotransmitters and other chemical messengers [43, 44]. These mechanoreceptors increase their response rate when gastric volume increases and remain active up to the point when gastric volume decreases which, in turn, decrease their response rate. The hypothesis that the stomach plays a role in satiation is supported by experimental studies utilizing reversible pyloric cuffs, which can inhibit the passage of chyme from the stomach to the small intestine [43, 44]. Such studies have revealed that gastric distension alone is capable of terminating ingestion, meaning that the response is not nutrient but volume-mediated [45, 46]. However, the volume of food

required for meal termination solely due to gastric distension would exceed the amount eaten in a typical meal [43].

Several other authors have demonstrated that distension of the stomach induces a satiating effect. In 1988, Geliebter [47] showed that upon inflating a gastric balloon with a volume of 400 mL or higher, energy intake including hunger ratings were reduced. Similar results were obtained from other balloon studies [48-50]. Also, findings from trials using ultrasound or magnet resonance imaging (MRI) showed a relationship between gastric distension and satiation in response to different liquid meals [51, 52]. Moreover, there is evidence that gastric distension-induced satiation can potentially be regulated by GI satiation hormones. Indeed, intravenous infusion of cholecystokinin (CCK) combined with gastric distension (water-filled balloon) enhanced the suppression of energy intake compared to CCK administration alone in humans [53]. In another example, glucagon-like peptide 1 (GLP-1) containing neurons in the nucleus of the solitary tract (NTS) were activated by gastric distension within the physiological range, supporting a role for GLP-1 in gastric distension-induced appetite signaling in rats [54].

1.1.1.2 Gastric Emptying

In addition to gastric distension, gastric emptying is an important process of the gastric phase. Gastric emptying is governed by meal volume, osmotic pressure, energy value, digestibility, and macronutrient content [27, 42]. When meals contain both liquids and solids, the two phases empty differently. Ingested liquids are distributed equally throughout the stomach and begin emptying almost immediately, directly proportional to the gastric volume and in an exponential process [42, 55]. However, liquids with a high energy value empty more slowly than those containing fewer calories per unit volume [56]. Solids, on the other hand, empty in a biphasic manner: they are initially restricted to the fundus and move gradually to the antrum by means of peristaltic contractions. There, they are mixed with gastric secretions, triturated, and reduced to chyme particles of 1-2 mm size to empty through the pylorus into the small intestine. The delay until the first emptying of solids occurs is known as the lag phase and depends on the physical characteristics of the meal [55, 57]. Once chyme enters the small intestine, multiple neural and GI hormone responses are initiated by a duodenal-gastric feedback mechanism [42]. Thus, gastric emptying affects not only the magnitude and duration of gastric distension but also determines the rate of appearance of liquids and solids in the small intestine and how they

interact with the stomach [27]. As a consequence, upon reaching the small intestine, the majority of ingested meals inhibit the secretion of the hunger hormone ghrelin and stimulate the release of GI satiation hormones, such as CCK, GLP-1, and peptide tyrosine tyrosine (PYY). In turn, ghrelin induces while CCK, GLP-1, and PYY inhibit gastric emptying (see **Figure 1**) [27, 42, 58-62].

1.1.1.3 Ghrelin

This thesis will focus on the hunger hormone ghrelin and its effects on appetite regulation, although there are also other signaling molecules produced in the stomach that might be involved.

Ghrelin is a 28-amino acid peptide hormone produced by closed-type EECs in the gastric fundus as well as by some small intestinal EECs, pancreatic-islet cells, and neurons in various brain areas, including the arcuate nucleus (ARC) of the hypothalamus [63-65]. Ghrelin is acylated by the ghrelin O-acyltransferase (GOAT) into its biologically active forms, octanoyl- and decanoyl-ghrelin to bypass the blood-brain-barrier (together referred to as acyl-ghrelin, in contrast to unacylated or des-acyl-ghrelin). Ghrelin binds to the growth hormone-secretagogue receptor-1A (GHSR1A) which is widely expressed both peripherally and centrally [65, 66]. Ghrelin is known for its short-term “meal initiator” role [67]. Consistent with this role, plasma concentrations of ghrelin increase before nutrient ingestion, rapidly decrease after ingestion, and then increase gradually until the next meal [68-70]. Sight, smell, and taste of food (i.e. the cephalic phase of eating control) were reported to induce and inhibit the release of ghrelin in humans [71-73]. Intravenous administration of ghrelin increased energy intake in humans [74, 75]. Moreover, appetite-related sensations such as feelings of hunger are closely associated with the rise and fall of ghrelin concentrations between meals [69, 76]. In addition, ghrelin concentrations at the beginning of a meal correlated with meal size [76]. Also, supraphysiological ghrelin infusions in humans increased the neural response to pictures of food, assessed by functional MRI, in brain areas that are involved in food reward [77, 78]. This supports a hypothesis, that ghrelin affects eating also via its effect on reward areas in the brain [42]. Ghrelin responses are dependent on caloric content and the type and composition of macronutrients. All three macronutrients inhibit ghrelin secretion in response to a meal [79, 80]. However, it seems that lipid ingestion suppresses ghrelin less effectively than proteins or

carbohydrates at isocaloric loads [79, 81]. CCK and PYY are potentially involved in inhibiting ghrelin secretion because intravenous administration of both hormones reduced plasma ghrelin concentrations in humans [82-84], whereas GLP-1 administration did not [83]. To date, only one study could demonstrate the role of CCK in inhibiting ghrelin secretion: administration of the CCK-1 receptor antagonist dexloxiglumide abolished the long-chain fatty acid-induced ghrelin inhibition in healthy men, suggesting that the mechanism involves CCK [85]. Other contributors involved in suppressing ghrelin concentrations are changes in plasma insulin levels, intestinal osmolarity, and enteric neural signaling, whereas gastric distension and the vagus nerve are not necessary [67, 86]. Besides ghrelin's short-term role in appetite regulation, the hunger hormone has a potential function in the long-term regulation of body weight because ghrelin concentrations increase in response to weight loss, and baseline levels are typically lower in patients with obesity [67].

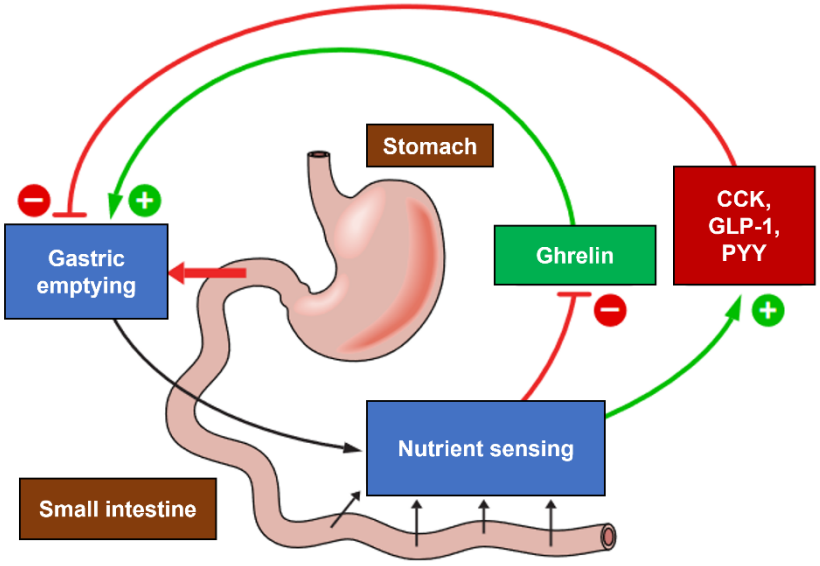

Figure 1 Duodenal-gastric feedback mechanism. Gastric emptying determines the rate of nutrients appearing into the small intestine. For most meals, small-intestinal nutrient sensing inhibits ghrelin secretion and stimulates CCK, GLP-1, and PYY secretion. In turn, ghrelin stimulates while CCK, GLP-1, and PYY inhibit gastric emptying, respectively. Adapted from [42].

1.1.2 Intestinal Phase

More than 100 years ago, Bayliss and Starling discovered secretin as the first GI hormone [87]. To date, more than twenty GI hormones are described that are released from EECs which are responsible for the regulation of energy intake [88]. EECs are specialized epithelial cells, which are interspersed among enterocytes in mucosal cells of the GI tract. They represent less than 1% of the entire gut epithelial population but constitute the largest endocrine organ of the human body [89]. The existence of different combinations of G protein-coupled receptors and transporters in these EECs, and their differential distribution along the gut, enable them to sense all major nutrients [90].

Figure 2 describes potential signaling ways of the GI satiation hormones CCK, GLP-1, and PYY. First, these GI satiation hormones act in the classical endocrine fashion, in which the hormones enter the systemic circulation, allowing them to act directly on other targets. The second mode is a neuroendocrine mode, in which the hormones activate vagal afferents which in turn, stimulate brain-mediated responses. Third, they act in a paracrine fashion, by acting on receptors on nearby cells, either neuroendocrine cells or other cell types. The fourth form of action displays hormone release from neuropod cells that form synapses with cells of the enteric nervous system and other cell types. This mode of action has been described for CCK and PYY cells [91-93] but may exist for other GI hormones as well. [42]

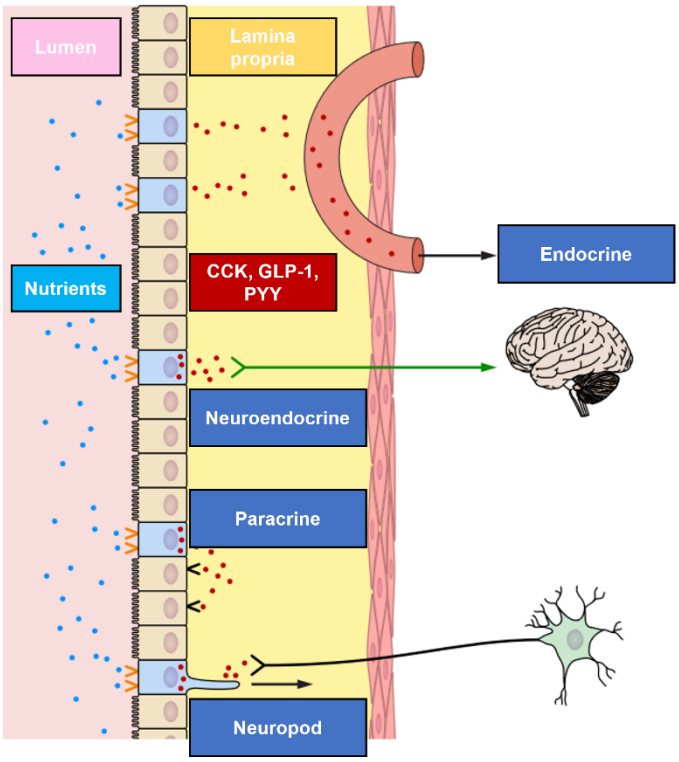

Figure 2 Overview of the potential modes of actions of CCK, GLP-1, and PYY adapted from [42].

Together with the parameters of the gastric phase, release of nutrient-stimulated GI satiation hormones from the intestine is considered to play a major role in the control of eating. There are, however, several criteria that must be fulfilled before such a GI satiation hormone can be accepted as a physiologically relevant signal in relation to energy intake [42, 94]: i) the concentration of the hormone should change based on the amount of nutrients ingested; ii) receptors for the hormones should be expressed at its site(s) of action; iii) exogenous administration of the hormone (in amounts that reproduce endogenous hormone secretion) should have the same physiological effects as the endogenous hormone; iv) administration of secretagogues for the hormone should produce effects similar to the effect of the hormone; v) the hormone's effect should not cause any abnormal behavioral, physiological, or subjective effects; vi) exogenous administration of substances that either activate (agonists) or block (antagonists) the hormone's receptors should produce effects that align with their known effects on the receptors.

The sections below introduce the relevant GI satiation hormones (CCK, GLP-1, and PYY) for this thesis.

1.1.2.1 Cholecystokinin (CCK)

CCK is a peptide hormone that is produced and secreted by open-type I-cells localized in the duodenum and jejunum mucosa that are in direct contact with the intestinal lumen where they can sense the luminal content [95-97]. I-cells and CCK can also be found in the remaining small intestine and even a few in the colon [95, 98, 99]. As mentioned above, **Figure 2** shows the potential local and endocrine modes of actions of CCK. It was the first gut hormone found to regulate food intake [100, 101]. CCK is released in response to food as soon as chyme is entering the duodenum [102], whereby plasma concentration increases 10 to 15 min after meal initiation. Dietary lipids and proteins are more potent stimulators compared to carbohydrates [102]. Nevertheless, the response to carbohydrates is still significant [103, 104]. CCK stimulates gallbladder contraction, release of pancreatic enzymes (e.g., somatostatin) and inhibits GI motility and gastric emptying [105, 106]. There are various bioactive forms of CCK, classified according to the number of amino acids they contain. CCK-8 and CCK-33 are mostly studied with regard to appetite [107]. There are two receptors identified for CCK, named CCK- 1 (originally named CCK-A “alimentary type”) and CCK-2 (originally named CCK-B “brain type”) receptors, respectively. CCK-1 receptors are mainly distributed in the GI tract whereas CCK-2 receptors predominate in the brain [105]. CCK-1 receptors are also expressed on afferent fibers of the vagus nerve through which CCK transfers satiation signals to the brain [108].

It is well known from the study by Gibbs and colleagues [100] that acute administration of intraperitoneal injections of CCK immediately before a meal, reduces meal size in a dose-dependent manner in rats, but also suppresses sham feeding [101]. As a result, these observations provided a rationale for the investigation of the effects of CCK on energy intake in humans. Multiple studies found that administering intravenous infusions of physiological doses of CCK led to reduced meal size in males as well as females, without any adverse events being reported [109-111]. Moreover, intravenous infusions of the CCK-1 receptor antagonist loxiglumide resulted in increased feelings of hunger before the meal, reduced fullness during the meal, increased meal size, and blocked the satiating effects of intraduodenal infusion of a fat emulsion [112-114]. All these acute human studies proved that CCK is one of the best-established GI satiation hormones.

Given the observed outcomes of CCK administration in acute studies, it has been proposed that sustained elevation of CCK plasma concentrations may lead to a reduction in energy consumption in the long-term, making it a promising therapeutic option for the treatment of obesity. However, results of animal studies showed that this is not the case [115-118]. For example, CCK infusions reduced meal size by 44% but the animals compensated the energy intake by increasing their meal frequency [118]. However, recent preclinical data on combinatorial therapies to treat obesity and obesity-related diabetes are more promising, especially the co-administration of GLP-1 and CCK-receptor agonists [119-123].

1.1.2.2 Glucagon-like peptide 1 (GLP-1)

GLP-1, together with glucose-dependent insulinotropic polypeptide (GIP), is known as an incretin hormone. It is synthesized from the 160 amino acid proglucagon, a prohormone produced in α-cells of the pancreas, enteroendocrine L-cells of the ileum and colon, and in the CNS. Differential cleavage of proglucagon by prohormone convertase 1 or 2 results in GLP-1, GLP-2, glucagon, glicentin or oxyntomodulin [124]. There are various forms of GLP-1: GLP-$1_{1\text{-}36}$, GLP-$1_{7\text{-}36}$, and GLP-$1_{7\text{-}37}$ with GLP-$1_{7\text{-}36}$ being the most common and biologically active form [125]. GLP-1 is rapidly degraded by dipeptidyl peptidase-IV (DPP-IV) and has a relatively short half-life (~1-2 min) [126]. The secretion of GLP-1 is stimulated by ingested food, especially fats and carbohydrates, and occurs in a biphasic manner. The first peak usually results within 5 to 30 min after meal initiation and a second more prolonged phase after 60 to 120 min [42]. Several mechanisms may contribute to the initial peak of GLP-1 release: i) L- cells are expressed in the proximal intestine (although the density is lower compared to the distal intestine) [127-129]; ii) the initial rate of gastric emptying of liquid solutions for example from carbohydrates, especially in participants on an empty stomach, may produce concentrations that exceed the absorption capacity of the proximal intestine, this way the liquids potentially reach more distal L-cells during the first period after meal initiation [130]; or iii) proximal to distal neuronal and/or humoral signals might affect the GLP-1 release [131]. The more prolonged second release occurs through contact with dietary nutrients from L-cells in the distal intestine [42, 126]. As an incretin hormone, GLP-1 increases glucose-dependent insulin release, β-cell growth and reduces glucagon secretion. Furthermore, it inhibits gastric acid secretion and delays gastric emptying, promoting the so-called “ileal brake” [42, 124, 132].

The “ileal brake” enables a moderate and consistent transit of nutrients from the stomach into the small intestine, thereby being part of a negative feedback mechanism to enhance efficient nutrient uptake [133].

In 1996, two groups simultaneously described the acute anorectic effects of centrally administered GLP-1 in rats [134, 135], with other studies confirming these results [136, 137]. Studies in rodents have shown that intracerebroventricular (icv) injection of GLP-1-receptor agonists suppressed energy intake in a dose-dependent manner and can be abolished by pretreatment with the GLP-1 receptor antagonist exendin$_{9\text{-}39}$ [134, 137]. Both of these effects are absent in GLP-1-receptor knockout mice [138]. Several studies reported that administration of GLP-1, intravenously and orally, reduced meal size in healthy humans, humans with obesity or T2DM [110, 133, 139-144], while other studies failed to suppress eating [145, 146]. The discrepancies of these results potentially arise from different study protocols, such as shorter GLP-1 administration, time of the study session, or the test environment that might affect behavioral responses of the participants. Administering the GLP-1 antagonist exendin$_{9\text{-}39}$ failed to increase meal size in healthy humans [147, 148]. Several effects might have led to this failure: other GI satiation hormones, such as CCK or PYY may have overridden a possible effect of exendin$_{9\text{-}39}$ or the discrepancy in results may also be attributed to variations in the study design [148].

The mechanism by which GLP-1 inhibits eating is not completely understood. GLP-1 acts via G-protein coupled receptors which are localized in the pancreas, intestine, stomach, heart, kidney, and several regions of the brain [42, 124]. Data from rat and mice studies suggests that intestinal GLP-1 acts locally on vagal afferents in the lamina propria of the intestine or in the brain [42, 126]. Indeed, meals failed to increase systemic GLP-1 concentrations in rats [149, 150]. Also, animal studies have identified a number of brain regions where GLP-1 potentially acts to suppress eating [42, 126]. GLP-1 may act in an endocrine mode of action in humans, because postprandial GLP-1 concentrations are relatively high in the systemic circulation and it is possible that endocrine GLP-1 does not affect these brain regions, due to its short half-life [42]. However, antagonist studies of GLP-1 have not yet confirmed this and a recent study suggested that glucose-induced endogenous GLP-1 affects the regulation of appetite by modulating the resting state functional connectivity between homeostatic and reward-related brain regions in healthy male participants [151]. In addition, some studies suggest that the

satiation effects of GLP-1 depend on synergism with other GI satiation hormones [152, 153]. Recently, Vana et al. [154] investigated whether CCK might influence the subsequent effect of endogenous GLP-1 in response to a meal in mice. They found that prior CCK secretion is important for GLP-1 to suppress eating and that the intestinal free fatty acid receptor 1 plays a pivotal role in inducing CCK release [154]. A previous study in humans has described a similar mechanism [152].

Patients with obesity display reduced amounts of EECs compared to lean individuals, and exhibit impaired secretion of GI satiation hormones that may contribute to the development of this metabolic disease [128]. Considering that GLP-1 reduces energy intake and stimulates glucose-dependent insulin release, several drugs have been developed for the treatment of obesity and T2DM during the last two decades. These include for example, GLP-1 analogues like exenatide, liraglutide, or semaglutide [126]. As mentioned earlier, other pharmacotherapies that combine GLP-1 with other GI hormones such as CCK are currently under clinical evaluation.

1.1.2.3 Peptide Tyrosine Tyrosine (PYY)

PYY belongs to the pancreatic polypeptide-fold family and was first characterized in 1980 [155, 156]. PYY is produced by open-type endocrine L-cells of the distal ileum, colon, and rectum but also in the CNS [125]. This peptide consists of 36 amino acids with two biological active forms: PYY_{1-36} and PYY_{3-36}. The biological activation requires a C-terminal amidation [157]. Plasma PYY is secreted as PYY_{1-36} and is rapidly degraded by DDP-IV to the most circulating form PYY_{3-36} [158]. As mentioned earlier, mouse intestinal PYY cells appear to release some PYY via neuropod EECs, which are specialized cell types that form a synaptic connection with the nervous system [93]. PYY is often co-secreted with GLP-1 in response to food ingestion with fat as a major stimulating factor [125]. The secretion follows a biphasic pattern, where concentrations generally begin to increase around 15 to 30 min after meals, reach a peak at approximately 60 to 90 min after meals, and remain elevated for several hours [42]. The stimulation of PYY secretion via nutrient receptors located on the membrane of EECs has been studied less extensively. Carbohydrates such as glucose might stimulate PYY secretion partly via the sweet taste receptors [159, 160]. However, equisweet artificial LCS binding to the receptor did not induce the release of PYY [28, 161, 162]. Another possibility that might

contribute to PYY release appears to be the calcium-sensing receptor (CASR) because PYY secretion was reduced upon administration of a CASR inhibitor [161]. Also, intravenous CCK infusions increased PYY concentrations in humans [83, 163], whereas administration of dexloxiglumide blocked the secretion of PYY in response to lipids [85]. Furthermore, GLP-1 infusion decreased [83] and exendin$_{9-39}$ increased PYY release [148, 164].

PYY slows down gastric emptying rate and is (together with GLP-1) involved as a major component of the ileal brake [61, 125, 165]. The role for PYY as a satiation hormone and thus reducing energy intake, is mediated by PYY$_{3-36}$ [43, 84, 166-168]. Intravenous infusions of PYY$_{3-36}$ inhibited eating more potently in rats and humans compared to PYY$_{1-36}$ whereas centrally administered PYY$_{1-36}$ triggered energy intake in rats [84, 169, 170]. Over twenty years ago, Batterham and colleagues [166] reported that peripheral and central administration of PYY$_{3-36}$ reduced energy intake in rodents and that intravenous infusions of postprandial PYY$_{3-36}$ decreased appetite and energy intake in humans by 33% over 24 hours. Several subsequent studies confirmed the satiating effects of PYY$_{3-36}$ in healthy humans and people with obesity [82, 84, 157, 168, 171].

Whether PYY$_{3-36}$ acts in a peripheral or central manner to reduce energy intake is unclear. PYY acts via five receptors from the neuropeptide Y (NPY) receptor family (Y1, Y2, Y4, Y5, Y6) which are coupled to inhibitory G-proteins [172]. In context of appetite regulation, PYY$_{1-36}$ triggers energy intake by activating the orexigenic Y1 and Y5 receptors, whereas PYY$_{3-36}$ induces its anorectic effects via Y2 receptors expressed in NPY and agouti-related peptide (AgRP) secreting neurons of the ARC [173, 174]. In support of a peripheral way of action, subdiaphragmatic vagotomy reduced or abolished the eating-inhibitory effect of peripherally administered PYY$_{3-36}$, and a Y2 receptor antagonist that was not able to cross the blood-brain barrier blocked it [175-177]. In favor of central action, injections of PYY$_{3-36}$ into the ARC decreased energy intake in rats [178]. Moreover, administration of the Y2 receptor antagonist reduced the inhibitory effect of PYY$_{3-36}$ in rats [178]. The inhibitory effect of PYY$_{3-36}$ was abolished in Y2 receptor knockout mice [166]. Also, PYY$_{3-36}$ suppressed eating in vagotomized mice [179].

Although PYY concentrations in humans with obesity are lower compared to lean individuals, there is still an anorectic effect of peripherally administered PYY$_{3-36}$ in this cohort, indicating

a therapeutic potential for PYY for the treatment of obesity [82, 168, 171, 180]. However, $PYY_{3–36}$-based therapies have not yet made it to clinic. This could be due to a number of factors including a short biological half-life of less than four hours or the observed adverse effects in humans such as nausea or abdominal discomfort in response to administered PYY_{3-36}, which may influence its appetite-inhibitory effects [84, 181]. Indeed, the interaction of PYY in the area postrema, known to house the vomiting center, would strengthen this view [175]. There is, however, new evidence from animal studies that PYY analogues in combination with other hormones such as GLP-1 and glucagon have potential as new obesity and T2DM treatments [182, 183].

In summary, the act of eating initiates a complexly coordinated series of GI responses that contribute to the control of eating through various mechanisms of action. The control of GI hormones by small-intestinal nutrient sensing is the basis of these functions. The GI hormones control exposure of the small intestine to nutrients via their effects on gastric functions, particularly gastric emptying, and thereby modulate their own secretion.

1.2 Conventional Sweeteners

1.2.1 Caloric Sugars

The term “sugar” is often misleading. In most cases, it refers to a specific type of sugar (sucrose), but it can also refer to all types of caloric sugars. In the human diet, sugar is mainly found in the form of the disaccharide sucrose or the monosaccharides glucose and fructose, while the proportion of other types of sugar, such as the disaccharide lactose, is relatively low. Various sugar products and syrups are marketed as healthier alternatives, but ultimately, they are made up of nothing more than fructose and glucose after being broken down in the small intestine. For example, agave and date syrup mainly contain fructose, coconut sugar and maple syrup consist of more than 95% sucrose, and honey is primarily a glucose-fructose mixture.

As mentioned earlier, excessive sugar consumption, especially in the form of SSBs, has risen over the past decades and may be contributing to the global obesity rates and associated NCDs. The common dietary caloric sugars glucose and fructose are absorbed and metabolized differently by the body. Prior studies have shown that these monosaccharides have different effects on hormones involved in the GI phase of eating control, which may significantly affect appetitive behavior and obesity risk. A limitation acknowledged by prior studies is that fructose and glucose are rarely consumed in isolation, and additional studies are necessary to examine how real-world sugars, such as sucrose, affect endocrine responses. We, therefore, used sucrose as a positive control in one study of the present thesis. The next paragraphs describe sucrose and its effects on several metabolic parameters relevant for this thesis.

1.2.1.1 Sucrose

The most frequently consumed and added sugar is sucrose, commonly referred to as table sugar, a disaccharide that consists of 50% glucose and 50% fructose (**Figure 3**). These two monosaccharides differ in their metabolism. After consumption of sucrose, it is broken down by digestive enzymes and the monosaccharides glucose and fructose are absorbed at the brush border of the intestinal cells. Glucose uses an active transport via the sodium-glucose dependent transporter- 1 (SGLT-1), while fructose uptake occurs in a facilitative way by glucose transporter 5 (GLUT5) [184, 185]. Both sugars are then transported out of the intestinal cells

via glucose transporter 2 (GLUT2) [184]. The complete digestion and absorption of sucrose makes it a highly caloric sugar (4 kcal/g). After intestinal uptake, glucose enters the bloodstream whereas fructose is partly converted to glucose by the liver and only partly enters the bloodstream in the form of glucose [19]. As a consequence, glucose increases blood glucose concentrations much more than fructose does and therefore elicits a stronger insulin response [186]. Uptake of circulating glucose by other tissues such as adipose or muscle tissue is stimulated primarily by insulin that signals to the GLUT transporters to move to the cell surface. This activates glucose uptake into the cells.

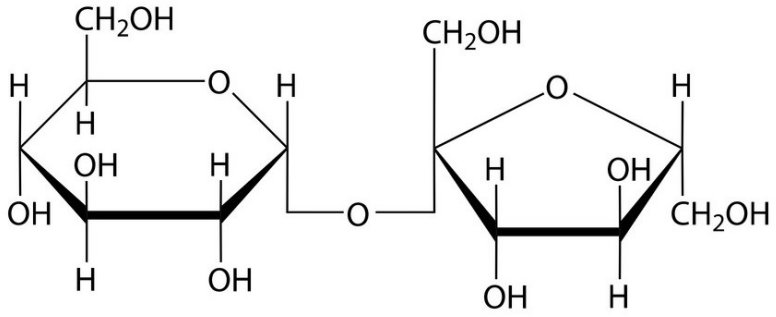

Figure 3 Chemical structure of sucrose.

1.2.1.1.1 Gastric Emptying

In 1994, Murry et al. [187] investigated several criteria of gastric emptying rates following ingestion of either 400 mL water or 6% solutions of glucose, sucrose, maltodextrin, and sucrose plus glucose in six resting participants. They found that the comparison of several criteria on gastric emptying (percentage of initial beverage volume remaining in the stomach, mean gastric emptying rates, and gross gastric volumes) provided different outcomes. For example, the mean gastric emptying rate of glucose was less than water, with no differences among the other drinks (e.g. sucrose versus water). A lack of effect for sucrose might be due to the small sample size of the study. In contrast, several other studies reported that sucrose reduces gastric emptying rates: Ma et al. [188] showed that intragastric infusions of 50 g sucrose dissolved in water emptied slower from the stomach compared to saline in seven healthy men. In another study, gastric emptying rates were reduced in response to an oral sucrose drink (300 mL water plus 100 g sucrose) compared to pure water in young adults [189]. Lavin et al. [190] compared oral

versus intragastric administration of solutions containing sucrose, maltose, and water as a control (for the oral experiment only). The results showed that, when administered orally, sucrose and maltose reduced gastric emptying rates compared to water. Sucrose emptied faster than maltose in response to both oral and intragastric administration [190]. Another study assessed the effect of four beverages containing different concentrations of glucose and sucrose on gastric emptying rates. The authors found that glucose resulted in a stronger inhibitory stimulus on gastric emptying compared to sucrose, possibly due to differences in osmolality and/or chemical structure of the sugars [191]. However, sucrose emptied slower from the stomach compared to the control (water) [191].

1.2.1.1.2 Gastrointestinal Hormone Secretion

A pilot study from Yau et al. [192] investigated the effects of oral sugar ingestion in seven healthy men. They found no differences in circulating GLP-1 in response to sucrose compared to glucose ingestion and the suppression of acyl-ghrelin was similar between both sugars. Compared to water, oral sucrose suppressed plasma ghrelin concentrations from t = 30 to 180 min post-ingestion in healthy humans [189]. In another study, 50 g of sucrose resulted in an increase in GLP-1 compared to sucralose or saline [188]. More recently, Yunker et al. [193, 194] performed two separate studies investigating the effects of sucrose on different metabolic parameters. In the first study, they investigated the effects of oral sucrose versus glucose (both 75 g) on appetite-regulating hormones in 69 adults [193]. They showed that sucrose compared to glucose intake led to reduced areas under the curve (AUC) for GLP-1 and PYY, whereas the AUC for acyl-ghrelin did not differ between sucrose and glucose [193]. The contrasting results compared to Yau et al. [192] are possibly due to the larger sample size and higher administered dose (75 g versus 36 g) as well as measuring active GLP-1 instead of total GLP-1. The second study assessed the effects of sucrose versus sucralose, with water as a control. There, sucrose led to an increased AUC of GLP-1, but not PYY, and a decreased AUC in acyl-ghrelin compared to water or sucralose [194]. Taken together, sucrose is able to stimulate the secretion of GI hormones although to a lesser extent compared to glucose. This is supported with previous findings where the effects of fructose compared to glucose resulted in a smaller GLP-1 release [195]. Several different mechanisms may be involved in the secretion of GI hormones. One of them is the sweet taste receptor located on EECs in the intestine, particularly those releasing

GLP-1 and PYY. In healthy humans, Gerspach et al. [196] demonstrated that lactisole, a competitive inhibitor of the sweet taste receptor, attenuated the glucose-stimulated secretion of GLP-1 and PYY. The secretion of CCK was unaffected by lactisole, indicating that its secretion is not mediated by the sweet taste receptor and, hence, that other glucose-sensing receptors must be involved. Other studies suggest that SGLT-1 triggers glucose-induced GLP-1 secretion [197]. A possible mechanism for GLP-1 secretion via GLUT5 has been suggested for fructose [198, 199]. Further mechanisms such as L-type Ca^{2+}- channels have been proposed to stimulate GLP-1 and CCK release upon glucose administration *in vitro* [200, 201]. Studies in humans are lacking so far and whether similar mechanisms are possible for sucrose needs to be investigated.

1.2.1.1.3 Energy Intake and Appetite-Related Sensations

Several studies found no differences on subsequent energy intake in response to sucrose compared to water. However, they failed to report the total energy intake [190, 202, 203]. Akhavan et al. [204] examined the effects of a sucrose and a sucralose containing preload on subsequent energy intake during an *ad libitum* test meal. They found no significant differences in energy intake during the test meal between both preloads but a significantly higher total energy intake when considering the caloric content of the sucrose preload [204]. These results are in line with previous findings, which state that the combination of sucrose with a meal lead to a higher total energy intake compared to water, as meal size did not differ between groups [205]. Appetite ratings were not different, except that sucrose decreased thirst to a lesser extent than water [205]. In a study in healthy women, ingestion of sucrose compared to high-fructose corn syrup (HFCS) resulted in a similar energy intake and hunger ratings [206]. Similarly, Yau et al. [192] found no differences in appetite-related sensations (hunger, fullness, and prospective food consumption) in response to sucrose compared to glucose. In contrast to the other studies mentioned above, a more recent preload study found that total energy intake was around 100 kcal lower following sucrose compared to water intake in healthy young participants [194]. The discrepancies of these results presumably arise from different doses administered and study designs.

1.2.1.1.4 Glycemic Control

Glycemic control aims at keeping blood glucose concentrations within a target range in order to reduce the risk of complications associated with high or low blood glucose. Oral sucrose consumption increases blood glucose and plasma insulin concentrations compared to water [189]. Similar effects were observed in response to 50 g of sucrose, which increased blood glucose and insulin concentrations compared to saline [188]. Testing the effects of sucrose compared to HFCS beverages in combination with meals in 34 participants resulted in no differences in 24-hour plasma profiles of glucose concentrations. There was, however, a significant increase in the insulin AUC in response to the sucrose-sweetened beverage [207]. Oral sucrose versus glucose led to reduced AUC for plasma glucose and insulin concentrations [193], but the AUCs were increased compared to water [194]. Moreover, two weeks of daily consumption of three servings of SSBs decreased insulin sensitivity, assessed by the Matsuda insulin sensitivity index, compared to beverages sweetened with aspartame [23]. When comparing the acute effects of sucrose, glucose, and fructose, the ingestion of sucrose and glucose resulted in a significant increase in blood glucose and insulin concentrations compared to fructose [21].

1.2.1.1.5 Blood Lipids, Uric Acid, and hsCRP

Comparison of acute administration of sucrose, glucose, and fructose resulted in higher cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels in response to fructose compared to the other two sugars [21]. There was no difference in triglycerides, and high-sensitive C-reactive protein (hsCRP) was significantly increased after fructose compared to glucose, but not to sucrose [21]. Sucrose and fructose, but not glucose, were linked to an increase in uric acid in nine healthy men [208]. More recently, Sigala et al. [23] showed that daily consumption of sucrose-sweetened beverages over two weeks increased hepatic lipid compared to baseline and aspartame. Furthermore, they observed an increase in 24-hour triglyceride AUC, a rise in fasting and postprandial LDL, and HDL as well as an increase in both fasting and total 24-hour uric acid AUC in response to sucrose compared to aspartame [23]. In addition, consumption of different SSBs during three weeks (fructose, glucose, and sucrose) increased hsCRP after all interventions [209]. The results of a meta-

analysis reported that the changes in hsCRP in response to HFCS were not significantly higher compared to sucrose [210].

1.2.1.1.6 Gastrointestinal Tolerance

GI symptoms in response to sucrose usually occur with an existing sucrose intolerance in form of carbohydrate malabsorption or other digestive conditions such as irritable bowel syndrome. In healthy humans, however, sucrose is well-tolerated as it is completely absorbed from the intestine. Studies assessing the effects of different carbohydrates (sugar alcohols, fiber, or resistant starch) on GI symptoms often use sucrose as a comparator [211]. For instance, several studies compared the effects of erythritol compared to sucrose on GI well-being, with more complaints of GI effects in response to erythritol than sucrose [212-214]. Another study investigated the GI response in 58 children consuming sweets sweetened with either sucrose or isomalt. The children experienced 44 bowel movements consisting of watery faeces following the intake of isomalt sweets compared to only five following sucrose sweets [215]. Moreover, there was a significant response in stomach-ache and abdominal-rumbling noises after ingestion of isomalt sweets compared with sucrose sweets. In general, the sucrose sweet was better tolerated, although the symptoms reported did not stop the children from eating the sweets [215]. Overall, sucrose is well-tolerated in adults and children without any GI malfunctions.

1.2.1.1.7 Other Health Aspects

The consequences of sugar consumption are not limited to metabolic health, but also play a role in general physical health. An underestimated issue lies in the development of dental caries. Regular sugar ingestion leads to an increase in cariogenic bacteria, which metabolize sugar into acids [216]. This results in a local decrease in pH and demineralization of the tooth surface [216]. As previously mentioned, excessive and regular sugar intake plays a significant role in the development of NCDs including obesity, T2DM, NAFLD, gout, CVD, and cancer [2-8]. Moreover, a systemic, irreversible harmful effect of high sugar consumption leads to the development of advanced glycation end products (AGEs) [217]. These are spontaneously and endogenously formed protein-sugar complexes, whose proper functioning is impaired through excessive sugar intake. In the case of the protein collagen for example − which is found in vessel walls, the skin, and lenses − these complexes lead to a significant loss of vascular

elasticity [218-220]. Long-term elevations in blood glucose concentrations are therefore associated with premature aging of wall vessels, the skin as well as with cataract [220]. Preserving vascular elasticity is of great importance for cardiovascular health because epidemiological studies have shown a significantly higher cardiovascular risk with increasing sugar consumption [221, 222]. There is also evidence that other diseases, such as cancer or cognitive disorders, are associated with an excessive intake of sugary foods and beverages [223-225].

In summary, sucrose is able to slow down gastric emptying rates and to stimulate the secretion of GI hormones, ultimately affecting subsequent energy intake. Despite its pleasurable taste, the excessive overconsumption of sugar has deleterious effects on metabolic health and associated disorders. Thus, reducing sugar intake is imperative in the human diet. One strategy is the replacement of sugars with alternative sweeteners. The sections will provide an overview of the various classifications of alternative sweeteners.

1.3 Alternative Sweeteners

As a strategy to reduce sugar consumption, the use of alternative sweeteners has increased drastically in the past decades. Artificial LCS are most frequently used to sweeten beverages and foods [226]. Despite the ongoing use, the potential health implications of LCS intake remain controversial. The current literature shows ambiguous results regarding the influence of LCS on hunger, body weight and glucose metabolism, with no conclusive agreement on whether LCS consumption has a positive or negative effect [1, 227]. As a result, two other groups, natural low-caloric bulk sweeteners and rare sugars are gaining popularity. The next sections describe the three different groups of alternative sweeteners (artificial LCS, low-caloric bulk sweeteners, and rare sugars) and their effects on several metabolic parameters.

1.3.1 Artificial Low-Caloric Sweeteners

To reduce the high in sugar consumption, more and more food products containing artificial LCS are being developed. Artificial LCS are much sweeter than sucrose and contain few or no calories but do not possess nutritive value [228, 229]. Seven LCS are approved by the US Food and Drug Administration (FDA) and marked as Generally Recognized As Safe (GRAS):

acesulfame-K, aspartame, advantame, neotame, saccharin, sucralose and stevia (the only naturally occurring LCS) [229-232]. Sucralose, aspartame, acesulfame-K and saccharin are the most widely used artificial LCS with sucralose becoming the most popular one [226].

Despite the promising purpose of artificial LCS, there is conflicting data, discussing whether artificial LCS consumption helps to reduce sugar intake, thus improving energy balance, body weight, metabolic diseases or cardiovascular risk factors [35, 228, 230-232]. Observational studies and results from prospective cohort studies associated artificial LCS consumption with the possibility of long-term harm with an increased risk of obesity, T2DM, metabolic syndrome, cardiovascular disease and mortality [233-237]. The detailed mechanisms of how artificial LCS increase the risk for T2DM and metabolic syndrome are still not fully understood and highly disputed, however, central mechanisms might be the influence on glucose homeostasis as well as alterations of the gut microbiota [230, 238]. However, the study population often consisted of risk groups for metabolic diseases or individuals which already suffer from these chronic diseases and/or the results might be derived from reverse causation [231, 232, 237]. Therefore, the data is difficult to interpret and further research is required. On the other hand, meta-analyses of randomized-controlled trials (RCTs) and prospective cohort studies suggest that artificial LCS probably have a neutral effect, i.e. neither gain nor loss, on long-term body weight regulation [233]. Notably, artificial LCS are metabolized and absorbed differently from each other due to different chemical properties and therefore might evoke different effects [233].

Given that artificial LCS lack nutritive value and are not able to induce the secretion of GI hormones [28, 104, 188], the assumption may drive that they lead to increased energy intake. Several reviews and meta-analyses investigated the effects of replacing sugars with artificial LCS on energy intake [233, 234, 237, 239]. Pang et al. [233] showed that in several acute and long-term RCTs, energy intake was decreased when sugar was replaced by artificial LCS. It was, however, not clear if the calories from sugars were considered in the energy intake or not. Lee et al. [239] investigated the effects of preloads sweetened with artificial LCS compared to caloric sugars on subsequent *ad libitum* energy intake. They found that the *ad libitum* energy intake was increased in response to artificially sweetened preloads. However, taking the calories from the caloric sugars into account, total energy intake was around 130 kcal lower in response to preloads sweetened with artificial LCS. Moreover, the recent report from the WHO showed that short-term artificial LCS intake reduced body weight and body mass index (BMI) in adults

(assessed in RCTs with low certainty of evidence) which possibly results from a decreased energy intake when sugars are replaced by artificial LCS. There was no difference in response to artificial LCS compared with water on body weight or energy intake. [14]

The current research data about artificial LCS is controversial. There is a lack of well-designed long-term intervention trials in humans which study the physiological effects of individual artificial LCS. So far, the suitability of artificial LCS as sugar replacer should be viewed critically. Therefore, research is currently being conducted into alternative sweeteners like low-caloric bulk sweeteners or rare sugars.

Sucralose was chosen as artificial LCS in this thesis because of its widespread use in the food industry and is described in more detail below.

1.3.1.1 Sucralose

Sucralose, 1',4',6'-trichloro-1',4,6'-trideoxygalactosucrose, is a disaccharide with the formula $C_{12}H_{19}Cl_3O_8$ made from sucrose by a chemical process exchanging the three hydroxyl groups with three chloride atoms (**Figure 4**). Sucralose is a white crystalline powder with a relative sweetness of 600 times that of sucrose, which explains its widespread use in the food industry [240, 241].

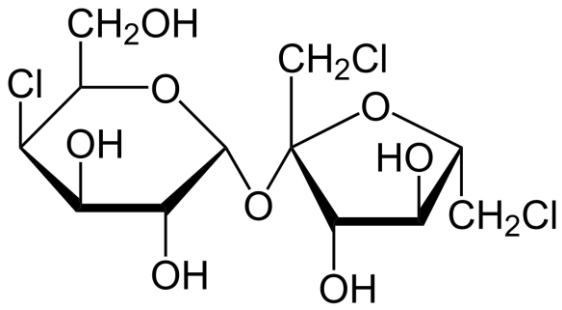

Figure 4 Chemical structure of sucralose.

The following information is available on the absorption, metabolization, and excretion of sucralose: peak plasma concentration was reached within 1.5 to 3 hours upon oral administration of sucralose, with only 15% of the total dose being absorbed [242]. Within five days, approximately 78.3% of sucralose was excreted in feces and 14.5% in urine. In the feces, only unchanged sucralose was found, whereas in the urine two minor metabolites identified as

glucuronide conjugates of sucralose were detected (around 2% of the total dose) [242]. However, glucuronidation is a metabolic process in the kidney for excretion. In summary, sucralose is poorly absorbed and not digested or metabolized for energy, thus delivers no calories.

1.3.1.1.1 Gastric Emptying

In healthy participants, intragastric administration of different doses of sucralose (80 mg or 800 mg dissolved in normal saline) did not slow down gastric emptying rates compared to sucrose [188]. Wu et al. [243] found a similar result in response to different oral preloads prior to a mashed potato meal. The gastric emptying rates were slower after the preloads containing 3-O-Methyl-D-glucose (3-OMG) and a tagatose/isomalt mixture compared to sucralose [243]. In another study, the gastric emptying rates of solutions containing different doses of sucralose were similar compared to water only [244]. Taken together, these studies show that sucralose does not slow down gastric emptying rates.

1.3.1.1.2 Gastrointestinal Hormone Secretion

In vitro studies discovered that sucralose has the potential to stimulate the secretion of GLP-1 and GIP via the G-protein coupled sweet taste-receptor [245, 246], whereas this is not the case in healthy humans [28]. In 2017 and 2020, Magnuson et al. [240] and Ahmad et al. [241], respectively, extensively reviewed the effects of sucralose administration on GI hormone release (mainly GLP-1 and GIP, but also ghrelin) on fasting levels as well as following the ingestion of a carbohydrate bolus (oral glucose tolerance test (OGTT), maltodextrin, or glucose infusion). They summarized that numerous studies found no acute effect of sucralose intake prior to or in combination with other carbohydrates on GI hormone concentrations in healthy participants [240, 241]. Three studies reported higher GLP-1 concentrations or GLP-1 AUC in response to sucralose compared to placebo (either water, water in combination with carbohydrates, or a placebo capsule) [244, 247, 248]. However, these observed effects are more likely related to the variations of experimental conditions, the route of administration (e.g. capsules), the previous and current exposure to sucralose, and other possible factors that might influence the secretion of GLP-1. In addition to healthy participants, the effect of sucralose (prior to a meal or OGTT) was also studied in patients with obesity, prediabetes or T2DM.

There were no effects on GI hormone secretion in acute clinical studies [240, 241]. More recent human studies reported similar results: a daily sucralose intake over two weeks was not associated with changes in GI hormone outcomes [249]. Yunker et al. [194] reported that sucralose administered in isolation and in a fasted state did not induce the secretion of GLP-1, acyl-ghrelin, PYY, or leptin. Although there are some studies that report GLP-1 release in response to sucralose, the majority of evidence from human trials shows that sucralose is not able to induce the secretion of GI hormones.

1.3.1.1.3 Energy Intake and Appetite-Related Sensations

The question is raised repeatedly whether sweet taste paired with the absence of calories (e.g. sucralose) and GI satiation hormone release, does increase appetite and motivate people to eat (more). Several studies assessed the effect of sucralose consumption on subsequent energy intake. Akhavan et al. [204] found that the energy intake during an *ad libitum* test meal was around 100 kcal higher (although not significant) after the sucralose preload compared to three different sugar preloads. However, the total energy intake containing the calories of the three sugar preloads was higher compared to the sucralose preload. Ford et al. [250] found no difference in energy intake and appetite ratings (desire to eat, hunger and prospective food consumption) at the buffet meal two hours after the administration of sucralose compared to water. Conducting two comparable crossover and parallel-groups studies, assessing the effects of a sucrose versus sucralose preload on energy intake, Gadah et al. [251] found that sucrose reduced subsequent energy intake compared to sucralose with no differences in appetite-related sensations. In the parallel-groups study, they also included a water arm, but the energy intake did not differ between sucralose and water [251]. A similar conclusion was drawn in the study by Woodend and Anderson [252]. Steinert et al. [28] observed that intragastric administration of artificial LCS such as sucralose increased ratings of satiety and fullness and reduced hunger to an amount that was intermediate between glucose and fructose and the water control. However, none of the results were statistically significant. Moreover, in a study conducted in children and adolescents, the glucose preload decreased subsequent energy intake compared to sucralose and subjective appetite feelings were increasing over time but were higher in response to sucralose [253]. Comparing three test foods sweetened with either 50 g sucrose, 120 mg sucralose, or 120 mg sucralose but matched in carbohydrate with 50 g maltodextrin, the

sucralose only test food resulted in a higher energy intake when food intake was recorded for the remaining day. Thus, the lower energy content in the test food was eventually fully compensated [254]. Higgins and Mattes [255] observed a decreased total energy intake in response to a daily sucralose drink over 12 weeks compared to a sucrose drink in adults with overweight or obesity. Here, the difference in energy intake was attributed to the calories of the sucrose drink, and when these calories were removed the effect was not statistically significant anymore. The study by Yunker et al. [194] reported that the participants consumed more total calories during the *ad libitum* buffet meal in response to the sucralose preload compared to the sucrose one, but with no total compensation for the sucrose preload. Interestingly, they found an interaction effect between sex and preload on total energy intake during the buffet meal. Female participants consumed more total calories after the intake of the sucralose preload compared with sucrose, whereas the total caloric intake did not differ for male participants [194].

Overall, there seems to be an increase in total energy intake in response to sucralose compared to sucrose, with a tendency to fully compensate the zero calories provided by sucralose. The energy intake compared to water seems to be similar.

1.3.1.1.4 Glycemic Control

From early on, sucralose was intended to be used as a sugar alternative for patients with both types of diabetes. Therefore, a lot of human intervention studies investigated the effects of acute and repeated sucralose consumption on glycemic outcomes in different cohorts. As reviewed by Magnuson et al. [240], repeated daily sucralose intake had neither an effect on glucose homeostasis (fasting and post-prandial glucose, C-peptide, and insulin) nor on glycated hemoglobin (HbA1c) in healthy participants as well as in participants with T2DM. The same result was observed with an acute administration of sucralose in absence of glucose or sucrose in healthy participants. Assessing the effects of sucralose following the ingestion of carbohydrates led to mixed results. Most of the studies demonstrated that the intake of sucralose prior to or in combination with other carbohydrates had no effect on glycemic outcomes in normoglycemic or hyperglycemic individuals [240, 241]. According to Temizkan et al. [247], ingestion of 24 mg sucralose before an OGTT led to lower blood glucose concentrations compared to water in healthy humans. However, no effect was observed on insulin and

C- peptide concentrations and additionally, the total AUCs were similar for all parameters between sucralose and water in patients with T2DM [247]. The results from Romo-Romo et al. [256], on the other hand, suggest that regular consumption (14 days) of 36 mg sucralose with addition of 958 mg glucose and 30 mg maltodextrin led to insulin resistance. This is supported by findings from Lertirt et al. [248], who found that insulin sensitivity decreased after consuming capsules containing 200 mg sucralose during four weeks compared to empty capsules. A more recent study showed that short-term consumption of sucralose (60 mg) in combination with carbohydrates increased glucose and insulin concentrations and decreased insulin sensitivity [257]. This setting is closer to real-life conditions, where artificial-sweetened beverages are often consumed together with a meal. Nevertheless, insulin sensitivity was not altered by sucralose or carbohydrate consumption alone [257]. In line with this observation were the glycemic outcomes in the study by Suez et al. [238]. Sucralose (102 mg) bulked with glucose, but not glucose alone, resulted in impaired glycemic responses, whereas elevated plasma insulin was only observed in participants supplemented with glucose alone [238]. Therefore, further studies are needed to investigate whether sucralose alone, but more importantly, in combination with other carbohydrates, affects glucose homeostasis. Future studies should also take into account if participants consume a carbohydrate-rich or restrictive diet as this might influence the glycemic outcomes.

1.3.1.1.5 Blood Lipids, Uric Acid, and hsCRP

Administration of sucralose in animal experiments showed mixed results in terms of blood lipids. While sucralose intake improved the lipid profile compared to sucrose in a study in non- diabetic mice [258], another study found an increase in total cholesterol and LDL-cholesterol but a decrease in triglycerides in response to sucralose, with no effect on uric acid concentrations [259]. Moreover, long-term intake of sucralose (18 weeks) increased total cholesterol, LDL-cholesterol and triglyceride levels compared to water in adult male rats [260].

In human studies, on the other hand, acute or chronic sucralose administration did not lead to differences in lipid metabolism (LDL, HDL, cholesterol, triglycerides) or an increase in uric acid concentrations [261-263]. More recently, addition of sucralose to a citrus-maqui juice increased HDL-cholesterol with no changes in LDL and triglycerides concentrations in participants with overweight [264]. Data on the effects on hsCRP are currently not available.

1.3.1.1.6 Gastrointestinal Tolerance

Brown et al. [265] found no effect in response to 6 g sucralose on GI well-being in healthy females. The same conclusion was drawn by Stellingwerff et al. [266] where sucralose did not lead to any GI symptoms. Although not specifically performed to asses GI symptoms, sucralose was well tolerated by healthy participants in single doses up to 10 mg/kg/day and repeated doses increasing to 5 mg/kg/day for 13 weeks [261]. The acceptable daily intake (ADI) for sucralose for the entire population is 5 mg/kg.

1.3.1.1.7 Other Health Aspects

Numerous toxicological and pharmacokinetic studies have been performed to evaluate the safety of sucralose [240]. Sucralose seems to be safe with no effects on survival, carcinogenicity, reproductive performance, fertility, neuronal development and mutagenic potential [240]. Moreover, sucralose has an anticariogenic potential because it suppressed planktonic growth, acid production, and biofilm formation of several species in the oral microbiome [267].

Nowadays, studies on the gut microbiome are of particular interest because sweeteners can influence our microbiome positively or negatively. Exposure to certain artificial LCS has shown to cause dysbiosis in the microbiome [233, 241]. Several animal experiments reported that sucralose consumption negatively affected the microbiome [231, 268-270]. However, contradictory results regarding the effects of sucralose on the gut microbiota composition have been found in human studies. According to Thomson et al. [271] and Ahmad et al. [272], sucralose ingestion over a period of 7 and 14 days, respectively, had no effect on the gut microbiome. In contrast, Méndez-García et al. [273] reported that a 10-week daily sucralose intake led to gut dysbiosis associated with altered glucose and insulin concentrations during an OGTT. This is in line with a more recent study by Suez et al. [238] that assessed the effects of several artificial LCS including sucralose on human metabolic health and the gut microbiome for two weeks. They found that sucralose supplementation compared to the non-supplemented control group impaired glycemic responses in healthy participants that were strictly non-artificial LCS consumers. Moreover, they showed that the intestinal gut microbiome was altered in response to sucralose. By performing fecal transplantation of human microbiomes into germ-

free mice, the authors could demonstrate that these impacts were causally associated with an elevated glycemic response [238]. Several of these contradictory results in human studies might potentially be explained by the way in which sucralose was administered, either as commercial sachets including glucose as fillers (as in the study by Suez et al.) or in isolation as shown by Thomas et al., Ahmad et al., or Méndez-García et al. Moreover, the duration of the artificial LCS exposure (acute vs. chronic) might play a role as well.

In summary, the majority of the evidence indicates that sucralose is not able to induce the release of GI satiation hormones and to slow down gastric emptying rates, which in turn has an impact on subsequent energy intake. Furthermore, the effects of sucralose on glucose homeostasis are controversial. The combination of sucralose and carbohydrates could have a negative effect on insulin sensitivity and the gut microbiome. However, more randomized controlled intervention studies in humans investigating the effects of sucralose over a longer period of time are needed to draw a definitive conclusion.

1.3.2 Low-Caloric Bulk Sweeteners

In recent years, low-caloric bulk sweeteners, also called “sugar alcohols” or “polyols” attracted attention as sugar alternatives. They are defined as “saccharide derivatives in which a ketone or aldehyde group is replaced by a hydroxyl group” [274]. Common polyols are sorbitol, mannitol, xylitol, erythritol, maltitol, lactitol, and isomalt. They naturally occur in small amounts in fruits and vegetables and are commercially produced from other carbohydrates such as glucose, sucrose, and starch via hydrogenation processes [274].

Low-caloric bulk sweeteners are extensively used in the food industry to replace sugar due to their low to non-caloric content (0−2.4 g/kcal). Moreover, several polyols have a relative sweetness similar to sucrose (e.g. xylitol 100%, maltitol 90%) [274]. This allows a volume-to-volume replacement of sugar, whereas for example sucralose, which has a much higher sweetness, needs fillers. Apart from the positive effects on oral and dental care, sugar alcohols offer a variety of health benefits including, low glycemic, low insulinemic, anti-hyperglycemic, anti-diabetic and anti-obesogenic effects [275]. A single oral dose of 25 g of xylitol lowered plasma glucose and insulin concentrations compared to glucose in healthy men [276]. Similar effects have been observed in response to sorbitol, mannitol, lactitol and isomalt in humans

[274]. A more recent study found a weak dose-dependent increase in blood glucose and insulin concentrations in response to 7, 17, and 35 g of xylitol compared to tap water [277]. Since the focus of human studies has been mainly on the investigation of the glycemic role and GI tolerance in response to low-caloric bulk sweeteners, there have not been many studies that assessed the effects on appetite control. Wölnerhanssen et al. [103] showed that xylitol stimulated the secretion of CCK and GLP-1 and induced a significant retardation in gastric emptying compared to tap water. Furthermore, Shafer et al. [278] evaluated the effects of various doses of xylitol as part of a solid-food complex meal on energy intake and found that the highest dose (25 g) resulted in a significant reduction in energy intake compared to water. Another study used different yogurt preloads with either xylitol, polydextrose, the combination of both, or sucrose (control) and assessed energy intake and appetite-related sensations [279]. They found that the preload containing xylitol suppressed energy intake slightly, but the difference was not statistically significant. However, they observed an increase in fullness compared to the yogurt with sucrose [279]. For erythritol, Overduin et al. [280] recently conducted a study on energy intake and found no difference between sucrose and erythritol. The study is described in more detail below.

Given the increasing use of low-caloric bulk sweeteners – especially erythritol and xylitol – questions arise regarding their metabolic effects in humans. This thesis focuses on erythritol with more details described below.

1.3.2.1 Erythritol

Erythritol, meso-1,2,3,4-butantetrol, is a monosaccharide polyol with the molecular formula of $C_4H_{10}O_4$ (**Figure 5**) [274, 281, 282]. Erythritol is present in nature in some mushrooms, fruits, vegetables as well as in fermented foods and drinks and is produced from glucose with yeast-like fungi [283, 284]. Polyol metabolism requires little or no insulin, with erythritol in particular having a very low insulinemic index, as well as a glycemic index of zero [274]. The latter is a stand-alone property of erythritol among the polyols and allows its usage as sweetener in specialized meals for patients with obesity or T2DM. Moreover, erythritol is the only polyol that is non-caloric and has a sweetness of 60-70%, according to some references even up to 80% relative to sucrose [274].

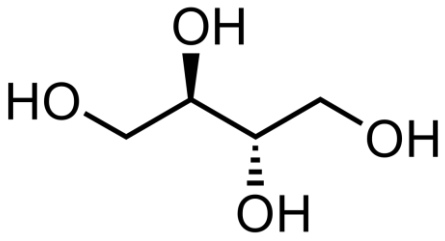

Figure 5 Chemical structure of erythritol.

Monosaccharide sugar alcohols such as erythritol are partly absorbed through passive diffusion along a concentration gradient in the small intestine [283]. Based on the studies by Bornet et al. in 1996 [214, 285], plasma and urine erythritol concentrations increased within two hours in proportion to the amount consumed. Depending on the ingested dose of erythritol, the total urinary excretion reached 60-78% after 22-24 hours and less than 20% remained unabsorbed [214, 285]. Munro et al. [283] summarized that erythritol is rapidly absorbed from the small intestine (> 90%) and quantitatively excreted unchanged with the urine. Whether the remaining 10% of erythritol are subject to colonic fermentation or excreted unchanged in the feces is unknown in humans. However, in an *in vitro* setting, it was shown that erythritol resisted colonic fermentation within 24 hours [286]. In 2019, Hootman et al. [287] proposed another pathway where 5-10% of consumed erythritol is oxidized into the sugar erythrose, which is in turn oxidized to erythronate. We recently showed that erythritol is absorbed in a dose-dependent and saturable manner in humans [288]. Moreover, we confirmed that erythritol is metabolized dose-dependently to erythronate but to a smaller extent (< 1%) than proposed by Hootman and colleagues [288].

1.3.2.1.1 Gastric Emptying

In an *in vivo* absorption study, Chukwuma et al. [289] demonstrated that erythritol delayed gastric emptying rates in normal and diabetic rats. Wölnerhanssen et al. [103] showed that a single bolus of 75 g of erythritol delayed gastric emptying rates in lean participants and in participants with obesity but without T2DM. In a subsequent dose-ranging study, the gastric emptying rates of the test solutions containing erythritol were slower compared to placebo (tap water) in normal-weight, healthy participants [290].

1.3.2.1.2 Gastrointestinal Hormone Secretion

Several human metabolic studies demonstrate that erythritol affects GI hormone secretion. We have recently shown that intragastric administration of different doses of erythritol induced an increase in the release of the GI hormones CCK, GLP-1, and PYY with no effect on GIP and motilin [103, 290, 291]. Overduin et al. [280] have reported that partial replacement of sucrose by erythritol in a test breakfast resulted in a similar secretion of GLP-1 and PYY compared to the sucrose-only test breakfast. Furthermore, Sorrentino et al. [292] observed a reduction in serum ghrelin concentrations at time points 20, 30, and 45 minutes post oral erythritol consumption (50 g) compared to the artificial LCS aspartame. They argue that the reduction in ghrelin was due to the high osmolarity of the erythritol beverage [292]. However, in the dose-ranging study by Wölnerhanssen et al. [290], the solution with the lowest erythritol concentration (10 g) was close to iso-osmolar and nonetheless clearly stimulated the release of CCK and GLP-1. Therefore, the underlying mechanisms of the GI satiation hormone secretion are currently unknown.

1.3.2.1.3 Energy Intake and Appetite-Related Sensations

The effect of erythritol consumption on energy intake is poorly understood until now. Overduin et al. [280] partially replaced sucrose by erythritol in a test breakfast which served as a preload in the study. Four hours after the test breakfast, the participants were served an *ad libitum* test meal and energy intake was assessed. No differences were found in postprandial hunger and fullness scores in the 4-hour period between the test breakfast and the *ad libitum* test meal. Moreover, the authors observed similar levels of energy intake during the *ad libitum* test, showing that, even though erythritol has zero calories, the intake was the same as for sucrose [280]. Wölnerhanssen et al. [103] assessed appetite-related sensations as well and found no statistically significant differences between the treatments erythritol, xylitol, glucose, and tap water. Given that the test breakfast used in the study by Overduin et al. was a mix of different nutrients and only partial replacement of sucrose, the effects of pure oral erythritol on subsequent energy intake are not known yet.

1.3.2.1.4 Glycemic Control

In 1994, Noda et al. [293] demonstrated that acute oral administration of erythritol (0.3 g/kg BW) in five healthy participants had no effect on serum glucose and insulin concentrations. A similar result was shown in a study with patients with T2DM (20 g erythritol) [294]. In addition, administration of erythritol for 14 days to 11 patients with T2DM decreased HbA1c levels over the time period [294]. In Korean participants with glucose intolerance, consumption of erythritol over two weeks did not affect glucose homeostasis [295]. More recently, the studies by Wölnerhanssen et al. [103, 290] assessed the effects of different single bolus doses on glycemic outcomes. In healthy participants, doses ranging from 10 g to 75 g of erythritol had no effect on glucose and insulin concentrations [103, 290]. Overduin et al. [280] assessed post-prandial glucose and insulin concentrations in response to a test breakfast where sucrose was partially replaced by erythritol. Compared to the sucrose test breakfast, the erythritol breakfast caused a smaller excursion in glucose and insulin concentrations [280]. Furthermore, the combination of different sugars including erythritol in a beverage resulted in lower post-prandial serum glucose concentrations in participants with T2DM compared to a glucose or sucrose beverage [296]. Regarding animal studies, erythritol reduces post-prandial glucose concentrations via inhibition of α-glucosidase in diabetic mice [297]. Moreover, Chukwuma et al. [289] reported from their experiments, that administering erythritol to diabetic rats reduced small intestinal glucose absorption. However, in a recent human study in participants with obesity, chronic intake of erythritol over five weeks did not affect intestinal glucose absorption [298].

1.3.2.1.5 Blood Lipids, Uric Acid, and hsCRP

Only a few studies have investigated the effects of erythritol on blood lipids and uric acid. Oral administration of 0.3 g/kg BW of erythritol to five healthy male participants had no effect on total serum cholesterol, triglycerides, and free fatty acids [293]. In a study by Ishikawa et al. [294], a solution containing 20 g of erythritol increased free fatty acids and 3-hydroxybutyric acid levels in 5 patients with T2DM. However, the levels decreased after ingestion of a meal. The increase was probably due to no energy supply in response to erythritol, placing the participants in a state of hunger [294]. In a more recent study, 50 g of erythritol had no effect on blood lipids (total cholesterol, triglyceride, LDL- and HDL cholesterol) and uric acid in 12

healthy participants [290]. Data on the effects of hsCRP in response to erythritol are currently lacking.

1.3.2.1.6 Gastrointestinal Tolerance

In 1996, Tetzloff et al. [212] studied the GI tolerance of repeated oral doses of erythritol (up to 1g/kg BW) in 12 healthy, male participants over the course of seven days. Erythritol was mixed into different foods and was consumed in five portions throughout the day. They found no significant GI side effects and concluded that doses of 1g/kg BW of erythritol are well tolerated by humans [212]. Storey et al. [213] investigated the effect of 20 g, 35 g, or 50 g erythritol (given as a single oral bolus) on GI tolerance in seventy healthy participants aged 18 to 24 years. Only the highest dose led to GI symptoms like nausea or borborygmi [213]. In 2015, a study with 184 children aged four to six years, showed that a rapid intake of erythritol up to 15 g in a beverage was well tolerated; suggesting that children are not more sensitive to GI symptoms in response to erythritol than adults on a g/kg BW basis [299]. In the first study by Wölnerhanssen et al. [103], the dose of 75 g of erythritol led to GI symptoms such as diarrhea in about 50% of the participants. In the more recent study, however, the doses up to 50 g were well tolerated [290]. In general, erythritol is well tolerated and the GI symptoms observed mainly occurred in trials with a rapid oral administration or via a nasogastric tube on an empty stomach which probably causes a great strain to the GI tract. Mäkinen et al. [300] summarized, that a safe dose of erythritol for a single bolus lies around 0.6−0.8 g/kg BW. The GI tolerance is high in individuals that are used to erythritol consumption [212]. Moreover, a slower ingestion or erythritol mixed with other nutrients seems to limit GI symptoms as well [212]. No ADI limits were established for erythritol, as there are no health concerns for its use.

1.3.2.1.7 Other Health Aspects

Numerous toxicological studies have been performed to evaluate the safety of erythritol. Acute toxicity studies have demonstrated that erythritol has no toxic effects after oral administration up to 18 g/kg BW. Furthermore, sub chronic studies have shown that erythritol has no effect on survival, carcinogenicity, reproductive performance, fertility and has no mutagenic potential [274, 282, 301]. Based on these toxicological safety data, erythritol is GRAS for its intended use in food by the US FDA [274, 301]. To date, erythritol is approved in more than 60 countries,

including Europe, the USA, Canada, Australia, and Japan [302]. Apart from the well-studied effects of erythritol being non-cariogenic and improving oral health [303], the non-caloric bulk sweetener got a lot of attention due to its anti-hyperglycemic and anti-diabetic potential. Flint et al. [304] reported that patients with T2DM profit from a daily intake of erythritol, as this natural non-caloric bulk sweetener reduced arterial stiffness and improved endothelial function. The pathogenesis of CVD in individuals with T2DM is partially attributed to endothelial dysfunction, which also serves as a predictor of cardiovascular events [305]. Based on the results by Flint et al., we recently performed a study in 42 participants with obesity but otherwise healthy. Consuming 36 g of erythritol three times per day (compared to 24 g of xylitol or no substance (control group)) over a period of five weeks had no effect on vascular function, abdominal fat, glucose tolerance, uric acid, hepatic enzymes, and creatinine [Bordier, Teysseire et al. unpublished].

Paradoxically, several metabolomic profiling studies have shown a positive association between circulating erythritol concentrations and NCDs, such as obesity, T2DM, or coronary heart disease [306]. A recent study conducted by Witkowski et al. [307] has reported similar results. In this study, circulating levels of multiple polyols, particularly erythritol, were associated with an increased risk of major adverse cardiovascular events such as death, nonfatal myocardial infarction, or stroke in patients undergoing cardiac risk assessment. This association was confirmed in two independent validation cohorts comprising patients with a high prevalence of CVD, including hypertension, obesity, and T2DM. The study also found that erythritol enhanced platelet reactivity and thrombosis formation in *in vitro* and *in vivo* studies, respectively. Lastly, a pilot intervention study (n = 8) showed that erythritol ingestion induced marked and sustained increases (over two days) in plasma erythritol concentrations in healthy participants. [307] However, the study had several limitations: a direct causal relationship between the consumption of erythritol and an increased risk of cardiovascular complications could not be shown, as dietary intake was not assessed in any of the cohorts studied. Witkowski et al. were thus unable to demonstrate a distinction between the endogenous production and the intake of erythritol from food. It is therefore unclear whether the participants in the study consumed erythritol at all, and it also raises the question of whether patients with an increased cardiovascular risk produce more erythritol from consumed sugar.

Diets rich in sugar, which are consumed at much higher levels than erythritol, can influence endogenous erythritol production and may associate with CVD [306]. Hyper- or impaired glycemia may underlie the elevated circulating erythritol observed in observational studies, as the pentose phosphate pathway (PPP) runs parallel to glycolysis, and many glycolytic/gluconeogenetic intermediate metabolites feed into it. The PPP is reported to be dysregulated in patients with obesity, insulin resistance, and T2DM [308, 309]. Moreover, this pathway modulates insulin sensitivity and obesity-induced inflammation in multiple tissues [308, 310]. Therefore, elevated erythritol concentrations may be a result of endogenous production via up-regulated PPP, which could have implications for hyper- or impaired glycemia [306]. However, whether high erythritol concentrations contribute to the pathogenesis of CVDs or serve as a marker of PPP dysregulation remains to be investigated.

In terms of the gut microbiome, there is currently little evidence about the effects of erythritol [311]. Initial *in vitro* studies show that erythritol increases the production of short-chain fatty acids (especially butyric acid), suggesting that they may have a positive impact on the human gut microbiome [312]. It is well known that short-chain fatty acids, especially butyric acid, can exert beneficial effects on glycemic control and blood lipids [313]. Further studies, especially *in vivo* human studies, are needed to determine the effects of regular consumption of erythritol on the gut microbiome.

In summary, erythritol displays several beneficial metabolic properties as a sugar alternative. The non-caloric bulk-sweetener induces the secretion of GI satiation hormones without affecting glucose and insulin concentrations. Moreover, erythritol slows down gastric emptying rates which potentially affects subsequent energy intake in humans. To explore the underlying mechanism of GI hormone secretion in response to erythritol and the effect of pure erythritol on subsequent energy intake, further studies are required. The current thesis will address these questions. Regarding the cardiovascular events in response to erythritol, more long-term safety studies are needed to confirm its role as predictor of metabolic risks.

1.3.3 Rare Sugars

A relatively new class of sugar substitutes are “rare sugars”. The International Society of Rare Sugars defines them as “monosaccharides and their derivates that are present in limited quantities in nature” [314]. There are over 40 different types of rare sugars with only small differences in their chemical structure compared to conventional mono-and disaccharides [315]. The most common representatives are D-allulose (D-psicose), tagatose, isomaltulose (palatinose), L-arabinose and trehalose. For example, D-allulose has a different arrangement of a hydrogen atom and hydroxyl group, compared to fructose. D-allulose differs from fructose at the third carbon atom (**Figure 6**).

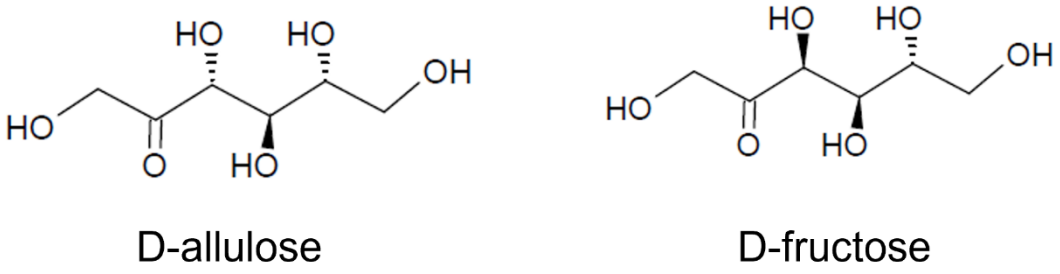

Figure 6 Chemical structures of D-allulose and D-fructose.

Although these rare sugars occur naturally in a variety of foods (including honey, vegetables, fruits, and wheat), they are mostly produced by genetically engineered enzymes with conventional sugars such as glucose as a start substrate [316-318]. This process allows the production of rare sugars in high quantities to study the health effects as well as the estimation of their caloric content in animals and humans compared to conventional sugars [319, 320]. Using rare sugars as alternative sweeteners has shown several beneficial physiological effects in *in vitro* and *in vivo* animal experiments as well as in human trials [315, 321]. For instance, in a human intervention study, which included participants with T2DM, a 1-year tagatose supplementation resulted in a body weight reduction of 5.1 kg and improved HDL levels compared to baseline values [322]. In another study with participants with and without T2DM, a dose of 75 g of tagatose had no effect on fasting glucose and insulin concentrations [323].

Moreover, tagatose reduced the increase in serum glucose after an OGTT, though the results were only significant in participants with T2DM [323]. There are only a few studies that investigated the effects of rare sugars on appetite or energy intake. Two animal experiments reported a reduction in energy intake in response to sorbose and D-allulose [324, 325]. In a study by Buemann et al. [326] sucrose was replaced by tagatose in a breakfast meal and resulted in reduced subsequent appetite and food intake at dinner on the same day. Furthermore, Wu et al. [243] observed, that a preload of 40 g tagatose and isomaltulose (compared to sucralose) administered prior to a test meal resulted in reduced glucose and insulin concentrations and slowed down gastric emptying rates. Similar effects on glycemic control have been observed in response to L-arabinose: L-arabinose is a potent sucrase inhibitor lowering post-prandial glycemic responses in rats and humans [327, 328]. Additionally, L-arabinose reduced waist circumference, body weight, fasting glucose, serum uric acid, and triglyceride levels in a six month intervention study in humans with metabolic syndrome [329].

Overall, rare sugars offer many beneficial physiological effects, ranging from weight reduction to improved glycemic responses. However, hardly any of these rare sugars are included in European Food Safety Authority (EFSA) claims because they lack high-quality randomized clinical trials with larger sample sizes and longer duration [315]. Among the rare sugars, D-allulose is the most extensively studied in humans and has the potential to be the most promising sugar replacement currently available.

1.3.3.1 D-allulose

D-allulose is a monosaccharide (C-3 epimer of fructose) with a molecular formula of $C_6H_{12}O_6$ and has been used as an alternative sweetener in Asia (Japan, China, and Korea) for more than a decade. D-allulose has about 70% of the sweetness of sucrose, but almost zero calories (0.2 kcal/g) and occurs naturally in small quantities in wheat and fruits (e.g., raisins, dried figs) [321]. Due to the very low caloric content of D-allulose and because it does not promote dental caries, it can be excluded from “Total sugars” and “Added sugars” on the Nutrition and Supplemental Facts label in the USA [330]. D-allulose can be synthesized from fructose by enzymatic epimerization [316]. D-allulose competes with fructose for binding to the glucose transporter GLUT5 and is further transported into the hepatic portal vein via GLUT2 [331, 332]. As a result, D-allulose is not completely absorbed (depending on the amounts ingested), but

reaches the large intestine. Both the human metabolism and the human microbiome metabolize D-allulose only slightly [333]. Therefore, D-allulose enters the circulation largely unchanged via the liver and is excreted as urine via the kidneys. In rats, the highest D-allulose concentrations after oral intake are found in the intestine, liver, kidney, and bladder [334]. No comparable distribution data is available for humans. However, in humans, administration of D-allulose up to 0.17 g/kg body weight (approx. 12 g at 70 kg) results in an excretion of about 80% unchanged in the urine within 48 hours. If higher doses are administered, the proportion excreted after 48 hours drops below 80% [333].

1.3.3.1.1 Gastric Emptying

It is known that hexose sugars such as glucose, fructose or the rare sugar tagatose slow down gastric emptying rates [335]. No data is, to best of our knowledge, currently available in animal experiments or human trials that investigated the effect of D-allulose on gastric emptying rates.

1.3.3.1.2 Gastrointestinal Hormone Secretion

To date, only animal studies have reported the effects of D-allulose on GI hormones. Two recent studies performed by the same research group investigated the effects of D-allulose on orexigenic (NPY / AgRP) and anorexigenic (cocaine- and amphetamine-regulated transcript (CART) / pro-opiomelanocortin (POMC)) neurons in the hypothalamic ARC in mice [336, 337]. Icv injection of D-allulose decreased calcium concentrations in ghrelin-responsive and NPY neurons [336] but increased calcium concentrations dose-dependently in the ARC neurons that respond to GLP-1 and POMC neurons [337]. The mechanisms underlying the action of D-allulose to inhibit and activate these neurons in the ARC remain unknown. In another mice study, D-allulose (1 and 3 g/kg) stimulated the release of active GLP-1 in a dose-dependent manner. However, total GIP, CCK, and PYY concentrations did not change in response to D-allulose [324]. The mechanism by which D-allulose selectively releases GLP-1 (and not PYY) remains to be investigated. Hayakawa et al. [338] have shown a dose-dependent release of GLP-1 in response to D-allulose in rats (0.5−2.0 g/kg body weight). In this study, GLP-1 secretion was higher after D-allulose compared to dextrin, fructose, or glucose. As a possible mechanism, the authors suggest that the secretion might be stimulated by GLUT5 because D-allulose-induced GLP-1 secretion was inhibited in the presence of xanthohumol (a GLUT5

inhibitor), whereas the SGLT-1 inhibitor phlorizin and the sweet taste receptor antagonist lactisole had no effect [338].

Data in human trials regarding the secretion of GI hormones as well as the underlying mechanisms are lacking.

1.3.3.1.3 Energy Intake and Appetite-Related Sensations

Oral administration of D-allulose at 1 and 3 g/kg significantly reduced energy intake in mice up to 6 hours. Cumulative energy intake at 24 hours after D-allulose administration returned to normal levels and body weight did not change as shown by Iwasaki et al. [324]. The effects of D-allulose on energy intake were attenuated or suppressed in the presence of the GLP-1 receptor antagonist, exendin$_{9\text{-}39}$ and GLP-1 receptor knock-out mice [324]. This suggests that GLP-1 receptor signaling is mandatory for the anorexigenic effect of D-allulose. The same research group also investigated the effect of D-allulose on energy intake in obese and diabetic mice. Energy intake as well as cumulative energy intake for 24 hours was suppressed in response to D-allulose without compensation for the following 24 to 48 hours, which resulted in a reduction in body weight gain [324]. Further experiments to understand the underlying mechanisms behind the suppression of energy intake in response to D-allulose indicated that the rare sugar activates afferent neural pathways notably via GLP-1 secretion, which in turn directly interacts with vagal afferent neurons [324]. Lastly, also icv-injected D-allulose decreased energy intake in mice [336, 337].

Van Opstal et al. [339] investigated brain activity and connectivity changes in response to shakes sweetened with glucose, fructose, D-allulose, or sucralose in humans. They found that D-allulose had no effect on Blood-Oxygen-Level Dependent (BOLD) signal or network connectivity and that the reporting of hunger post-ingestion correlated with the functional brain measures for the D-allulose stimuli. Unfortunately, they did not assess other appetite-related sensations such as satiety after the ingestion of the different shakes.

Animal studies show promising effects of D-allulose and its effects on energy intake. There is, however, a need for human studies investigating whether these observed effects in animal experiments are translatable to humans.

1.3.3.1.4 Glycemic Control

As mentioned earlier, rare sugars became popular sugar alternatives because of their potential to improve glycemic outcomes.

In the study by Hossain et al. [340] in Otsuka Long-Evans Tokushima fatty (OLETF) rats, 5% of D-allulose (compared to 5% glucose) resulted in a 43% smaller increase in glucose and insulin concentrations after a glucose challenge. In 2008, Iida et al. [341] showed that consumption of D-allulose (5 g and 7.5 g) prior to a maltodextrin challenge suppressed glucose concentrations dose-dependently compared with the consumption of only maltodextrin in healthy humans. Kimura et al. [342] reported from their study, that intake of 5 g of D-allulose dissolved in 150 mL of water 30 min prior a meal tolerance test, reduced plasma glucose concentrations compared to aspartame. In a randomized, controlled, crossover trial with 24 healthy participants it was shown that 5 g or 10 g D-allulose had no effect on post-prandial blood glucose in response to a 75 g OGTT in healthy humans [343]. However, when compared to the assigned control (0 g D-allulose) in a sensitivity analysis, there was a significant decrease in plasma glucose in response to 5 g D-allulose [343]. The same intervention conducted in patients with T2DM showed that 10 g of D-allulose compared to 0 g resulted in an 8% reduction of plasma glucose concentrations [344]. Furthermore, Franchi et al. [345] demonstrated that, in healthy participants, an acute intake of D-allulose together with 50 g of sucrose leads to a dose-dependent reduction in glucose and insulin concentrations compared to sucrose alone. In a long-term study (12 weeks) in 17 patients with borderline T2DM, 5 g of D-allulose (3x/day) had no effect on glucose or insulin concentrations [346]. A recent systematic review and meta-analysis of acute feeding trials has shown that D-allulose (< 30 g) significantly reduced postprandial incremental AUC glucose response by 10%, while the results of the incremental AUC of insulin were not significant [347]. The authors reported that the certainty of evidence was moderate for D-allulose and that more long-term RCTs are needed.

1.3.3.1.5 Blood Lipids, Uric Acid, and hsCRP

Another important contributor to the beneficial metabolic effects is the impact of D-allulose on blood lipids. A 4-week exposure to 5% D-allulose in Wistar rats resulted in improved blood lipid profiles compared to 5% glucose [348]. Moreover, administering 200 mg/kg body weight

of D-allulose, fructose, or glucose to diabetic mice for 28 days ameliorated liver triglycerides and cholesterol levels compared to the monosaccharides [349]. In addition, lower triglycerides levels have been observed in OLETF rats upon D-allulose administration compared to glucose [340]. A 12-week trial in patients with overweight and obesity showed no adverse effect on blood lipids [350]. In assessing the effects of D-allulose in patients with hypercholesteremia for 48 weeks, Tanaka et al. [351] showed that the intake of 5 g or 15 g of D-allulose had no effect on blood lipids or hsCRP. Moreover, a 12-week trial with a daily intake of D-allulose in tea with a standard meal did not affect uric acid concentrations [346].

1.3.3.1.6 Gastrointestinal Tolerance

Examining the GI tolerance in healthy and young adults, Han et al. [352] identified the maximum single dose of D-allulose to be 0.4 g/kg body weight (about 25 g) without side effects. Doses of more than 0.5 g/kg body weight resulted in severe diarrhea. Comparing 0.5 g/kg body weight of D-allulose to the same amount of sugar, participants reported an increase in diarrhea, abdominal distention, and abdominal pain in response to D-allulose. However, their results cannot be transferred to other populations such as patient with metabolic or GI disease as they might experience more GI symptoms in response to D-allulose ingestion [352].

1.3.3.1.7 Other Health Aspects

In 2016 and 2017 D-allulose received the GRAS status by the US FDA [321]. The approval in the European Union (EU) is still pending. The EFSA has several applications for D-allulose that are currently being evaluated. A possible reason that D-allulose has not yet been approved in Europe (including Switzerland) might be due to the question whether the use of D-allulose as a sugar substitute may pose a health risk as stated by the German Federal Institute for Risk Assessment (BfR) [353]. The reason is the increase in infections with the virulent germ *Clostridium difficile* in the USA, which was described in 2018 in relationship with the use of the rare sugar trehalose [354]. Based on the data currently available, it cannot be conclusively assessed whether similar health risks are justified with the use of D-allulose as a food ingredient.

Nevertheless, toxicological studies performed in rats and dogs showed no or no severe toxic effects in response to D-allulose [315]. Dogs receiving 4 g/kg experienced diarrhea, however, they remained active and had good appetite throughout the study [355]. A dose of 0.2 g/kg over

12 weeks caused no harmful effects in dogs [356]. Furthermore, a dose of 2000 mg/kg of D-allulose had no effect on reproductive toxicity in rats [357].

Other recent studies on metabolic effects, especially on body weight and fat mass reduction in response to D-allulose, have been studied as well. In a study with OLETF rats, substitution of 5% glucose supplementation with 5% of D-allulose in drinking water for 13 weeks resulted in a significantly reduced body weight and abdominal fat mass. Moreover, D-allulose reduced fat accumulation and stimulated glycogen synthesis via translocation of glucokinase from the nucleus to the cytoplasm in hepatocytes [340]. A 15-week supplementation with 5% of D-allulose in leptin deficient mice significantly decreased body and liver weight by 20% and 15%, respectively [358]. Human studies reported similar effects. Han et al. [350] found that D-allulose (compared to sucralose) reduced abdominal and subcutaneous fat areas in Korean participants. Moreover, a single dose of 5 g of D-allulose compared to 10 mg of aspartame enhanced post-prandial fat oxidation by 9% in healthy humans, indicating that fat oxidation might be a contributor to the weight-reducing effects of D-allulose [342]. In addition, there is currently insufficient evidence to determine the effects of regular consumption of D-allulose on the gut microbiome [359].

In summary, short- and long-term animal and human studies show that the consumption of D-allulose may lead to several metabolic improvements, making it a promising sugar alternative. However, to date, only animal studies have investigated the secretion of GI hormones and the underlying mechanisms in response to D-allulose. In addition, more safety parameters are warranted for D-allulose to be approved in the EU. The PolyAlluLac study (see 4.1 and 4.2) of the present thesis focuses on these topics.

Given the high demand on sugar alternatives due to public health reasons and the conflicting results of artificial LCS, more studies on low-caloric bulk sweeteners and rare sugars are needed. Initial studies of these substances show promising results. This thesis deals with erythritol and D-allulose as sugar alternatives and aimed at closing the knowledge gap of a possible mechanism underlying the GI satiation hormone secretion as well as the influence of these hormones on subsequent energy intake. The next chapter describes the aims of the thesis in detail.

Chapter 2

Aims of the Thesis

2 Aims of the Thesis

A widely adopted strategy to reduce sugar intake, as proposed by the WHO guideline, is to substitute sugar with alternative sweeteners, such as artificial LCS (e.g. sucralose), low-caloric bulk sweeteners (e.g. erythritol), or rare sugars (e.g. D-allulose). The overall aim of the present thesis was to study the effects of replacing sugar with erythritol and D-allulose in healthy humans. This overall aim was pursued in two studies with the specific aims outlined below.

2.1 First Aim

The first aim was to investigate the importance of the sweet taste receptor T1R2/T1R3 for the release of CCK, GLP-1, and PYY in response to intragastric administration of erythritol and D-allulose by assessing the effect of lactisole on these responses.

In **PolyAlluLac – Part I** (see 4.1), we used the T1R2/T1R3 antagonist lactisole to investigate whether the secretion of GI satiation hormones (CCK, GLP-1, and PYY) is mediated via T1R2/T1R3. Moreover, we aimed to study the effect of the T1R2/T1R3 blockade on gastric emptying, appetite-related sensations, and GI symptoms.

In **PolyAlluLac – Part II** (see 4.2), we studied the metabolic effects and safety aspects of acute intragastric administration of erythritol and D-allulose on glucose, insulin, ghrelin, blood lipids, uric acid, and hsCRP concentrations.

2.2 Second Aim

Knowing that erythritol provides zero calories, similar to artificial LCS, but in contrast induces the release of GI satiation hormones, we wanted to investigate whether this GI satiation hormone release affects energy intake. To this purpose, the second aim of the present thesis was to investigate the effect of oral administration of erythritol on subsequent energy intake.

In **PolyFoodIntake**, (see 4.3) we compared the effects of oral administration of erythritol to sucrose, sucralose, or tap water on energy intake during a subsequent *ad libitum* test meal. In addition, we examined the release of CCK, glucose and insulin concentrations, and appetite-related sensations in response to these substances.

Chapter 3

General Methods

3 General Methods

3.1 Conducting Clinical Studies

A clinical study investigates whether new interventions such as drugs, medical devices, procedures, or diets have an effect on human health and involve human volunteers (study participants) [360]. Numerous laws, regulations, and guidelines create a defined framework to conduct clinical studies. The rights and integrity of the study participants must be protected and unnecessary examinations avoided. The goal of these requirements is the highest possible safety as well as the best methodological quality and transparency. To this end, every study must be reviewed by an ethics committee.

Both study protocols of the present thesis (PolyAlluLac and PolyFoodIntake) were approved by the Ethics Committee of Northwestern- and central Switzerland (EKNZ). The trials were conducted in accordance with the current version of the Declaration of Helsinki, the guidelines of good clinical practice (GCP) issued by the International Council on Harmonisation (ICH), the Swiss Law and Swiss regulatory authority's requirements [361]. Both trials were categorized as risk category A studies (only minimal risks) according to the Clinical Trials Ordinance (ClinO) Article 61 [362]. All participants provided verbal and written informed consent after receiving a full explanation of the study procedures. The studies were registered at ClinicalTrials.gov.

The Declaration of Helsinki, founded in 1964, lays down the ethical principles of experimental research on humans and was developed by the World Medical Association [363]. GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting trials involving human participants. The GCP was issued by the ICH with the aim of providing a unified standard for the EU, Japan, and the United States. [364].

In 1949, the WHO and the United Nations Educational, Scientific and Cultural Organization (UNESCO) established an international, non-governmental, non-profit organization called the Council for International Organizations of Medical Sciences (CIOMS). The CIOMS has a mandate to promote public health by advising on health research and policy, including ethics, medical product development and safety. [365]

3.2 Study Participants

For both studies, healthy participants with a normal weight (BMI 19.0 – 24.9 kg/m^2), age between 18 – 55 years were recruited through advertisements at the University of Basel. Additional requirements for study inclusion were: stable body weight, normal eating habits (omnivore and no diets), no medical or drug abuse, no acute or chronic infection or illness, no illness affecting the GI tract, no regular use of products containing artificial LCS and/or low-caloric bulk sweeteners (> once per week, assessed during the pre-screening), no pregnancy and involvement in another study with an investigational drug within 30 days preceding and/or during the studies. Both studies were performed in the morning after an overnight fast. More specific in-and exclusion criteria are given within the respective projects.

3.3 Experimental Procedure

In our experiments, participants received intragastric or oral solutions of sucrose, alternative sweeteners (sucralose, erythritol, or D-allulose) or tap water (placebo). The rationale for intragastric administration of the solutions was to bypass oro-sensory exposure (e.g. taste and intensity) directly affecting brain mechanisms that may influence physiological/endocrine responses [366, 367]. The doses of substances used in both trials and the experimental procedures are described in detail within the respective project.

3.4 Blood Sample Collection and Laboratory Analysis

In the present thesis, blood samples for the analysis of CCK, GLP-1, PYY, ghrelin, glucose and insulin were collected on ice into tubes containing ethylenediaminetetraacetic acid (EDTA) (6 µmol/L blood), a protease-inhibitor cocktail (Complete, EDTA-free, 1 tablet/50mL blood, Roche, Mannheim, Germany), and a DPP-IV inhibitor (for the measurement of CCK, GLP-1, and PYY, 10 µL/mL blood, Millipore Corp., St. Charles, Missouri, USA). Blood lipids, uric acid, and hsCRP blood samples were collected on ice into serum tubes. After centrifugation (4°C at 3000 rpm for 10 min, centrifuge MPW-352R, MPW Med. Instruments, Poland), the samples were processed into different aliquots (for the ghrelin samples, 150 µL of 1N hydrochloric acid (HCl) was added) and stored at −80°C until analysis. The techniques used in this thesis to detect the different parameters are described below.

Plasma CCK, GLP-1, PYY, and ghrelin were analyzed in collaboration with the Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Copenhagen in Denmark (for CCK), the Department of Biomedical Sciences and Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen in Denmark (for GLP-1 and PYY) and the Laboratory for Brain-Gut Axis Studies, Translational Research Center for Gastrointestinal Disorders, Department of Chronic Diseases and Metabolism, KU Leuven, Leuven in Belgium (for ghrelin).

Plasma CCK, GLP-1, and ghrelin were measured using specific radioimmunoassay (RIA) kits. In brief, radioactive antigen (labeled antigen, tracer) is added to the antibody, followed by the addition of non-radioactive antigen (unlabeled antigen, standard, or sample). The formed antigen-antibody complexes are separated from the unbound tracer either by a washing step or by precipitation using a precipitating reagent (anti-immunoglobulin, secondary antibody). After an appropriate reaction time and centrifugation, the unbound tracer is in the supernatant and can be removed. The amount of antibody-tracer complexes is counted by a radioactivity counter; it decreases as the concentration of unlabeled antigen increases. The amount of unlabeled antigen in samples can be derived by interpolation from a standard calibration curve generated in the same assay with reference standards of known concentrations of the antigen. More details on the specific RIA kits is available in the respective projects.

Plasma PYY was measured using a specific sandwich enzyme-linked immunosorbent assay (ELISA). The basic steps include: i) binding of human PYY molecules in the sample by rabbit anti-human PYY Immunoglobulin G (IgG) and immobilization of the resulting complex to the wells of a microtiter plate coated by a pre-titrated amount of anti-rabbit IgG antibodies, ii) and the simultaneous binding of a second biotinylated antibody to the PYY, iii) wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, iv) wash away of free enzyme, and v) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3', 5,5'-tetra-methylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured human PYY in the unknown sample, the concentration

of total PYY can be derived by interpolation from a reference curve generated in the same assay with the reference standards of known concentrations of human PYY.

The analysis of the following parameters was performed by Rothen Medizinische Laboratorien AG, Basel, Switzerland. Plasma glucose, serum blood lipids, serum uric acid, and serum hsCRP were analyzed with specific enzymatic assays. Plasma insulin was quantified by electrochemiluminescence (ECLIA). ECLIA follows similar steps as the ELISA. The main difference is that upon inserting the ECLIA plates into the reader, an electric pulse initiates the substrate conversion, resulting in chemiluminescence. For further details see the respective projects.

3.5 Assessment of Gastric Emptying

Scintigraphy is the current "gold standard" to determine gastric emptying rates and was introduced by Griffith et al. in 1966 [368]. This technique, however, requires radiation exposure and is only used by some specific research facilities. There are several other possible methods to quantify gastric emptying rates including ultrasonography, paracetamol absorption test, MRI, or wireless motility capsules [27, 369]. However, these methods are often expensive, time-consuming, and only used for scientific purposes. Since the introduction of stable isotope ^{13}C-breath tests in the 1970s, they have been widely used among others to measure gastric emptying rates [370]. The main reasons are that the ^{13}C-breath tests are relatively simple to perform and cost-effective, they are non-invasive and most importantly they show excellent correlations in comparison with scintigraphy. Furthermore, the study participants or patients are not exposed to radiation. [27, 369] The ^{13}C-breath tests can be used to measure gastric emptying of liquids and solids. Liquids are typically labeled with ^{13}C-sodium acetate which is more water-soluble, whereas the medium-chain fatty acid, ^{13}C-octanoic acid, or the edible blue-green algae, ^{13}C-*Spirulina platensis*, is used for labeling solids [371-373].

In PolyAlluLac – Part I (see 4.1), the gastric emptying rate was determined using the ^{13}C- sodium acetate test. For the exact procedure of the assessment, see the respective project.

3.6 Assessment of Appetite-Related Sensations

Human studies allow us to study both objective (physiological, unconditioned) and subjective (learned, conditioned) components of appetite control. Appetite is divided into two parts: hunger and satiety. “Hunger” describes the drive to eat inferred from objective conditions or the urge to eat due to physical sensations in our body (light-headedness, emptiness in stomach). “Satiety” is separated into two different terms: “Satiation” describes the state of meal termination during eating (intra-meal satiety) influenced by satisfaction and stimulation of the brain by expanding the volume of the stomach. “Satiety”, on the other hand, refers to the feeling of fullness due to nutritional and physiological signals (between-meal satiety), preventing further eating i.e. lack of appetite or hunger. [39-41]

A widely adopted and validated method to measure pre- and postprandial appetite-related sensations (hunger, prospective food consumption, satiety, and fullness) is the use of visual analogue scales (VAS). VASs consist of a horizontal, unstructured, 10-cm line representing the minimum (0.0 points) to the maximum (10.0 points). They are easy to design and use, and simple to explain to the participants. Several studies have acknowledged the reproducibility, reliability, and validity of VASs under controlled conditions [40, 374-377]. Moreover, VASs are a sensitive, reliable and valid way to study appetite-related sensations, when used within an appropriate study design (e.g. within-subject, randomized, crossover and enough time to allow a wash-out between two treatments) [40, 377].

To this purpose, participants were instructed to assign a vertical mark across the line to indicate the magnitude of their subjective sensations at specific time points during the study in response to the following questions:

- How hungry are you?
- How much do you think you could eat?
- How satiated are you?
- How full are you?

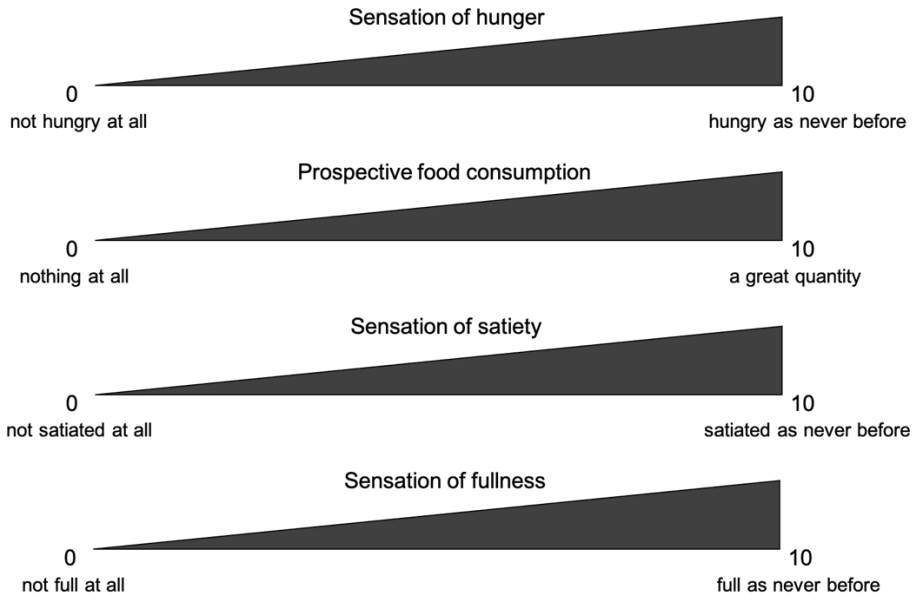

Figure 7 Visual analogue scales for the sensation of hunger, satiety, fullness, and the prospective food consumption.

The measurements were quantified by the distance from the left end of the line (minimum rating) to the participant's vertical mark. Of note, the participant's subjective appetite-related sensation ratings are not an unavoidable outcome of underlying physiological processes, it is rather their own interpretation of sensations, which are influenced by a number of physiological and unconditioned components of appetite control. Therefore, a strong correlation between feeding behavior and subjective appetite-related sensation ratings should not always be expected [377].

3.7 Assessment of Perceived Sweetness and Liking

Appetite is also governed by "sensory and hedonic" processes. To this purpose, two additional scales were used during the PolyFoodIntake study to rate sweetness (sensory) and liking (hedonic). The perceived sweetness of the preloads was rated from 0 to 100 using the Global Sensory Intensity Scale (GSIS). The scale goes from 0 (no sensation) to 100 (strongest sensation of any kind ever experienced). Perceived liking of the preloads and test meal were rated from −100 (most intensive disliking ever experienced) to 100 (most intensive liking ever experienced) using the Global Hedonic Intensity Scale (GHIS). The center is marked 0 as a neutral point [378].

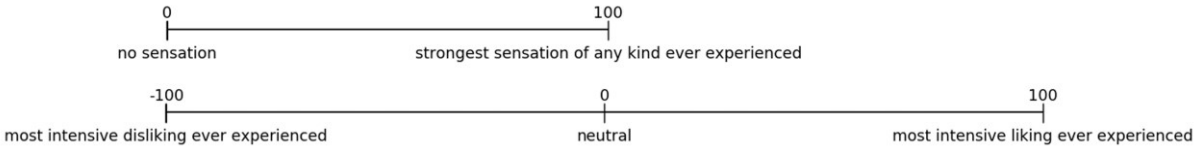

Figure 8 Global Sensory Intensity Scale (GSIS) and Global Hedonic Intensity Scale (GHIS).

The participants were verbally instructed that these scales cover sensory and hedonic experiences of all kinds. Prior to taking part in the study days, the participants were asked to write down the sensory and hedonic experiences that defined their scale boundaries as mentioned before [379]. Importantly, the boundaries selected are not related to the sensation of interest (preload and test meal). The sheet of paper on which the participants wrote these experiences was kept on the table in front of them during the study visits of the PolyFoodIntake study. The participants were asked to draw a tick on the scale with a pen at specific time points. For more details see 4.3.

The advantage of the GSIS and GHIS compared to VASs is that they allow across-group comparisons because the scale boundaries denote the same absolute perceived intensity, on average, to all groups [380, 381].

3.8 Materials

The purchase of the different substances administered during the studies is described in the respective projects.

3.9 Statistical Analysis

All outcome variables were analyzed using (generalized) linear mixed models in SAS (Statistical Analysis System) 9.4 (SAS Institute, Cary, NC, USA). Data are presented as mean ± SEM or mean ± SD, unless otherwise stated. A two-tailed p-value < 0.05 was considered statistically significant. For a detailed description including the specific hypotheses see respective projects.

Chapter 4

Projects

4 Projects

4.1 The role of D-allulose and erythritol on the activity of the gut sweet taste receptor and gastrointestinal satiation hormone release in humans: a randomized, controlled trial

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4.1.1 Abstract

Background: Glucose induces the release of gastrointestinal (GI) satiation hormones, such as glucagon-like peptide-1 (GLP-1), and peptide tyrosine tyrosine (PYY) in part via the activation of the gut sweet taste receptor (T1R2/T1R3).

Objective: The primary objective was to investigate the importance of T1R2/T1R3 for the release of cholecystokinin (CCK), GLP-1 and PYY in response to D-allulose and erythritol by assessing the effect of the T1R2/T1R3 antagonist lactisole on these responses and as secondary objectives to study the effect of the T1R2/T1R3 blockade on gastric emptying, appetite-related sensations and GI symptoms.

Methods: In this randomized, controlled, double-blind, cross-over study, 18 participants (five men, mean ± SD BMI: 21.9 ± 1.7 kg/m^2, age: 24 ± 4 y) received an intragastric administration of 25 g D-allulose, 50 g erythritol, or tap water, with or without 450 parts per million (ppm) lactisole, respectively, in six different sessions. ^{13}C-sodium acetate was added to all solutions to determine gastric emptying. At fixed time intervals, blood and breath samples were collected, and appetite-related sensations and GI symptoms were assessed. Data were analyzed with linear mixed model analysis.

Results: D-allulose and erythritol induced a significant release of CCK, GLP-1 and PYY compared to tap water (all $P_{Holm} < 0.0001$, $d_z > 1$). Lactisole did not affect the D-allulose- and erythritol-induced release of CCK, GLP-1, and PYY (all $P_{Holm} > 0.1$). Erythritol significantly delayed gastric emptying, increased fullness and decreased prospective food consumption compared to tap water ($P_{Holm} = 0.0002$, $d_z = -1.05$, $P_{Holm} = 0.0190$, $d_z = 0.69$ and $P_{Holm} = 0.0442$, $d_z = -0.62$, respectively).

Conclusions: D-allulose and erythritol stimulate the secretion of GI satiation hormones in humans. Lactisole had no effect on CCK, GLP-1, and PYY release, indicating that D-allulose- and erythritol-induced GI satiation hormone release is not mediated via T1R2/T1R3 in the gut.

Keywords

D-allulose, erythritol, gut sweet taste receptor, lactisole, gastrointestinal satiation hormones, gastric emptying, appetite-related sensations

4.1.2 Introduction

The increasing prevalence of obesity and diabetes mellitus type 2 (T2DM) and associated metabolic and cardiovascular disorders create serious health problems worldwide [382]. Sugar consumption has been shown to have harmful effects on the development of these diseases [7, 23]. The World Health Organization (WHO) strongly recommends to reduce free sugar intake to less than 10% of total energy intake, preferably less than 5% [32]. Partial substitution of sugar with natural, low-caloric sweeteners such as D-allulose and erythritol is one possible way to achieve the WHO recommendations.

Enteroendocrine cells (EECs) form the largest endocrine organ in the body, although they represent only 1% of the epithelial cells in the gut [88]. Scattered along the gastrointestinal (GI) tract, they are responsible for nutrient sensing, resulting in the release of GI satiation hormones such as cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide tyrosine tyrosine (PYY) [383]. These hormones signal retardation of gastric emptying, increases in satiety and fullness, as well as reduction in food intake [42, 59, 60, 384-386]. In humans, glucose can induce the release of GI satiation hormones via the activation of the sweet taste receptor (T1R2/T1R3) located on EECs [159], whereas this is not the case for artificial sweeteners, such as sucralose, acesulfame K or cyclamate [28, 188, 387]. Lactisole, a competitive inhibitor of the T1R3 subunit, attenuates glucose-stimulated release of GLP-1 and PYY in humans [159, 388].

D-allulose (C-3 epimer of D-fructose), also known as D-psicose, is a natural sugar with zero calories [319] and 70% of the sweetness of sucrose. In nature, it only occurs in small amounts, but it is industrially produced by enzymes catalyzing the conversion of D-fructose into D-allulose [316]. Moreover, D-allulose seems to have beneficial effects regarding fat and glucose metabolism in humans [341, 344, 345, 350]. Animal studies have indicated GLP-1 release upon D-allulose administration [324, 338]. The effect of D-allulose on GI satiation hormone release and on gastric emptying is not yet known in humans.

Erythritol is a naturally occurring sugar-alcohol without calories, 70% of the sweetness of sucrose, which can be commercially produced by yeast fermentation of glucose. Besides the preventive effect on caries [389], erythritol has a glycemic index of zero [281]. Recently, we

demonstrated that intragastric administration of erythritol induced the release of CCK, GLP-1 and PYY similar to glucose in healthy subjects. Furthermore, erythritol leads to a significant retardation of gastric emptying [290, 390]. Whether D-allulose induces the release of GI satiation hormones and if yes, whether their secretion is mediated via T1R2/T1R3 has not been studied in humans. Also, whether the erythritol-induced GI satiation hormone secretion is mediated via the gut sweet taste receptor is not yet known.

The primary objective of this study was therefore to investigate the importance of T1R2/T1R3 for the release of CCK, GLP-1 and PYY in response to intragastric administration of D-allulose and erythritol in healthy humans by assessing the effect of lactisole on these responses. The secondary objectives aimed to study the effect of the T1R2/T1R3 blockade on gastric emptying, appetite-related sensations and GI symptoms. More specifically, we hypothesize that CCK, GLP-1 and PYY will be released in response to D-allulose and erythritol compared to tap water. We also hypothesize that GLP-1 and PYY, but not CCK release will be reduced by lactisole. Gastric emptying rates will be reduced in response to D-allulose and erythritol compared to tap water, without an effect of lactisole. Satiety/fullness and hunger/prospective food consumption will be increased and reduced, respectively, in response to D-allulose and erythritol compared to tap water, without an effect of lactisole.

4.1.3 Methods

Participants

A total of 18 normal weight, healthy participants (mean ± SD body mass index (BMI): 21.9 ± 1.7 kg/m^2, range 19.1 – 24.3 kg/m^2, five men and 13 females; age: 24 ± 4 years, range 19-39 years) completed the study. See participant flowchart **Figure 9**.

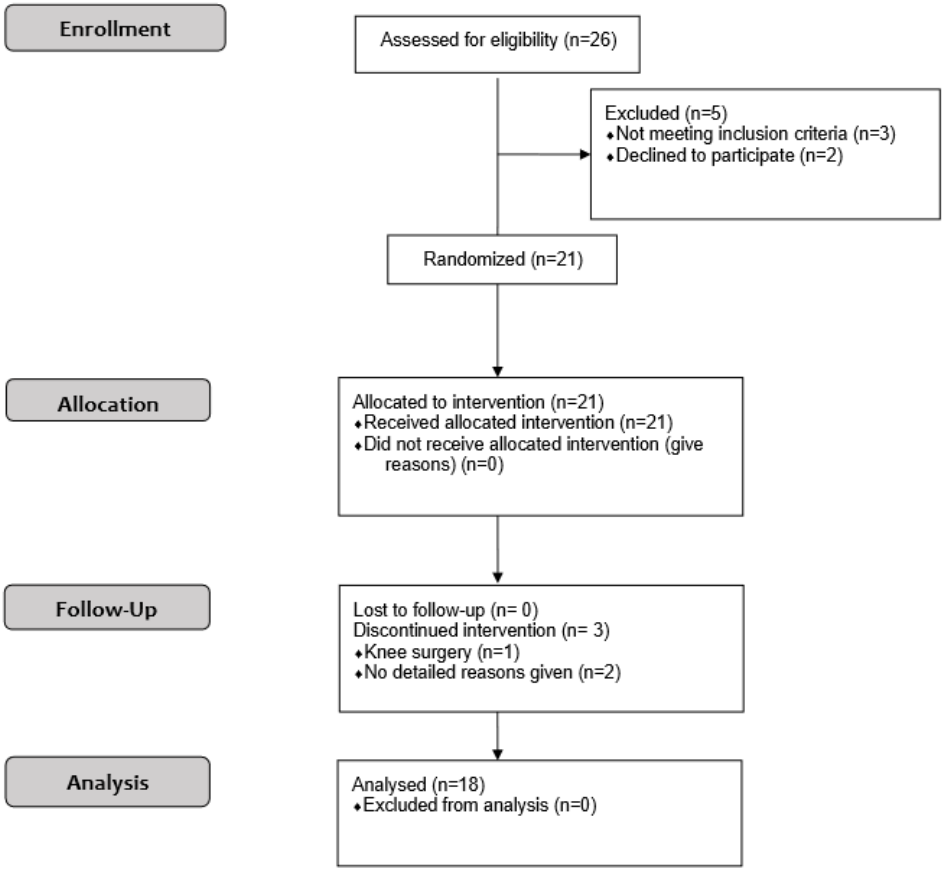

Figure 9 CONSORT flow diagram.

Overall study design

The study was conducted as a randomized (counterbalanced), placebo-controlled, double-blind, cross-over trial. The protocol was approved by the Ethics Committee of Basel, Switzerland (Ethikkomission Nordwest- und Zentralschweiz (EKNZ): 2019-01111) and conducted in accordance with the principles of the Declaration of Helsinki (version October 2013), the ICH-GCP and national legal and regulatory requirements. Recruitment of participants and follow-

up took place over a period of 12 months (September 2019 to September 2020). Each participant gave written informed consent for the study. The study was registered at ClinicalTrials.gov (NCT04027283). Exclusion criteria included substance and alcohol abuse, acute infections, chronic medical illness or illnesses affecting the GI system. None of the participants had a history of food allergies, dietary restrictions or pre-existing consumption of D-allulose and/or erythritol more than once a week. Weight, height, BMI, heart rate and blood pressure were recorded for all participants. On six separate test sessions, at least three days apart and after a ten hour overnight fast, participants were admitted to the St. Clara Research Ltd at ~0830 AM. An antecubital catheter was inserted into a forearm vein for blood collection. Participants swallowed a polyvinyl feeding tube (external diameter 8 French). The tube was introduced via an anesthetized nostril. The rationale for intragastric administration of the test solutions was to bypass oro-sensory cues to provide information on the isolated post-oral effects, which is crucial to increase the understanding of the role of the GI tract in the short-term control of appetite without confounding effects of cephalic and oral phases of ingestion, triggering hedonic responses and cognitions.

Experimental procedure

After taking blood samples (t = -10 and -1 min) and breath samples (t = -10 min) in the fasting state, and recording of appetite-related sensations and GI symptoms, participants received one of the following test solutions (at t = 0 min) directly into the stomach over two minutes in a randomized order:

- 50 g erythritol dissolved in 300 mL tap water
- 50 g erythritol and 450 ppm lactisole dissolved in 300 mL tap water
- 25 g D-allulose dissolved in 300 mL tap water
- 25 g D-allulose and 450 ppm lactisole dissolved in 300 mL tap water
- 300 mL tap water (placebo)
- 300 mL tap water and 450 ppm lactisole (placebo)

Concentrations were chosen based on the following considerations: 50 g erythritol induces GI satiation hormone release reliably without GI side effects and corresponds to around 33.5 g sucrose typically found in sweet beverages [390]. The effect of D-allulose on GI satiation hormones has not been investigated so far. The recommended maximal single dose – where no GI side effects are observed – is 25 g [352]. In a previous study design 450 ppm lactisole reliably induces a blockade of the gut sweet taste receptor [159]. The effectiveness of lactisole has been tested before in a pre-test oral taste experiment. Lactisole was able to block the D-allulose- and erythritol-induced sweet taste on the tongue. The results are in line with previous observations of other sweeteners [391]. To determine gastric emptying rates, 50 mg of ^{13}C-sodium acetate was added to the different test solutions. The intragastric test solutions were freshly prepared each morning of the study and were at room temperature when administered. The participants and the personnel involved in performing the study days and blood analysis were blinded regarding the content of administered test solutions.

After the administration of the test solution, blood samples (at t = 15, 30, 45, 60, 90, 120, and 180 min), for analysis of plasma CCK, GLP-1 and PYY, and end-expiratory breath samples (at t = 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min), for analysis of gastric emptying rates), were taken.

Appetite-related sensations (hunger, prospective food consumption, satiety and fullness) were assessed at t = 15, 30, 45, 60, 90, 120, and 180 min using visual analogue scales (VASs) as previously described [40, 375]. The ratings were recorded to one decimal point (e.g. 2.1).

Participants were also asked to rate GI symptoms (no symptoms (0 points), mild (1 point) or severe symptoms (2 points)) at t = 30, 60, 90, 120, 150, 180, and 240 min after the administration of the test solutions. The list included the following symptoms: a) abdominal pain, b) nausea, c) vomiting, d) diarrhea, e) borborygmus, f) abdominal bloating, g) eructation and h) flatulence.

Vital signs (blood pressure, heart rate) were measured at the beginning and at the end of each study day.

Materials

Erythritol was purchased from Mithana GmbH (Switzerland) and ^{13}C-sodium acetate from ReseaChem (Switzerland). D-allulose was purchased from Tate&Lyle (USA). Lactisole was a friendly gift of Domino Sugar Corporation, New York (USA).

Blood sample collection and processing

CCK, GLP-1, and PYY blood samples were collected on ice into tubes containing EDTA (6 µmol/L blood), a protease-inhibitor cocktail (Complete, EDTA-free, 1 tablet/50mL blood, Roche, Mannheim, Germany), and a dipeptidyl peptidase IV inhibitor (10 µL/mL blood, Millipore Corp., St. Charles, Missouri, USA). After centrifugation (4°C at 1409 RCF for 10 min), plasma samples were immediately processed into different aliquots and stored at -80°C until analysis.

Assessment of gastric emptying

The gastric emptying rate was determined using a ^{13}C-sodium acetate test, an accurate, non-invasive method for measuring gastric emptying, without radiation exposure, and a reliable alternative to scintigraphy, the current "gold standard" [372]. Test solutions were enriched with 50 mg of ^{13}C-sodium acetate, a compound readily absorbed in the proximal small intestine, transported to the liver where it is metabolized to $^{13}CO_2$, which is then exhaled rapidly [372]. At t = -10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min, end-expiratory breath samples were taken into a 100 mL foil bag. The ^{13}C-exhalation was determined by non-dispersive infrared spectroscopy using an isotope ratio mass spectrophotometer (Kibion® Dynamic Pro; Kibion GmbH, Bremen, Germany), and expressed as the relative difference (δ ‰) from the universal reference standard (carbon from Pee Dee Belemnite limestone). ^{13}C-enrichment was defined as the difference between pre-prandial ^{13}C-exhalation and post-prandial ^{13}C-exhalation at defined time points, δ over basal (DOB, ‰). Delta values were converted into atom percent excess and then into percent of administered dose of ^{13}C excreted per hour (% dose/h (%)).

Laboratory analysis

<u>*Plasma CCK*</u> was measured with a sensitive radioimmunoassay using a highly specific antiserum (No. 92128) [392]. The intra- and inter-assay variability is below 15%, respectively.

The appropriate range of this assay is 0.1 to 20 pmol/L. *Plasma GLP-1* samples were extracted in a final concentration of 70% ethanol before GLP-1 analysis. Total GLP-1 was measured as described by Ørskov *et al*. [393] using a radioimmunoassay (antibody code no 89390) specific for the C-terminal part of the GLP-1 molecule and reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite. The intra-assay variability is below 10% and the sensitivity of this assay is below 1 pmol/L. *Plasma PYY* was measured using Millipore human total PYY Elisa (cat no. EZHPYYT66K, Millipore, USA). The intra- and inter-assay variability is below 5.78% and 16.5%, respectively. The dynamic range of this assay is 14 pg/mL to 1800 pg/mL when using a 20 µL sample size.

Statistical analysis

In previous data on GI satiation hormone responses to intragastric infusion of 50 g erythritol versus tap water [290], the smallest proposed sample size (N = 18) yields 100% power to detect the hypothesized difference in the CCK, GLP-1, and PYY response between erythritol and tap water in linear mixed model analyses. Based on previous data on lactisole inhibition of glucose-induced hormone secretion [159], N = 18 yields >80% power to detect the hypothesized inhibitory effect of lactisole on GLP-1 and PYY secretion. Data were analyzed in SAS 9.4 (SAS Institute, Cary, NC, USA) and shown as mean ± SEM unless otherwise stated. A two-tailed *P*- value ≤ 0.05 was considered significant and Cohen's d_z for paired t-tests was reported as a measure of effect size.

For all analyses, if the assumption of normally distributed residuals was violated (based on a significant *P*-value of the Shapiro-Wilk test), natural logarithmic transformations of the dependent variables were used to normalize this distribution. Analysis was performed on transformed data. Logarithmic transformation of the dependent variables adequately normalized the residual distribution. Visit number was included to control for putative order effects in all models. All outcome variables were analyzed using (generalized) linear mixed models on changes from baseline (average of pre-infusion time point(s)). "Test solution" (intragastric D-allulose, D-allulose+lactisole, erythritol, erythritol+lactisole, tap water and tap water+lactisole) and "time" were included as within-subject independent variables in the models (including their main effects and the interaction). All the models were controlled for baseline values. To follow up on significant main or interaction effects, planned contrast

analyses were performed to test our specific hypotheses, with stepdown Bonferroni (Holm) correction for multiple testing. To test the hypothesis that D-allulose or erythritol induce an increase in GI satiation hormones and retard gastric emptying compared to tap water, we compared post-infusion GI satiation hormone levels and gastric emptying (change from baseline) between tap water on the one hand and D-allulose or erythritol on the other hand. To test the hypothesis that D-allulose or erythritol increase satiety/fullness and decrease hunger/prospective food consumption compared to tap water, respectively, we compared post-infusion appetite-related sensations between tap water on the one hand and D-allulose or erythritol on the other hand. To test the hypothesis that addition of lactisole does (not) decrease GI satiation hormones, retard gastric emptying, change appetite-related sensations in response to D-allulose or erythritol, we compared post-infusion GI satiation hormone levels and gastric emptying (change from baseline) to each of the substances with and without added lactisole.

For the associations, the difference between the test solutions of the significant planned contrasts at each timepoint were calculated and used as a dependent variable in the model with the same difference at each timepoint for the GI satiation hormones as independent variable in addition to time.

4.1.4 Results

Twenty-one participants were recruited for the study. There were three drop-outs (one participant had to withdraw due to a knee surgery and two withdrew for personal reasons). Therefore, 18 participants completed the six treatments. Complete data from all 18 participants were available for analysis.

GI satiation hormones

Plasma CCK, GLP-1, and PYY

CCK, GLP-1 and PYY secretion in response to D-allulose and erythritol are depicted in **Figure *10*** and **Table 1**. Both, D-allulose and erythritol induced a significant increase in GI satiation hormones compared to tap water. Adding lactisole had no effect on the secretion. Planned contrast analyses showed that the increase of CCK, GLP-1, and PYY was greater for D-allulose and erythritol vs. tap water (comparisons of the changes from baseline, all $P_{Holm} < 0.0001$, $d_z > 1$), with no significant difference for D-allulose+lactisole and erythritol+lactisole compared to the test solutions without lactisole (all $P_{Holm} > 0.1$). The main effect of test solution was significant for CCK, GLP-1, and PYY ([F(5,65) = 14.08, $P < 0.0001$], [F(5,60) = 12.85, $P < 0.0001$], and [F(5,54) = 28.68, $P < 0.0001$], respectively), indicating a difference in GI satiation hormone concentrations between the six test solutions over all time points. Further, the test solution-by-time interaction effect was significant for CCK, GLP-1, and PYY ([F(30,264) = 7.73, $P < 0.0001$], [F(30,267) = 1.66, $P = 0.0203$], and [F(30,271) = 5.26, $P < 0.0001$], respectively), indicating that the difference between test solutions differs between time points.

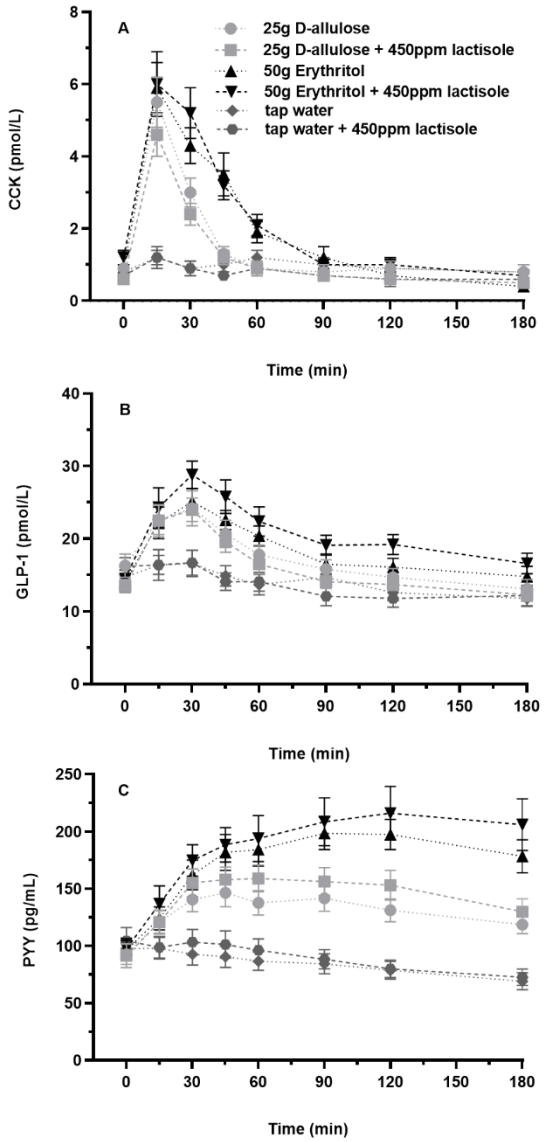

Figure 10 A) CCK, B) GLP-1, and C) PYY release after intragastric administration of solutions containing 25g D-allulose, 25g D-allulose + 450ppm lactisole, 50g erythritol, 50g erythritol + 450ppm lactisole, tap water and tap water + 450ppm lactisole to 18 healthy adults. Data are expressed as mean ± SEM, absolute values are reported. N=18 (5 men, 13 females). Statistical tests: Linear mixed-effects modeling followed by planned contrasts with Holm correction for multiple testing. The increase of CCK, GLP-1, and PYY was greater for D-allulose and erythritol vs. tap water (comparisons of the changes from baseline, all $P_{Holm} < 0.0001$. $d_z > 1$), with no significant difference for D-allulose+lactisole and erythritol+lactisole compared to the test solutions without lactisole (all $P_{Holm} > 0.1$). CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; PYY, peptide tyrosine tyrosine.

Table 1 Estimates from linear mixed models, results from planned contrast analyses and effect sizes in response to intragastric administration of solutions containing 25g D-allulose, 25g D-allulose + 450ppm lactisole, 50g erythritol, 50g erythritol + 450ppm lactisole, tap water and tap water + 450ppm lactisole to 18 healthy adults[1].

	Test solutions				**P-values**	
	D-allulose vs. tap water	**D-allulose vs. D-allulose+lactisole**	**erythritol vs. tap water**	**erythritol vs. erythritol+lactisole**	**Main effect of test solution**	**Test solution-by-time interaction**
CCK [pmol/L]	0.77 ± 0.12	-0.29 ± 0.16	1.58 ± 0.19	0.03 ± 0.23	$P < 0.0001$	$P < 0.0001$
P_{Holm}	< 0.0001	0.1703	< 0.0001	0.8800		
d_z	1.48		1.94			
GLP-1 [pmol/L]	4.08 ± 0.76	0.55 ± 1.09	7.41 ± 0.96	2.40 ± 1.34	$P < 0.0001$	$P = 0.0203$
P_{Holm}	< 0.0001	0.6136	< 0.0001	0.1594		
d_z	1.27		1.83			
PYY [pg/mL]	64.4 ± 6.15	9.48 ± 6.35	104 ± 9.21	13.5 ± 8.68	$P < 0.0001$	$P < 0.0001$
P_{Holm}	< 0.0001	0.2502	< 0.0001	0.2502		
d_z	2.47		2.67			
Gastric emptying [dose/h(%^{13}C)]	0.10 ± 0.16	-0.22 ± 0.28	-0.37 ± 0.08	0.08 ± 0.17	$P = 0.0003$	$P < 0.0001$
P_{Holm}	1	1	0.0002	1		
d_z			-1.05			
Hunger [cm]	-0.14 ± 0.24	0.56 ± 0.31	-0.49 ± 0.25	0.16 ± 0.28	$P = 0.2020$	$P = 0.0400$
P_{Holm}	0.2283	1	0.2283	1		
Pfc [cm]	0.06 ± 0.21	0.39 ± 0.32	-0.61 ± 0.24	-0.08 ± 0.27	$P = 0.0615$	$P = 0.1784$
P_{Holm}	0.6811	1	0.0442	1		
d_z			-0.62			
Satiety [cm]	-0.23 ± 0.26	0.18 ± 0.27	0.47 ± 0.29	0.41 ± 0.36	$P = 0.1206$	$P = 0.1521$
P_{Holm}	0.7695	0.7695	0.4533	0.7695		

	Test solutions				**P-values**	
	D-allulose vs. tap water	**D-allulose vs. D-allulose+lactisole**	**erythritol vs. tap water**	**erythritol vs. erythritol+lactisole**	**Main effect of test solution**	**Test solution-by-time interaction**
Fullness [cm]	-0.18 ± 0.22	0.16 ± 0.33	0.71 ± 0.24	0.62 ± 0.33	$P = 0.0011$	$P = 0.3473$
P_{Holm}	0.8714	0.8714	0.0190	0.2071		
d_z			0.69			

[1] N = 18 (5 men, 13 females). Estimates are expressed as means ± standard error and represent the changes from baseline for D-allulose and erythritol vs. tap water and the changes from baseline for lactisole within D-allulose and erythritol. Statistical tests: Linear mixed-effects modeling followed by planned contrasts with Holm correction for multiple testing and Cohen's d_z for paired t-tests is reported as a measure of effect size. CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; ppm, part per million; PYY, peptide tyrosine tyrosine.

Gastric emptying

Changes in gastric emptying in response to D-allulose and erythritol are depicted in **Figure 11** and **Table 1** Erythritol induced a significant retardation of gastric emptying compared to tap water, whereas D-allulose had no effect. Adding lactisole did not retard gastric emptying. Planned contrast analyses showed that gastric emptying was retarded for erythritol vs. tap water but not for D-allulose vs. tap water (comparisons of the changes from baseline, $P_{Holm} = 0.0002$, $d_z = -1.05$ and $P_{Holm} = 1$, respectively), with no significant difference for D-allulose+lactisole and erythritol+lactisole compared to the test solutions without lactisole (all $P_{Holm} = 1$). The main effect of test solution was significant [$F(5,39) = 6.13$, $P = 0.0003$], indicating a difference in gastric emptying between the six test solutions over all time points. Further, the test solution-by-time interaction effect was significant [$F(15,102) = 10.43$, $P < 0.0001$], indicating that the difference between test solutions differs between time points.

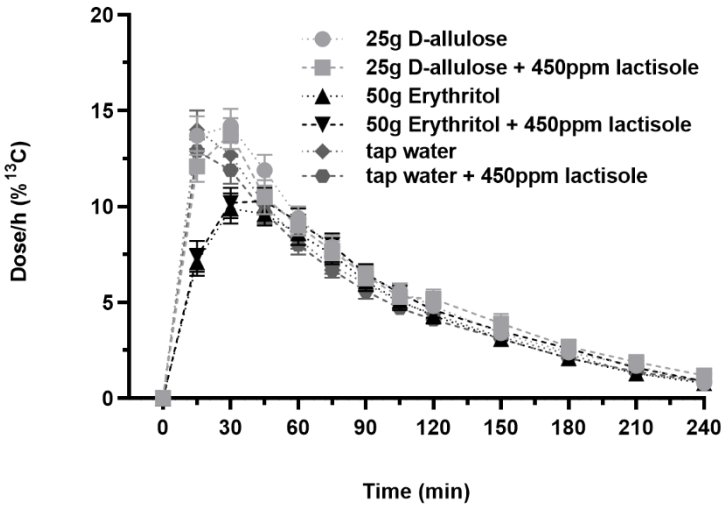

Figure 11 Gastric emptying after intragastric administration of solutions containing 25g D-allulose, 25g D-allulose + 450ppm lactisole, 50g erythritol, 50g erythritol + 450ppm lactisole, tap water and tap water + 450ppm lactisole to 18 healthy adults. Data are expressed as mean ± SEM. Change from baseline values are reported. N=18 (5 men and 13 females). Statistical tests: Linear mixed-effects modeling followed by planned contrasts with Holm correction for multiple testing. Gastric emptying was retarded for erythritol vs. tap water but not for D-allulose vs. tap water (comparisons of the changes from baseline, $P_{Holm} = 0.0002$, dz = -1.05 and $P_{Holm} = 1$, respectively), with no significant difference for D-allulose+lactisole and erythritol+lactisole compared to the test solutions without lactisole (all $P_{Holm} = 1$).

Appetite-related sensations

Hunger

Sensations of hunger in response to D-allulose and erythritol are depicted in **Figure 12A** and **Table 1**. Neither D-allulose nor erythritol affected the sensations of hunger compared to tap water. Adding lactisole had no effect. None of the planned contrast analyses were significant. The main effect of test solution was not significant. The main effect of test solution was not significant [$F(5,57) = 1.51$, $P = 0.2020$], indicating no difference in hunger between the six test solutions over all time points. Further, the test solution-by-time interaction effect was significant [$F(30,277) = 1.54$, $P = 0.0400$].

Prospective food consumption

Sensations of prospective food consumption in response to D-allulose and erythritol are depicted in **Figure 12B and Table 1**. Erythritol decreased the sensations of prospective food consumption compared to tap water, whereas D-allulose had no effect. Adding lactisole had no effect. Planned contrast analyses showed that prospective food consumption was lower for erythritol vs. tap water but not for D-allulose vs. tap water (comparisons of the changes from baseline, $P_{Holm} = 0.0442$, $d_z = -0.62$ and $P_{Holm} = 0.6811$, respectively), with no significant difference for D-allulose+lactisole and erythritol+lactisole compared to the test solutions without lactisole (both $P_{Holm} = 1$). Neither the main effect of test solution [$F(5,61) = 2.24$, $P = 0.0615$] nor the test solution-by-time interaction effect [$F(30,278) = 1.25$, $P = 0.1784$] was significant.

Satiety

Sensations of satiety in response to D-allulose and erythritol are depicted in **Figure 12C** and **Table 1**. Neither D-allulose nor erythritol affected the sensations of satiety compared to tap water. Adding lactisole had no effect. None of the planned contrast analyses were significant.

Neither the main effect of test solution [$F(5,51) = 1.84$, $P = 0.1206$] nor the test solution-by-time interaction effect [$F(30, 283) = 1.29$, $P = 0.1521$] was significant.

Fullness

Sensations of fullness in response to D-allulose and erythritol are depicted in **Figure 12D** and **Table 1**. Erythritol increased the sensations of fullness compared to tap water, whereas D-allulose had no effect. Adding lactisole had no effect. Planned contrast analyses showed that fullness was greater for erythritol vs. tap water but not for D-allulose vs. tap water (comparisons of the changes from baseline, $P_{Holm} = 0.0190$, $d_z = 0.69$ and $P_{Holm} = 0.8714$, respectively), with no significant difference for D-allulose+lactisole, and erythritol+lactisole compared to the test solutions without lactisole ($P_{Holm} = 0.9814$ and $P_{Holm} = 0.2071$, respectively). The main effect of test solution was significant [$F(5,55) = 4.76$, $P = 0.0011$], indicating a difference in fullness between the six test solutions over all time points. Further, the test solution-by-time interaction effect was not significant [$F(30,280) = 1.09$, $P = 0.3473$].

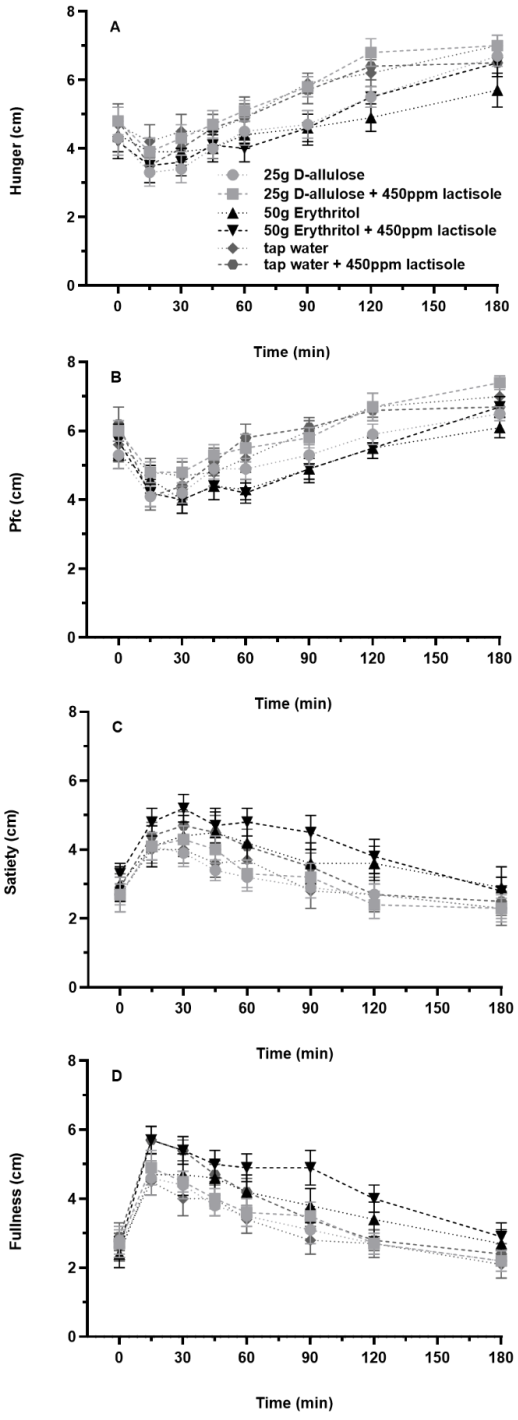

Figure 12 A) Hunger, B) Pfc, C) Satiety, and D) Fullness after intragastric administration of solutions containing 25g D-allulose, 25g D-allulose + 450ppm lactisole, 50g erythritol, 50g erythritol + 450ppm lactisole, tap water and tap water + 450ppm lactisole to 18 healthy adults. Data are expressed as mean ± SEM, absolute values are reported. N=18 (5 men and 13 females). Statistical tests: Linear mixed-effects modeling followed by planned contrasts with Holm correction for multiple testing. Pfc was lower for erythritol vs. tap water but not for D-allulose vs. tap water (comparisons of the changes from baseline, $P_{Holm} = 0.0442$, $d_z = -0.60$ and $P_{Holm} = 0.6811$,

respectively), with no significant difference for D-allulose+lactisole and erythritol+lactisole compared to the test solutions without lactisole (both $P_{Holm} = 1$). Fullness was greater for erythritol vs. tap water but not for D-allulose vs. tap water (comparisons of the changes from baseline, $P_{Holm} = 0.0190$, $d_z = 0.69$ and $P_{Holm} = 0.8714$, respectively), with no significant difference for D-allulose+lactisole, and erythritol+lactisole compared to the test solutions without lactisole ($P_{Holm} = 0.9814$ and $P_{Holm} = 0.2071$, respectively). No significant results for hunger and satiety. Pfc, prospective food consumption.

Associations between GI satiation hormones and gastric emptying

The difference in GLP-1 levels between erythritol and tap water was significantly associated with the respective difference in gastric emptying [β ± SE, 0.05 ± 0.02, F(1,101) = 7.33, $P = 0.0080$ $d_z = 0.64$]. The differences in CCK and PYY levels between erythritol and tap water were not associated with the respective difference in gastric emptying [0.04 ± 0.06, F(1,101) = 0.4, $P = 0.5301$ and 0.001 ± 0.003, F(1,101) = 0.59, $P = 0.4449$, respectively].

Associations between GI satiation hormones and appetite-related sensations

The difference in GLP-1 levels between erythritol and tap water was significantly associated with the respective difference in prospective food consumption [-0.06 ± 0.02, F(1,101) = 5.60, $P = 0.0199$, $d_z = -0.64$]. The differences in CCK and PYY levels between erythritol and tap water were not associated with the respective difference in prospective food consumption [- 0.06 ± 0.07, F(1,101) = 0.88, $P = 0.3501$ and 0.00002 ± 0.004, F(1,101) = 0.00, $P = 0.9956$, respectively]. The differences in CCK, GLP-1, and PYY levels between erythritol and tap water were not associated with the respective difference in fullness [0.07 ± 0.06, F(1,101) = 1.08, $P = 0.3009$, 0.008 ± 0.02, F(1,101) = 0.11, $P = 0.7458$, and 0.003 ± 0.004, F(1,101) = 0.85, $P = 0.3597$, respectively].

Gastrointestinal symptoms

All participants tolerated the study well. None of the participants had to withdraw from the study due to GI related symptoms. The symptoms were mild and short-lasting. Details are listed in **Table 2.**

Table 2 Assessment of GI symptoms after intragastric administration of solutions containing 25g D-allulose, 25g D-allulose + 450ppm lactisole, 50g erythritol, 50g erythritol + 450ppm lactisole, tap water and tap water + 450ppm lactisole to 18 healthy adults[1].

Symptom	Participants with symptom[2]	Reported severity[3]
Abdominal pain		
D-allulose	3	1.0
D-allulose + lactisole	4	1.0
Erythritol	6	1.0
Erythritol + lactisole	7	1.0
Tap water	2	1.0
Tap water + lactisole	3	1.0
Nausea		
D-allulose	3	1.0
D-allulose + lactisole	3	1.0
Erythritol	9	1.0
Erythritol + lactisole	10	1.1
Tap water	1	1.0
Tap water + lactisole	3	1.3
Vomiting		
D-allulose	0	0
D-allulose + lactisole	0	0
Erythritol	2	1.5
Erythritol + lactisole	0	0
Tap water	0	0
Tap water + lactisole	0	0
Diarrhea		
D-allulose	2	1.0
D-allulose + lactisole	0	0.0
Erythritol	5	1.0
Erythritol + lactisole	3	1.3
Tap water	0	0
Tap water + lactisole	0	0
Bowel sounds		
D-allulose	11	1.1
D-allulose + lactisole	13	1.1
Erythritol	14	1.0
Erythritol + lactisole	14	1.1
Tap water	11	1.0
Tap water + lactisole	8	1.0
Bloating		
D-allulose	3	1.0
D-allulose + lactisole	3	1.0
Erythritol	5	1.0
Erythritol + lactisole	4	1.0
Tap water	2	1.0
Tap water + lactisole	0	1.0
Eructation		
D-allulose	4	1.0
D-allulose + lactisole	4	1.0
Erythritol	4	1.0
Erythritol + lactisole	7	1.3
Tap water	2	1.0
Tap water + lactisole	3	1.0
Flatulence		
D-allulose	3	1.0
D-allulose + lactisole	2	1.0
Erythritol	5	1.0

Symptom	Participants with symptom[2]	Reported severity[3]
Erythritol + lactisole	3	1.0
Tap water	0	0
Tap water + lactisole	0	0

[1] N = 18 (5 men, 13 females). GI, gastrointestinal; ppm, parts per million.

[2]GI symptoms were assessed by the use of a list. Participants were asked to choose between "no symptom" (0 points), "mild symptoms" (1 point), and "severe symptoms" (2 points) for each item.

[3]Reported severity was calculated by the sum of the points divided by the participants with symptom.

4.1.5 Discussion

The results of the current study can be summarized as follows: D-allulose and erythritol induced a statistically significant release of CCK, GLP-1 and PYY compared to tap water. Lactisole did not affect the D-allulose- and erythritol-induced release of CCK, GLP-1, and PYY. Erythritol led to a statistically significant retardation of gastric emptying, an increase in fullness and a decrease in prospective food consumption compared to tap water. Doses of 25 g of D-allulose and 50 g of erythritol were well tolerated.

The increase in obesity and T2DM is related to sugar consumption, especially in the form of sugar-sweetened beverages [7, 23]. WHO and other national health institutions have formulated guidelines encouraging consumers to limit their sugar intake [394, 395]. A possible way to achieve such reductions in sugar consumption is substitution of sugar with natural, low-caloric sweeteners such as D-allulose and erythritol. Both D-allulose and erythritol may have beneficial effects on glucose metabolism; in addition, both have been shown to stimulate the release of GI satiation hormones [290, 324, 338, 390]. Of particular interest are CCK, GLP-1 and PYY, which induce a retardation of gastric emptying, an increase in satiety and fullness, as well as a reduction in food intake [42, 59, 60, 384-386]. In humans, glucose can induce the release of CCK, GLP-1 and PYY [159], whereas this is not the case for artificial sweeteners, such as sucralose, acesulfame K or cyclamate [28, 188, 387]. Here we have shown that intragastric administration of the naturally occurring, low-caloric sweetener D-allulose induces the release of CCK, GLP-1 and PYY in healthy humans, translating rodent studies to humans [324, 338].The previously demonstrated effect of erythritol on the secretion of CCK, GLP-1, and PYY was confirmed in the present study: intragastric administration of 75 g erythritol solution stimulated the secretion of CCK and GLP-1 in healthy subjects [390]. The findings are in line with the results of Overduin *et al.* where the partial replacement of sucrose by erythritol in a test breakfast lead to equal secretion of GLP-1 and PYY [280].

In humans, glucose has been reported to induce release of GI satiation hormones in part via the activation of T1R2/T1R3; lactisole, a competitive inhibitor of the T1R3 subunit, attenuated the glucose-stimulated release of GLP-1 and PYY, whereas CCK release is unaffected [159]. The inhibitory effect of lactisole is specific to humans and other primates [388]. We therefore hypothesized that GLP-1 and PYY but not CCK release would be reduced by lactisole in response to D-allulose and erythritol. However, lactisole had no effect on the D-allulose- and

erythritol-induced GI satiation hormone release in the current study. The knowledge about the T1R3 blockade in this study is based on the observations made by Schiffmann *et al*. for the sweet taste receptor on the tongue [391]. The sweet intensity of different sweeteners (including sucrose and glucose) were significantly blocked at concentrations of 250 and 500 ppm lactisole. The inhibition was only observed when sweeteners and lactisole were mixed prior to tasting and not when lactisole was introduced prior to these respective substances [391]. Therefore, a lack of effect based on mixing the sweeteners with lactisole prior to the intragastric administration can be excluded. Moreover, the use of 450 ppm lactisole is based on previous intragastric studies where glucose-stimulated secretion of GLP-1 and PYY were significantly reduced [159, 160]. In both studies glucose and lactisole were mixed prior to the intragastric administration. Apart from these studies with lactisole, Karimian Azari *et al*. used a comparable study design to evaluate the metabolic effects with lactisole in response to an oral glucose load in healthy lean participants with a comparable outcome [396]. Another potential factor which could have interfered with the effectiveness of lactisole inhibition is the relative absorption rates of the test solutions used. D-allulose and erythritol are absorbed with around 80% and 90% efficiency, respectively, while lactisole is rapidly absorbed [285, 331, 397]. Based on this, lactisole could have effectively blocked the natural sweeteners at proximal intestine, but not at distal which may have contributed to the lack of inhibition. However, the distribution and density of GLP-1 cells, although largely distributed in the terminal ileum, is also present in the duodenum [128]. Therefore, lactisole should have effectively blocked the sweeteners at the proximal GLP-1 secreting cells, which was not the case.

The lack of effect of lactisole suggests that D-allulose and erythritol induce the release of GI satiation hormones via other receptor/transporter mechanisms. There is evidence suggesting that SGLT-1 is the main driver of glucose-induced GLP-1 secretion [199]. The pharmacological SGLT-1 inhibitor phlorizin or the comparison between wild-type and *Sglt1*$^{-/-}$ mice reduced glucose-induced GLP-1 release [197, 201, 398]. However, mice lacking SGLT-1 have an increase in the later phase of GLP-1 secretion after glucose administration alone, suggesting that in the absence of SGLT-1 other pathways are active [399]. One hypothesis is that the increased delivery of glucose into the distal intestine possibly involves its fermentation into short chain fatty acids which in turn may trigger GLP-1 release [399]. Although up to 20% of erythritol is unabsorbed and available for colonic fermentation [285], it is unlikely that this might be a reason for the erythritol-induced GLP-1 release because we have an increase in GLP-1 after 30 minutes in this study. Furthermore, phlorizin did not reduce D-allulose-induced

GLP-1 release in rats, which also contradicts the hypothesis that SGLT-1 plays a role in the GI satiation hormone release [338]. In the same study, the authors also used xanthohumol – an inhibitor of the glucose/fructose transporter (GLUT5) – which inhibited D-allulose-induced GLP-1 secretion, suggesting that the secretion might be stimulated via GLUT5. The authors explain this by the fact that D-allulose and fructose are epimers and that a possible mechanism for GLP-1 secretion via GLUT5 has been suggested for fructose [198, 199]. Data in humans are lacking so far.

Gastric emptying is regulated by several feedback mechanisms, including GI satiation hormone release such as CCK, GLP-1 and PYY [42, 57]. Here erythritol retarded gastric emptying confirming our previous findings [290, 390]. Lactisole had no effect on the erythritol-induced retardation of gastric emptying. The latter findings extend our previous results: Gerspach *et al.* showed that the retardation of gastric emptying neither was affected by lactisole after glucose nor after mixed liquid meal administration [159]. We had anticipated that D-allulose would retard gastric emptying – especially in view of the observed effect on the GI satiation hormones – but we were unable to confirm our hypothesis.

Both increased concentrations of GI satiation hormones and prolonged gastric emptying are associated with feelings of fullness and satiation [400, 401]. In this trial, erythritol induced an increase in fullness and decrease in prospective food consumption. The findings are most likely related to the observed release of GI satiation hormones and the retardation of gastric emptying. In contrast to erythritol, D-allulose did not affect appetite-related sensations despite the marked increase in GI satiation hormones. As discussed above, changes in gastric emptying play an important role in the regulation of hunger and satiety feelings. The missing effect on gastric emptying observed in response to D-allulose is in line with this observation.

The mild and short-lasting symptoms of the present study for D-allulose are in line with a previous GI tolerance study [352]. There was a slight increase in symptoms after the erythritol-containing solutions compared to our most recent study [290]. However, participants familiarized to erythritol intake show a higher GI tolerance [212]. The participants in this trial were not used to these substances and the test solutions were rapidly applied (over two minutes) immediately into the stomach which probably causes the greatest stress for the GI tract.

Some limitations of our study require consideration: First, we studied acute effects of single bolus doses of D-allulose and erythritol with and without lactisole applied in a liquid solution

to participants with a BMI between 19.0 and 24.9 kg/m^2 who were not used to these substances. Differential effects of long-term exposure on the secretion of GI satiation hormones and gastric emptying rates need to be investigated, as adaptive processes cannot be ruled out. Second, we measured total GLP-1 which may imply less sensitivity towards detecting a small size effect for the gut sweet taste receptor inhibition than active GLP-1. Third, the substances used in this trial may behave differently when included in a food matrix with other nutrients rather than administered in isolation. Moreover, effects on subsequent food intake were not measured. Forth, appetite-related sensations could have been affected by the presence of the feeding tube, although in the present study it was only used for a short period of time and immediately removed after the administration of the test solutions.

In conclusion, D-allulose and erythritol stimulate the secretion of GI satiation hormones in humans. Lactisole had no effect on CCK, GLP-1, and PYY release, indicating that D-allulose and erythritol induced GI satiation hormone release is not mediated via the gut sweet taste receptor (T1R2/T1R3). The mechanism remains to be determined.

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Author contributions

ACMG, BKW, LVO, NW, and CB designed research; FT and VB conducted research; FT, ACMG, JFR., JJH, BH, LVO, NW, AB, and BKW analyzed data; FT, ACMG, and BKW wrote paper; FT, ACMG and BKW had primary responsibility for final content. All authors have read and approved the final manuscript.

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Conflict of Interest Statement

The authors have nothing to disclose.

4.2 Metabolic effects and safety aspects of acute administration of D-allulose and erythritol in healthy subjects

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4.2.1 Abstract

The rapid increase in sugar consumption is associated with various negative metabolic and inflammatory effects; therefore, alternative sweeteners become of interest. The aim of this study was to investigate the metabolic effects and safety aspects of acute D-allulose and erythritol on glucose, insulin, ghrelin, blood lipids, uric acid, and high-sensitive C-reactive protein (hsCRP). In three study visits, 18 healthy subjects received an intragastric administration of 25 g D-allulose or 50 g erythritol, or 300 mL tap water (placebo) in a randomized, double-blind and crossover order. To measure the aforementioned parameters, blood samples were drawn at fixed time intervals. Glucose and insulin concentrations were lower after D-allulose compared to tap water ($p = 0.001$, $d_z = 0.91$ and $p = 0.005$, $d_z = 0.58$, respectively); however, Bayesian models show no difference for insulin in response to D-allulose compared to tap water, and there was no effect after erythritol. An exploratory analysis showed that ghrelin concentrations were reduced after erythritol compared to tap water ($p = 0.026$, $d_z = 0.59$), with no effect after D-allulose; in addition, both sweeteners had no effect on blood lipids, uric acid and hsCRP. This combination of properties identifies both sweeteners as excellent candidates for effective and safe sugar alternatives.

Keywords: D-allulose; erythritol; sweeteners; glycemic control; ghrelin; blood lipids; uric acid; hsCRP; healthy subjects

4.2.2 Introduction

Fructose, also known as fruit sugar, is typically found in fruits, sucrose, honey, and high fructose corn syrup (HFCS). The excessive consumption of foods and beverages containing HFCS or sucrose are, however, associated with various risk factors such as insulin resistance, elevated blood lipids and uric acid, as well as an increase in systemic inflammation [21, 31, 402, 403]. These negative metabolic effects lead to an increased risk of non-communicable diseases such as obesity, diabetes mellitus type 2 (T2DM), cardiovascular diseases (CVD), and hyperuricemia [3, 8, 404, 405]. From a preventive perspective, HFCS and sucrose consumption should be reduced; therefore, there is growing interest in the use of efficacious and safe alternative sweeteners.

D-allulose and erythritol, two naturally occurring sweeteners, are interesting alternatives. Both have a sweetness of approximately 60-80% of sucrose and are associated with several positive health effects.

It was shown that D-allulose, a C_3 epimer of D-fructose, does not affect blood glucose in response to an oral glucose tolerance test (OGTT) in healthy humans [343]. More importantly, Franchi et al. [345] have reported that an acute intake of D-allulose in combination with 50 g of sucrose leads to a dose-dependent reduction in glucose and insulin concentrations compared to sucrose alone. In addition, several studies have found that D-allulose reduces postprandial blood glucose concentrations compared to either maltodextrin, a tea without D-allulose, or fructose in healthy participants and participants with prediabetes as well as T2DM [341, 344, 346]. Similar to fructose, D-allulose induces the release of gastrointestinal (GI) hormones such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide tyrosine tyrosine (PYY), thereby modulating appetite [28, 104, 406]. In mice, it has been shown that central injection of D-allulose inhibited ghrelin-responsive neurons in the arcuate nucleus (ARC) in the hypothalamus [336]. Whether D-allulose affects orexigenic ghrelin concentrations in humans is currently unknown. Furthermore, it has been shown that D-allulose compared to sucralose reduces body mass index (BMI) including abdominal and subcutaneous fat areas in a 12─week trial including participants with overweight and obesity, with no adverse effect on blood lipids [350]. Administering D-allulose to patients with high low-density lipoprotein (LDL) cholesterol levels for 48 weeks did not increase blood lipids or high-sensitive C-reactive

protein (hsCRP) [351]. Finally, daily intake of D-allulose in tea with a standard meal over 12 weeks did not affect uric acid concentrations [346].

Whether an acute intragastric administration of 25 g of pure D-allulose is efficacious and safe in regulating glucose, insulin, and ghrelin concentrations, as well as blood lipids, uric acid and hsCRP has not been investigated yet.

Erythritol, for its part, is a four-carbon sugar alcohol with the formula $C_4H_{10}O_4$ and occurs naturally in fruits, vegetables, and fermented food and drinks [274]. Erythritol does not affect glucose and insulin concentrations and seems to have protective effects on endothelial function in patients with T2DM [103, 290, 294, 304]. Although erythritol provides zero calories, it induces the release of CCK, GLP-1, and PYY similar to glucose and sucrose [103, 290]. A recent study indicates that ghrelin concentrations are suppressed in response to oral erythritol in healthy participants [292]. In a pilot dose-ranging study, acute ingestion of erythritol did not affect blood lipid and uric acid concentrations [290]. Based on toxicological and safety data, erythritol is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) in the United States for its intended use in foods [407]. However, the effect of erythritol on hsCRP is not known yet.

The aim of this study was to investigate the metabolic effects of acute intragastric administration of 25 g D-allulose or 50 g erythritol on glucose, insulin, and ghrelin concentrations as well as to assess safety aspects of both alternative sweeteners on blood lipids, uric acid, and hsCRP concentrations. The rationale for intragastric administration of the solutions was to bypass oro-sensory exposure. We hypothesized that glucose and insulin concentrations will be similar, and ghrelin will be reduced in response to D-allulose and erythritol compared to tap water, respectively.

4.2.3 Subjects and Methods

Approval

The Ethics Committee of Northwestern-and central Switzerland (EKNZ): 2019-01111 approved the trial. The trial was conducted in accordance with the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by the International Council on Harmonisation (ICH) and the Swiss law and Swiss regulatory

authority's requirements. All participants gave their informed consent for inclusion before inclusion in the study. The study was registered at ClinicalTrials.gov (NCT04027283).

Subjects

Twenty-one subjects were recruited via advertisement at the local university. Subjects were eligible for the study when meeting all of the subsequent inclusion criteria: age between 18−55 years, BMI of 19.0–24.9 kg/m^2 and normal eating habits (no diets, no dietary changes). Exclusion criteria were medical or drug abuse including alcohol dependence, acute or chronic infection or illness, illnesses affecting the GI tract, pre-existing consumption of D-allulose and/or erythritol more than once a week, pregnancy and involvement in another study with an investigational drug within 30 days preceding and/or during the current study.

Design and procedure

The study used a double-blind, placebo-controlled, cross-over design and was conducted between September 2019 and September 2020. Part of the results and the sample of this study have been reported elsewhere [406]. Each subject took part in three separate study visits as follows: 25 g D-allulose, 50 g erythritol, or 300 mL tap water (placebo). The solutions were dissolved in 300 mL tap water. The rationale for the doses was chosen for the primary outcome of this study (GI satiation hormone release) and was based on the following considerations previously described [406]: 50 g erythritol induces CCK, GLP-1, and PYY release reliably without GI side effects and corresponds to around 33.5 g sucrose typically found in sweet beverages [103]. The effect of D-allulose on GI satiation hormones has not been investigated before in humans. The recommended maximal single dose – where no GI side effects are observed – is 25 g [352]. . The order of the study visits was randomized and counterbalanced between subjects. The study visits took place at least three days apart and after a 10-hour overnight fast. All study visits started at ~0830 in the morning and upon arrival, a cannula was inserted into a forearm vein for blood collection. Next, a nasogastric feeding tube (external diameter 8 French) was inserted into the stomach. The rationale for intragastric administration of the solutions was to bypass oro-sensory exposure (e.g. taste and intensity) directly affecting brain mechanisms that may influence physiological/endocrine responses [366, 367].

After taking blood samples in a fasting state (t = -10 and -1 min) subjects received one of the solutions (at t = 0 min) via the nasogastric feeding tube over two minutes.

More blood samples were taken at t = 15, 30, 45, 60, 90, 120, and 180 min, for analysis of glucose, insulin, and ghrelin, and at t = 30, 60, and 120 min, for analysis of blood lipids, uric acid and hsCRP (**Figure 13**). Blood pressure and heart rate were measured at the beginning and at the end of each study visit. The subjects including the personnel performing the study visits and blood analysis were blinded regarding the content of administered solutions.

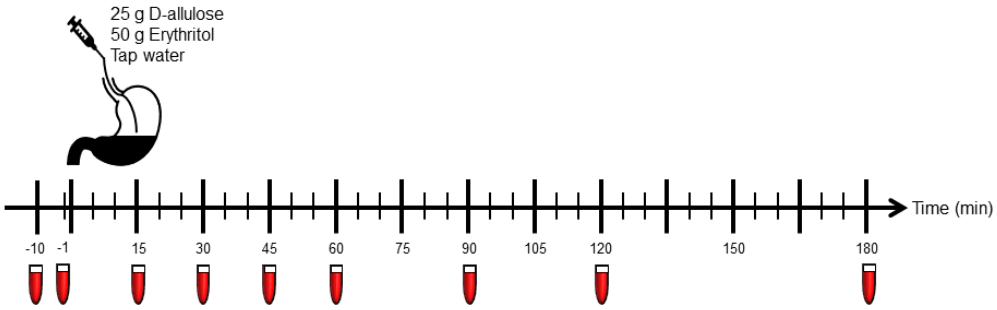

Figure 13 Study timeline: Intragastric administration of the solutions at t = 0 min to 18 healthy subjects in a randomized, double-blind, crossover order, in three different study visits after an overnight fast. The red tubes indicate blood sample collection.

Blood Sample Collection and Processing

Blood samples for glucose, insulin and ghrelin were collected on ice into tubes containing EDTA (6 µmol/L blood) and a protease-inhibitor cocktail (complete, EDTA-free, 1 tablet/50 mL blood, Roche, Mannheim, Germany). Blood lipids, uric acid and hsCRP blood samples were collected on ice into serum tubes. After centrifugation (4 °C at 3000 rpm for 10 min), the samples were processed into different aliquots (for the ghrelin samples, 150 µL of 1N hydrochloric acid was added) and stored at −80 °C until analysis.

Materials

D-allulose was purchased from Tate & Lyle (Decatur, IL, USA) and erythritol from Mithana GmbH (Zimmerwald, Switzerland).

Laboratory Analysis

Plasma glucose and insulin were measured with an enzymatic assay from Beckman-Coulter and an electrochemiluminescence immunoassay (ECLIA) (Rothen Medizinische Laboratorien AG, Basel, Switzerland), respectively. The intra- and inter-assay variability is below 0.7% and 0.9% (glucose) and below 4.3% and 5.3% (insulin). The appropriate range of the assays are 0.6 to 45 mmol/L (glucose) and 0.4 to 1000 µU/mL (insulin). Plasma octanoylated ghrelin was

measured by a radioimmunoassay with 125l [Tyr24] human ghrelin [1–23] as a tracer and a rabbit antibody against human ghrelin [1–8] (final dilution 15/100000), which does not cross-react with desoctanoylated ghrelin, as described previously in more detail [408]. Serum blood lipids, uric acid and hsCRP were measured with enzymatic assays from Beckman-Coulter (Rothen Medizinische Laboratorien AG, Basel, Switzerland). The intra- and inter-assay variability is below 0.7% and 0.8% (cholesterol), below 2.26% and 2.71% (LDL), below 0.85% and 1.92% (HDL), below 1.06% and 1.76% (triglycerides), below 1.55% and 2.44% (uric acid) and below 5% and 7.5% (hsCRP). The appropriate range of the assays are 0.5 to 18.0 mmol/L (cholesterol), 0.3 to 10.3 mmol/L (LDL), 0.05 to 4.65 mmol/L (HDL), 0.1 to 11.3 mmol/L (triglycerides), 89 to 1785 µmol/L (uric acid) and 0.2 to 80.0 mg/L (hsCRP).

Statistics

The sample size calculation to detect a difference between GI hormones in response to both alternative sweeteners compared to tap water was previously reported [406]. For the metabolic effects (glucose, insulin and ghrelin) and safety aspects (blood lipids, uric acid and hsCRP) parameters, no sample size calculations were performed. However, in a sensitivity power calculation, the sample size of 18 participants yields 80% power to detect a medium effect size (Cohen's d = 0.65) for the comparison of D-allulose and erythritol with tap water using a one-tailed paired t-test with Holm multiple testing correction ($\alpha = 0.0375$). The one-tailed test is justified by the directional nature of our hypothesis regarding the effects on ghrelin (see below).

Statistical analysis was conducted using SAS 9.4 (SAS Institute, Cary, NC, USA). Data is presented as the mean ± SEM unless otherwise stated. A two-tailed p-value < 0.05 was considered significant and Cohen's d_z for paired t-tests was presented for effect sizes. Kolmogorov-Smirnov testing and quantile-quantile plots were used to assess normality; for instance, if necessary, natural logarithmic transformations of the data were used to normalize distributions. The visit number was included to control for putative order effects in all models. The metabolic and safety outcome variables were analyzed using linear mixed models on changes from baseline (average of pre-infusion time point(s) for the metabolic parameters) and absolute values for the safety aspect parameters. "Solution" and "time" were included as within-subject independent variables in the models (including their main effects and the interaction). The metabolic outcome models controlled for baseline values. To follow up on significant main

or interaction effects, planned contrast analyses were performed to test the specific hypotheses, with stepdown Bonferroni (Holm) correction for multiple testing.

To test the hypotheses that glucose and insulin concentrations, in response to D-allulose and erythritol, will be similar to tap water and that ghrelin will be reduced in response to D-allulose and erythritol compared to tap water, respectively, we compared the post-infusion glucose, insulin and ghrelin concentration changes from the baseline between tap water and D-allulose or erythritol. We did not formulate any a priori hypotheses about the safety outcomes.

Given our hypothesis about glucose and insulin concentrations being similar for the two solutions compared to tap water, we complemented our frequentist statistical analysis with Bayesian analyses in two complementary ways. First, we ran Bayesian equivalents of the abovementioned linear mixed model analyses for these two outcomes using SAS PROC BGLIMM with 10,000 burn-in samples followed by 100,000 Markov chains. A weakly informative normal prior ($\mu = 0$, $\sigma = 2$) was used for the fixed effects coefficients, while an uninformative uniform prior with upper limit 1000 was used for the variance parameter of the covariance matrix for the random substance effect, to downplay the role of a relatively informative prior on the posterior distribution. Diagnostics (trace, autocorrelations and density plots and effective sample sizes) were used to confirm Markov chain convergence. Second, to the best of our knowledge, Bayes factors were not implemented in the context of the Bayesian linear mixed model analysis outside the context of Bayesian model selection [409], and we calculated Bayes factors in a one-way repeated measures ANOVA analysis with the AUC of glucose or insulin concentrations as the dependent variable and the solution as the sole independent variable as implemented in the JASP 0.16.4.0 software [410].

Since the AUC of total serum ghrelin was not significant in the study by Sorrentino et al. [292] between erythritol and aspartame consumption, but time points t = 20, 30, and 45 min after erythritol consumption were, we further explored the time points t = 30 and 45 min of ghrelin concentrations in response to D-allulose and erythritol, compared to tap water, in the current study.

4.2.4 Results

Twenty-one subjects were randomized. There were three drop-outs (one subject withdrew due to knee surgery and two withdrew for personal reasons). A total of 18 subjects (5 males and 13 females, mean ± SD (range), age: 24 ± 4 (19–35) years and BMI 21.9 ± 1.7 (19.1–24.3) kg/m^2 completed the three study visits. Complete data sets from all 18 subjects were available for analysis.

Plasma Glucose

D-allulose decreased plasma glucose, whereas erythritol had no effect compared to tap water (**Figure 14A**). The main effect of the solution and the solution-by-time interaction effect were significant ((F (2, 41) = 8.86, p = 0.001) and (F (12, 166) = 3.20, p = 0.0004), respectively). Planned contrast analyses show that plasma glucose was lower after D-allulose vs. tap water, but not after erythritol vs. tap water (p = 0.001, d_z = 0.91 and p = 0.787, respectively). These results were corroborated by Bayesian linear mixed model analysis, showing a difference between D-allulose and tap water (estimate ± standard deviation (SD): −0.202 ± 0.078, highest probability density (HPD) interval −0.356 − −0.048), but not between erythritol and tap water (−0.018 ± 0.055, −95% HPD 0.12–0.097).

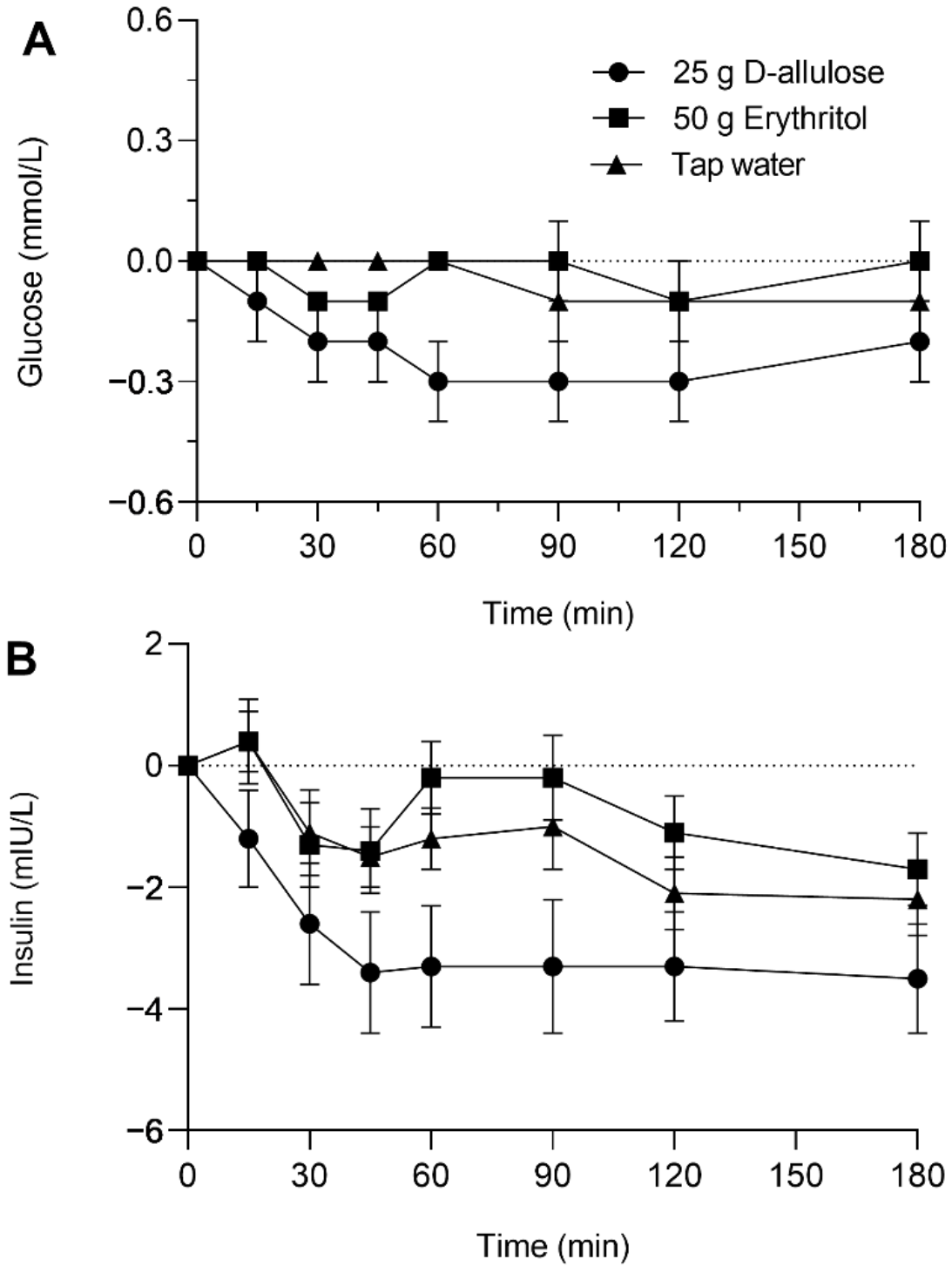

Figure 14 Glucose (A) and insulin (B) concentrations in response to intragastric administration of solutions containing 25 g D-allulose, 50 g erythritol or tap water to 18 healthy subjects. Data are expressed as mean ± SEM, and changes from baseline values are shown.

The Bayesian repeated measures ANOVA on the AUC yielded moderate evidence in favor of a difference between the three solutions in the omnibus test (BF_{10} = 7.50, R^2 = 0.42 [0.26–0.57]), as well as for the D-allulose vs. tap water post-hoc comparison (BF_{10} = 4.14). Moderate evidence was found in favor of erythritol being no different from tap water (BF_{10} = 0.243) (**Figure 15A**).

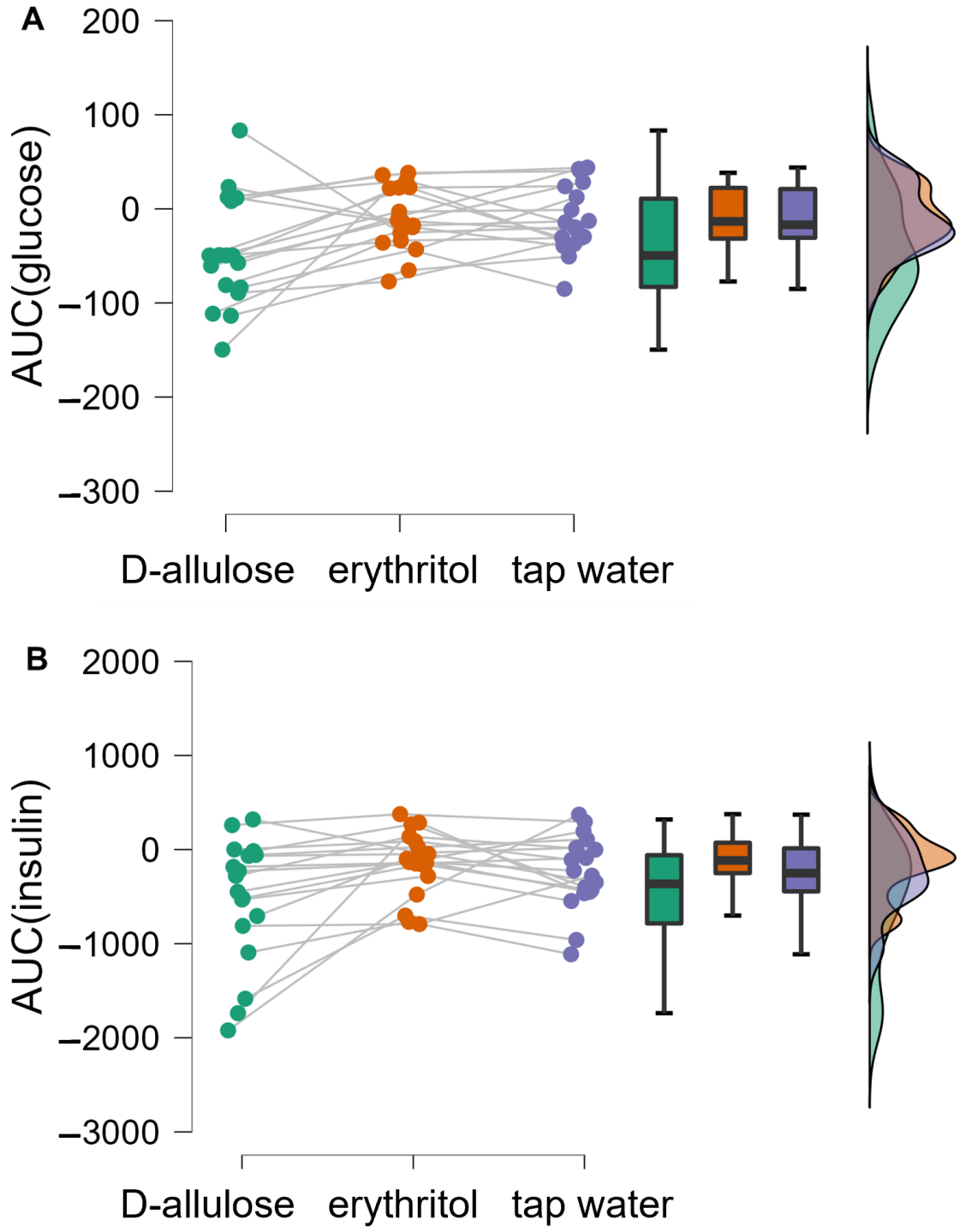

Figure 15 Raincloud plots showing the Bayesian repeated measures ANOVA on the AUC of glucose (A) or insulin (B) concentrations in response to the intragastric administration of solutions containing 25 g D-allulose (green), 50 g erythritol (orange) or tap water (purple) to 18 healthy subjects.

Plasma Insulin

D-allulose decreased plasma insulin, whereas erythritol had no effect compared to tap water (**Figure 14B**). The main effect of the solution was significant ($F (2, 37) = 6.15$, $p = 0.005$). The solution-by-time interaction effect was not significant ($F (12, 170) = 0.59$, $p = 0.848$). Planned contrast analyses show that plasma insulin was lower after D-allulose vs. tap water, but not after erythritol vs. tap water ($p = 0.005$, $d_z = 0.58$ and $p = 0.320$, respectively). The difference between D-allulose and tap water was not confirmed in a Bayesian linear mixed model analysis (0.020 ± 0.755, −95% HPD 1.457–1.495); however, the lack of difference between erythritol and tap water was corroborated (0.027 ± 0.756, 95% HPD −1.444–1.521).

The Bayesian repeated measures ANOVA on the AUC yielded moderate evidence in favour of a difference between the three solutions in the omnibus test ($BF_{10} = 4.77$, $R^2 = 0.41$ [0.25–0.56]), with the evidence for the D-allulose vs. tap water and erythritol vs. tap water post-hoc comparisons being inconclusive ($BF_{10} = 1.06$ and 0.42, respectively) (**Figure 15B**).

Plasma Octanoylated Ghrelin

D-allulose and erythritol had no effect on ghrelin compared to tap water (**Figure 16**). Neither the main effect of the solution nor the solution-by-time interaction effect were significant (($F (2, 39) = 2.14$, $p = 0.132$) and ($F (12, 156) = 0.86$, $p = 0.591$), respectively). None of the planned contrast analyses were significant. However, further exploration of the time points at 30 min and 45 min post D-allulose and erythritol administration show a decrease of ghrelin in response to erythritol at 30 min ($p = 0.026$, $d_z = 0.59$), with no effects in response to D-allulose ($p = 1$).

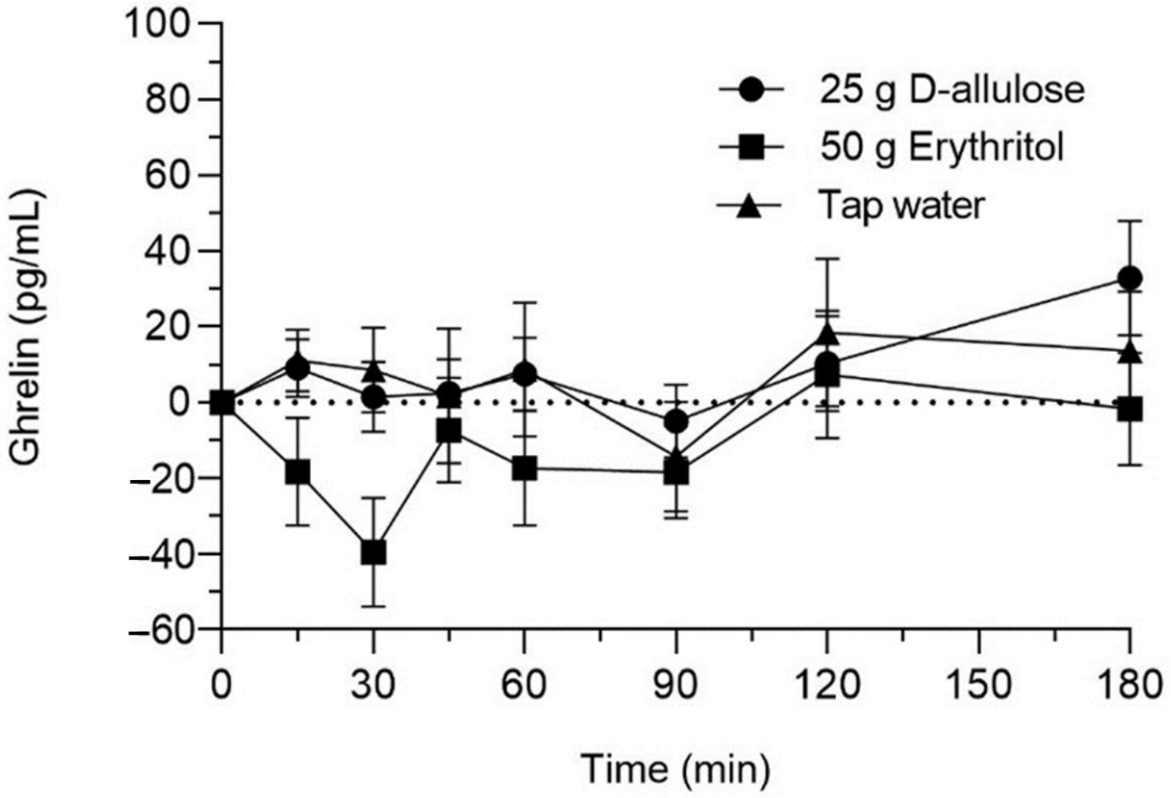

Figure 16 Ghrelin concentrations in response to intragastric administration of solutions containing 25 g D-allulose, 50 g erythritol or tap water to 18 healthy subjects. Data are expressed as mean ± SEM, and changes from baseline values are shown.

Serum Total Cholesterol

D-allulose and erythritol had no effect on total cholesterol compared to tap water. The main effect of the solution was not significant ($F(2, 26) = 0.03$, $p = 0.967$). The solution-by-time interaction effect was significant ($F(6, 83) = 3.28$, $p = 0.006$). None of the planned contrast analyses were significant.

Serum LDL Cholesterol

D-allulose and erythritol had no effect on LDL cholesterol compared to tap water. The main effect of the solution was not significant ($F(2, 21) = 0.12$, $p = 0.886$). The solution-by-time interaction effect was significant ($F(6, 40) = 2.99$, $p = 0.016$). None of the planned contrast analyses were significant.

Serum HDL Cholesterol

D-allulose and erythritol had no effect on HDL cholesterol compared to tap water. The main effect of the solution was not significant (F (2, 21) = 0.58, p = 0.568). The solution-by-time interaction effect was significant (F (6, 85) = 3.66, p = 0.003). None of the planned contrast analyses were significant.

Serum Triglycerides

D-allulose and erythritol had no effect on triglycerides compared to tap water. Neither the main effect of the solution nor the solution-by-time interaction effect were significant ((F (2, 29) = 0.61, p = 0.550) and (F(6, 81) = 2.08, p = 0.064), respectively). None of the planned contrast analyses were significant.

Serum Uric Acid

D-allulose and erythritol had no effect on uric acid compared to tap water. The main effect of the solution was not significant (F (2, 17) = 0.08, p = 0.925). The solution-by-time interaction effect was significant (F (6, 35) = 9.91, p = 0.001). None of the planned contrast analyses were significant.

Serum hsCRP

D-allulose and erythritol had no effect on hsCRP compared to tap water. Neither the main effect of the solution nor the solution-by-time interaction effect were significant ((F (2, 23) = 0.51, p = 0.606) and (F (6, 83) = 1.21, p = 0.309), respectively). None of the planned contrast analyses were significant.

4.2.5 Discussion

This study aimed to investigate the metabolic effects and safety aspects of the acute intragastric administration of either 25 g D-allulose or 50 g erythritol on glucose, insulin, ghrelin, blood lipid, uric acid and hsCRP concentrations. The results show that: (i) glucose and insulin concentrations did not increase in response to D-allulose and erythritol, compared to tap water; (ii) ghrelin concentrations decreased in response to erythritol (exploratory analysis), but not to D-allulose, compared to tap water; (iii) blood lipids, uric acid and hsCRP were not affected in response to D-allulose and erythritol compared to tap water.

The linear mixed model analysis shows that glucose and insulin concentrations were lower in response to D-allulose, but not erythritol, compared to tap water. However, both Bayesian models did not show evidence of a difference in insulin concentrations in response to D-allulose compared to tap water. The results of D-allulose and erythritol on glucose and insulin concentrations are therefore in line with previous human studies and support the anti-diabetic effects (i.e., no increase in glucose or insulin concentrations) [103, 290, 293, 341, 343-346]. In contrast to the intragastric administration of 25 g of D-allulose given in isolation in our study, these previous studies used oral doses between 2.5−10 g with the addition of either maltodextrin [341], an OGTT [344] or an oral sucrose load [345] assessing post-prandial blood glucose and insulin concentrations. To date, however, the mechanisms underlying the anti-diabetic effects of D-allulose and erythritol are not clear. A study in rats has suggested hepatic glucokinase changes in response to a rare sugar syrup containing D-allulose for 10 weeks as a possible mechanism for the reduction of post-prandial blood glucose [411]. However, it merits further investigation if this mechanism applies to pure and acute D-allulose administration. For erythritol, ameliorated insulin-mediated muscle glucose uptake and reduced intestinal glucose absorption was proposed as a mechanism in diabetic rats [289]. However, chronic intake of erythritol had no effect on intestinal glucose absorption in a recent human study [298]. At least for now, acute ingestion of both natural sweeteners seems to be a helpful alternative compared to sugar, especially for patients with obesity or T2DM.

Anorexigenic and orexigenic hormones play an important role in regulating appetite and satiation. Common mediators are CCK, GLP-1, PYY and ghrelin [412]. Unlike the other hormones, ghrelin is known as a “hunger hormone” and promotes food intake and increases gastric emptying [43, 413]. It was shown that ghrelin concentrations were not affected in

response to acute or chronic (2 weeks) artificial low-caloric sweeteners such as sucralose or aspartame [194, 249, 292]. We observed a similar acute effect in this study for D allulose. In contrast the exploratory analysis, erythritol induced a reduction of ghrelin at time point 30 min. The finding for erythritol is in line with the acute pilot study from Sorrentino et al. [292]. Moreover, the results of ghrelin in response to both alternative sweeteners reflect the gastric emptying rates recently reported, with no effect in response to D-allulose and a slowing down in response to erythritol [406]. Thus far, the results from the study in mice by Rakhat et al. [336], where a reduction in ghrelin-responsive neurons in the ARC was reported in response to D-allulose, are not translatable to humans. The sample size of the current study was rather small and further studies are needed to investigate the effects of D-allulose and erythritol on orexigenic hormones.

The potential side effects associated with high sugar intake, especially fructose, are changes in blood lipids, uric acid or hsCRP [21, 31]. For both sweeteners, we and others show that D-allulose and erythritol have no clinically relevant effects on blood lipids [290, 293, 350, 351]. Our findings are in line with these studies. However, more long-term studies with D-allulose and erythritol in different patients are needed to investigate the effects on blood lipids on long-term safety.

High uric acid concentrations and inefficient excretion thereof are often associated with hyperuricemia. Besides purine-rich food, other factors such as high fructose or alcohol consumption can trigger this metabolic disease [8, 25, 405, 414]. Since D-allulose is a stereoisomer of fructose, we examined uric acid concentrations. Our finding for acute administration of D-allulose is in line with the study by Hayashi et al. [346] where no effect was found on uric acid during a 12-week period. Of note, the administered dose in the current study was 10 g higher and without the influence of any other nutrients. No effects on uric acid were observed in response to erythritol. This is in line with a recent dose-ranging study where the highest dose of erythritol (50 g) had no effect on uric acid concentrations [290]. However, further studies are needed to test if chronic consumption of D-allulose and erythritol influence uric acid concentrations.

CRP is an acute-phase protein biomarker indicating inflammatory processes in the body [415], whereas the hsCRP is specific to CVD [416]. It was reported that acute and chronic fructose consumption increased hsCRP, possibly leading to systemic inflammation [21, 209]. Our

results for D-allulose are in line with the study by Tanaka et al. [351] who examined the long-term effects of D-allulose in participants with high LDL cholesterol levels on hsCRP and found no increase during the 48-week trial. To the best of our knowledge, no studies have investigated the effects of erythritol on hsCRP, and in the current study, no acute effect on hsCRP was found. This suggests that the acute administration of both sweeteners does not cause pro-inflammatory effects in the body.

Some limitations need to be considered. First, the design of this acute trial does not allow the investigation of chronic effects of D-allulose and erythritol. Second, additional, at this stage not identified side effects could occur under long-term treatment. Third, the study involved the intragastric administration of two alternative sweeteners by bypassing oro-sensory cues, which may limit translational inferences that can be drawn from the 'real-life' consumption of sweeteners (especially over the longer term). Fourth, a comparison of D-allulose and erythritol to a sucrose solution would be informative.

In conclusion, this study shows that the acute intragastric administration of the two alternative sweeteners D-allulose and erythritol, has beneficial physiological effects regarding glycemic control and ghrelin, and exhibits a clinically favorable safety profile with respect to blood lipids, uric acid and systemic inflammation. This combination of properties identifies D-allulose and erythritol as excellent candidates for effective and safe sugar alternatives.

Author Contributions: Conceptualization, A.C.M.-G., B.K.W., L.V.O., N.W. and C.B.; methodology, A.C.M.-G., B.K.W. and C.B.; formal analysis, F.T., A.C.M.-G., L.V.O., N.W. A.B., and B.K.W.; investigation, F.T. and V.B.; resources, A.C.M.-G. and B.K.W.; data curation, F.T., L.V.O. and A.C.M.-G.; writing—original draft preparation, F.T., A.C.M.-G., B.K.W. and C.B.; writing—review and editing, all authors; visualization, F.T., A.C.M.-G. and B.K.W.; supervision, A.C.M.-G. and B.K.W.; project administration, A.C.M.-G. and B.K.W.; funding acquisition, A.C.M.-G., B.K.W. and L.V.O. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the regional Ethics Committee of Basel, Switzerland (Ethikkomission Nordwest- und Zentralschweiz (EKNZ): 2019-01111 on the 17 July 2019.

Informed Consent Statement: Informed consent was obtained from all participants involved in this study.

Data Availability Statement: The data presented in this study are available on https://github.com/labgas/proj_erythritol_1.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

4.3 Oral Erythritol Reduces Energy Intake during a Subsequent *ad libitum* Test Meal: A Randomized, Controlled, Crossover Trial in Healthy Humans

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4.3.1 Abstract

The impact of oral erythritol on subsequent energy intake is unknown. The aim was to assess the effect of oral erythritol compared to sucrose, sucralose, or tap water on energy intake during a subsequent *ad libitum* test meal and to examine the release of cholecystokinin (CCK) in response to these substances. In this randomized, crossover trial, 20 healthy volunteers received 50 g erythritol, 33.5 g sucrose, or 0.0558 g sucralose dissolved in tap water, or tap water as an oral preload in four different sessions. Fifteen minutes later, a test meal was served and energy intake was assessed. At set time points, blood samples were collected to quantify CCK concentrations. The energy intake (*ad libitum* test meal) was significantly lower after erythritol compared to sucrose, sucralose, or tap water ($p < 0.05$). Before the start of the *ad libitum* test meal, erythritol led to a significant increase in CCK compared to sucrose, sucralose, or tap water ($p < 0.001$). Oral erythritol given alone induced the release of CCK before the start of the *ad libitum* test meal and reduced subsequent energy intake compared to sucrose, sucralose, or tap water. These properties make erythritol a useful sugar alternative.

Keywords: energy intake; erythritol; sucrose; sucralose; gastrointestinal satiation hormone; cholecystokinin; healthy participants; low-caloric sweeteners

4.3.2 Introduction

Around 30% of people worldwide have overweight or obesity [417], representing a major susceptibility for metabolic diseases such as type 2 diabetes mellitus (T2DM). Several studies have reported that the number of sugar-sweetened beverages (SSBs) consumed correlates with the body mass index (BMI) as well as T2DM [3, 418, 419]. Consequently, the World Health Organization (WHO) recommends reducing sugar consumption [32].

Substituting sugar with artificial low-caloric sweeteners (LCS), such as sucralose or aspartame, might be a strategy for reducing calories while preserving sweet taste. However, the use of artificial LCS has not achieved the expected results. This may be in part because artificial LCS, given in isolation, have no effect on the release of gastrointestinal (GI) hormones and gastric emptying, possibly explaining the lack of effects on satiety, fullness, and digestive mechanisms [28, 188, 232, 265, 387]. Moreover, a review of in vitro and in vivo animal studies reported the negative effects of artificial LCS on glucose homeostasis [233], and an observational study in postmenopausal women indicated that artificial LCS consumption might increase the risk of developing T2DM [235]. However, the results of reviews and meta-analyses investigating the relationship between artificial LCS and glucose homeostasis are controversial [233, 236, 420, 421]. These discrepancies presumably arise from differences in chemical properties and the biological fate of the artificial LCS, as well as the intake of artificial LCS in isolation or in a combination with other nutrients [233, 422]. Dalenberg et al. [257] observed that the combination of sucralose with carbohydrates (e.g., in a meal) alters insulin sensitivity and glucose tolerance possibly because of the up-regulation of glucose transporters observed in animal models in response to artificial LCS consumption [246, 423].

Because of the controversial data on artificial LCS in humans, low-caloric bulk sweeteners are attractive alternatives. Erythritol, a sugar alcohol with zero calories and a relative sweetness of 60–70% relative to sucrose, and according to some references even up to 80% [282, 301, 424], is associated with several positive physiological effects. In humans, acute ingestion of erythritol leads to an increase in GI satiation hormones (cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide tyrosine tyrosine (PYY)), slows down gastric emptying without affecting glucose and insulin concentrations, as well as blood lipids [103, 290, 293, 406]. Regarding the effects on energy intake following erythritol consumption, the one human study to investigate partial sucrose replacement by erythritol in a test breakfast reported similar levels of energy

intake, GI satiation hormone release (GLP-1 and PYY), and satiety between erythritol and sucrose [280]. However, no pure erythritol was used, and hence the effect of oral erythritol on subsequent energy intake is still unknown.

The primary objective of this study was to investigate the effect of oral erythritol compared to sucrose, sucralose, or tap water on energy intake during a subsequent *ad libitum* test meal in healthy participants. As a secondary aim, we examined the release of the GI satiation hormone CCK, glycemic control, and appetite-related sensations in response to these substances.

We hypothesized that erythritol will lead to a similar subsequent energy intake during the *ad libitum* test meal as sucrose and to a lower energy intake compared to sucralose or tap water.

4.3.3 Participants and Methods

Participants

Twenty-six healthy participants were recruited via advertisement at the University of Basel and were eligible for the study when fulfilling all of the following inclusion criteria: age between 18–55 years, BMI of 19.0–24.9 kg/m2, and normal eating habits (eating breakfast, no diets, no dietary changes, no vegetarians/vegans, no intolerances/allergies). The exclusion criteria were working night shifts, fructose intolerance, substance and alcohol abuse, acute or chronic infections, chronic medical illness, illnesses affecting the GI system, pre-existing consumption of erythritol and/or sucralose more than once a week, pregnancy, and participation in another study with an investigational drug within 30 days preceding and during the present study.

Ethical Approval

The trial was approved by the regional Ethics Committee of Basel, Switzerland (Ethikkommission Nordwest-und Zentralschweiz (EKNZ): 2020-02847) and conducted in compliance with the current version of the Declaration of Helsinki and national legal and regulatory requirements. Each participant gave written informed consent for trial participation. The study was registered at ClinicalTrials.gov (NCT04713137).

Study Design and Procedure

The study utilized a randomized, placebo-controlled, double-blind, four-way crossover design. Participants attended four test sessions at least 1 week apart. All sessions were conducted

between February and June 2021. During the 24 h preceding each visit, participants were asked to refrain from physical activities, alcohol, and caffeine. After a standardized dinner (consisting of pasta, a chocolate bar, and a soup; total 783 kcal), participants had to do an overnight fast from 08:00 PM until admission to the St. Clara Research Ltd., Basel, Switzerland, the next morning. All studies started at 08:00 AM to account for the circadian rhythm of GI satiation hormones. A cannula was inserted into a forearm vein for blood collection. After taking a fasting blood sample (t = −16 min), participants received one of the equisweet preloads (at t = −15 min) in a randomized order and had 2 min to consume it:

- 50 g erythritol;
- 33.5 g sucrose;
- 0.0558 g sucralose;
- 300 mL tap water.

Erythritol, sucrose, and sucralose were dissolved in 300 mL tap water. The doses of erythritol, sucrose, and sucralose were matched regarding sweetness. Based on previous studies, 50 g erythritol releases GI satiation hormones without side effects and accounts to a relative sweetness of 67% of sucrose (33.5 g, a typical sweet beverage of around 300 mL) [290]. Sucralose, on the other hand, is 600 times sweeter than sucrose and corresponds to a dose of 0.0558 g. The preloads were freshly prepared each morning of the test session by an uninvolved colleague to ensure blinding of the personnel conducting the study day and were administered at room temperature. The personnel involved in conducting the test sessions and blood analyses, as well as the participants, were blinded regarding the content of the preloads.

Fifteen minutes (t = 0 min) after the administration of the preload, a standard sol-id test meal was consumed, and ad libitum energy intake was measured. Blood samples (for CCK response and glycemic control) were collected and appetite-related sensations were assessed at t = −1, 15, 30, 60, 90, 120, 150, and 180 min. Appetite-related sensations were recorded using visual analogue scales (VASs) [40, 375]. At t = −10 min, subjects were asked to rate the perceived sweetness and liking of the preload and at t = 180 min, the perceived liking of the test meal with the Global Sensory Intensity Scale (GSIS) and Global Hedonic Intensity Scale (GHIS), respectively [378]. Vital signs (blood pressure, heart rate) were measured at the beginning and at the end of each study day.

Materials

Erythritol, sucrose, and sucralose were purchased from regional suppliers (erythritol, Schweizer Edelzucker AG, St. Gallen, Switzerland; sucrose, Hänseler AG, Herisau, Switzerland; sucralose, Sigma-Aldrich, Buchs, Switzerland).

Composition and Conduction of the Test Meal

The test meal was freshly prepared every morning of the test session by the study personnel and consisted of ham sandwiches (78.5 g, 233.6 kcal per sandwich), cups of chocolate cream (50 g, 64.5 kcal per cup), and glasses of water and cooled orange juice (250 mL, 100 kcal per bottle). Each ham sandwich consisted of two slices of toast (56 g, 145.6 kcal), butter (10 g, 74.2 kcal), and one slice of ham (12.5 g, 13.8 kcal) and was cut to make four sandwich squares (19.6 g, 58.4 kcal per sandwich square). The chocolate cream, butter, ham, and orange juice were stored in the fridge at 7 °C. Participants were asked to eat and drink as much as they wanted but not for more than 20 min. However, the test meal ended as soon as the participant had stopped eating and/or drinking for more than 5 min because of maximum satiation. At irregular time intervals, food and drinks were served and refilled in excess to reduce the participant's awareness of the amount of food consumed.

Assessment of Energy Intake

To assess the energy intake, the number of sandwich squares and chocolate cream cups consumed were recorded, and the volume (mL) of water and orange juice was measured before and after the test meal. Afterwards, the (total) energy intake was calculated.

Blood Sample Collection and Processing

Blood samples for the analysis of CCK were collected on ice into tubes containing ethylenediaminetetraacetic acid (EDTA) (6 µmol/L blood), a protease-inhibitor cocktail (Complete, EDTA-free, 1 tablet/50 mL blood, Roche, Mannheim, Germany), and a di-peptidyl peptidase IV inhibitor (10 µL/mL blood, Millipore Corp., St. Charles, MO, USA). Blood samples for the analysis of glucose and insulin were collected on ice into tubes containing EDTA (6 µmol/L blood) and a protease-inhibitor cocktail (Complete, EDTA-free, 1 tablet/50 mL blood, Roche, Mannheim, Germany). After centrifugation (4 °C, g force 1409, 10 min),

plasma samples were immediately processed into different aliquots and stored at −80 °C until analysis.

Laboratory Analysis

Plasma CCK was measured with a sensitive radioimmunoassay using a highly specific antiserum (No. 92128) [392] (intra- and inter-assay variability below 15%; range of assay, 0.1 to 20 pmol/L). Plasma glucose was measured by a glucose oxidase method (Rothen Medizinische Laboratorien AG, Basel, Switzerland; range of assay, 0.6 to 45.0 mmol/L). Plasma insulin was quantified using a chemiluminescent microparticle immunoassay (chemiflex reagent kit (#8k41; Abbott), the relative light units detected by the ARCHITECT optical system (model: CI4100; Abbott), assay precision be-low 7% total CV; range of assay, 1.0 to 300.0 µU/mL).

Statistical Analysis

Data on a pure oral erythritol preload were not available. Based on a medium effect size (f = 0.31) for the difference in *ad libitum* energy intake after a sucrose versus sucralose preload [251], we determined that $n = 20$ yields 83% power to detect a similar difference. This sample size yields 80% power to detect a small effect size (f = 0.22) in the omnibus test of the mixed ANOVA comparing *ad libitum* energy intake after each of the four preloads, and 80% power to detect a medium effect size (d = 0.73) for the paired *t*-tests testing the specific hypotheses that erythritol will lead to a similar subsequent energy intake during an *ad libitum* test meal as sucrose and to a lower energy intake compared to sucralose and tap water, respectively, with multiple testing correction.

Data were analyzed in SAS 9.4 (SAS Institute, Cary, NC, USA) and reported as mean ± standard deviation (SD); the significance level was set at <0.05. Cohen's d_z for paired *t*-tests was presented for effect sizes. For all analyses, the natural log-transformations of the dependent variables were used to normalize the distribution if the assumption of normally distributed residuals was violated (based on a significant *p*-value of the Shapiro–Wilk test). The visit number was included in all models to control for putative order effects. All outcome variables were analyzed using (generalized) linear mixed models on absolute values (energy intake, sweetness, and liking) or changes from baseline (CCK, glycemic control, and appetite-related sensations). "Preload" (energy intake, sweetness, and liking) and "time" (CCK, glycemic

control, and appetite-related sensations) were included as within-subject independent variables in the models (including their main effects and the interaction). All models for CCK, glycemic control, and appetite-related sensations were controlled for the total energy intake. Planned contrast analyses were performed to test our specific hypotheses using Student's t-tests with Tukey (for energy intake, sweetness, and liking) and stepdown Bonferroni–Holm correction for multiple testing (for CCK, glycemic control, and appetite-related sensations):

Comparison of energy intake between erythritol and sucrose, sucralose, or tap water to test the hypothesis that erythritol will lead to a similar subsequent energy intake as sucrose and to a lower energy intake compared to sucralose or tap water.

Comparison of post-preload administration time point −1 min versus baseline values for each substance to test the hypotheses that: (i) CCK will be released in response to erythritol and sucrose, but not in response to sucralose or tap water, (ii) the glucose and insulin concentrations will be increased in response to sucrose, but not in response to erythritol, sucralose, or tap water, and (iii) hunger/prospective food consumption will be decreased and satiety/fullness will be increased in response to erythritol and sucrose, but not in response to sucralose or tap water.

Comparison of post-preload administration time point −1 min versus baseline values between erythritol and sucrose, sucralose, or tap water to test the hypotheses that: (i) CCK in response to erythritol will be similar to sucrose, but higher compared to sucralose or tap water, (ii) glucose and insulin concentrations will be lower in response to erythritol compared to sucrose, but similar between erythritol and sucralose or tap water, and (iii) hunger/prospective food consumption and satiety/fullness, respectively, in response to erythritol will be similar to sucrose, but lower and higher, respectively, compared to sucralose or tap water.

Comparison of post-preload administration time point 15 min (during the ad libitum test meal) versus baseline values between erythritol, sucrose, sucralose, or tap water to explore CCK, glycemic control, and appetite-related sensations. No hypotheses were formulated beforehand.

Comparison of perceived sweetness and liking of the preloads, and perceived liking of the test meal between erythritol and sucrose, sucralose, or tap water to test the hypothesis that erythritol will have a similar perceived sweetness as sucrose and sucralose, but higher compared to tap water. No differences will be observed in the perceived liking of the preloads and test meal between erythritol compared to sucrose, sucralose, or tap water.

To explore putative associations between CCK and energy intake, the differences between CCK concentrations between erythritol, sucrose, sucralose, or tap water at post-preload administration time point −1 min were correlated to the respective difference in energy intake by non-parametric Spearman's correlation coefficient, P.

4.3.4 Results

Twenty-one participants were allocated to the intervention. One dropped out due to personal reasons. Therefore, 20 participants (10 males and 10 females; mean ± SD (range), age: 29.3 ± 10.9 (21−54) years, BMI: 22.3 ± 1.6 (19.6–24.8) kg/m^2) completed the study and complete data from 20 participants were available for analysis (**Figure 17**). All preloads were well tolerated.

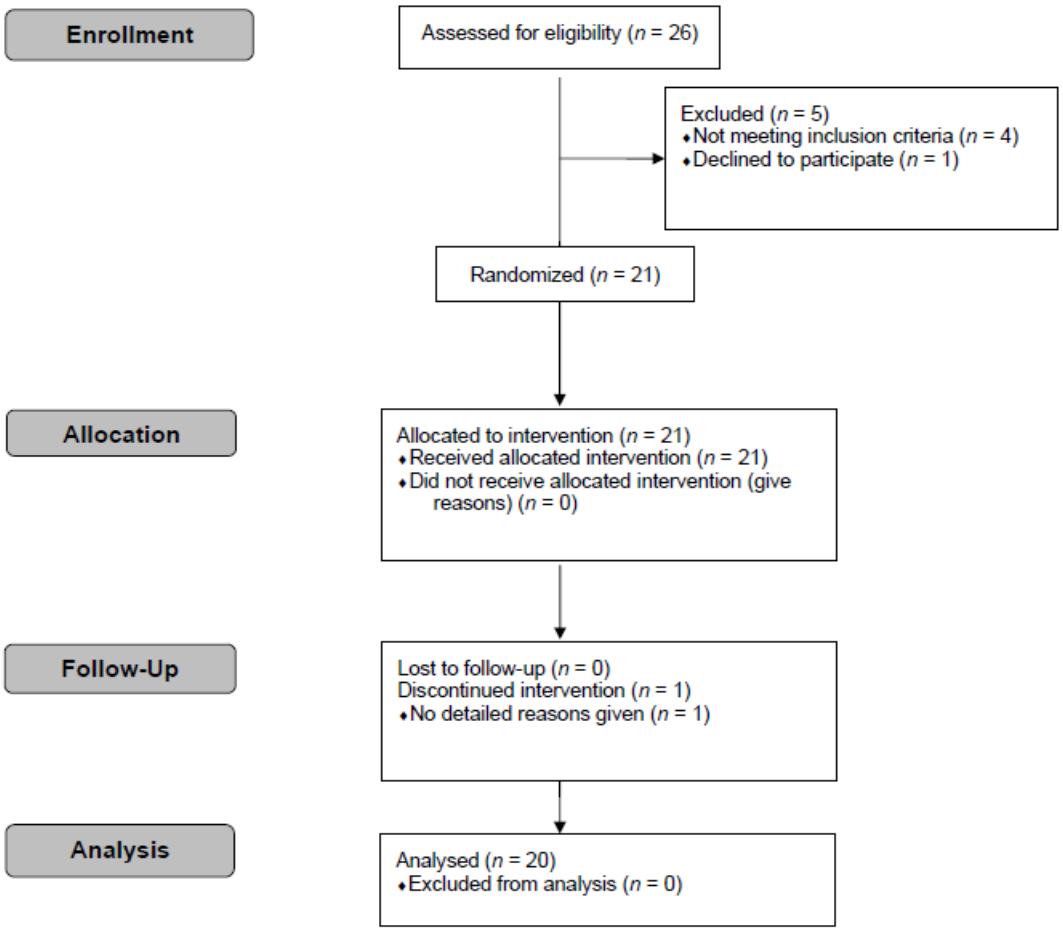

Figure 17 CONSORT flow diagram.

Energy Intake and Total Energy Intake

A significant main effect of preload was found for the energy intake ($F(3, 19) = 8.10$, $p = 0.001$) and total energy intake ($F(3, 19) = 16.67$, $p < 0.001$). Planned contrast analyses showed that energy intake and total energy intake were lower after oral erythritol compared to sucrose, sucralose, or tap water (for all comparisons, $p_{Tukey} < 0.05$ and $d_z \geq 0.68$). **Figure 18 and Table 3** show the energy intake from the *ad libitum* test meal and the total energy intake (preload and *ad libitum* test meal).

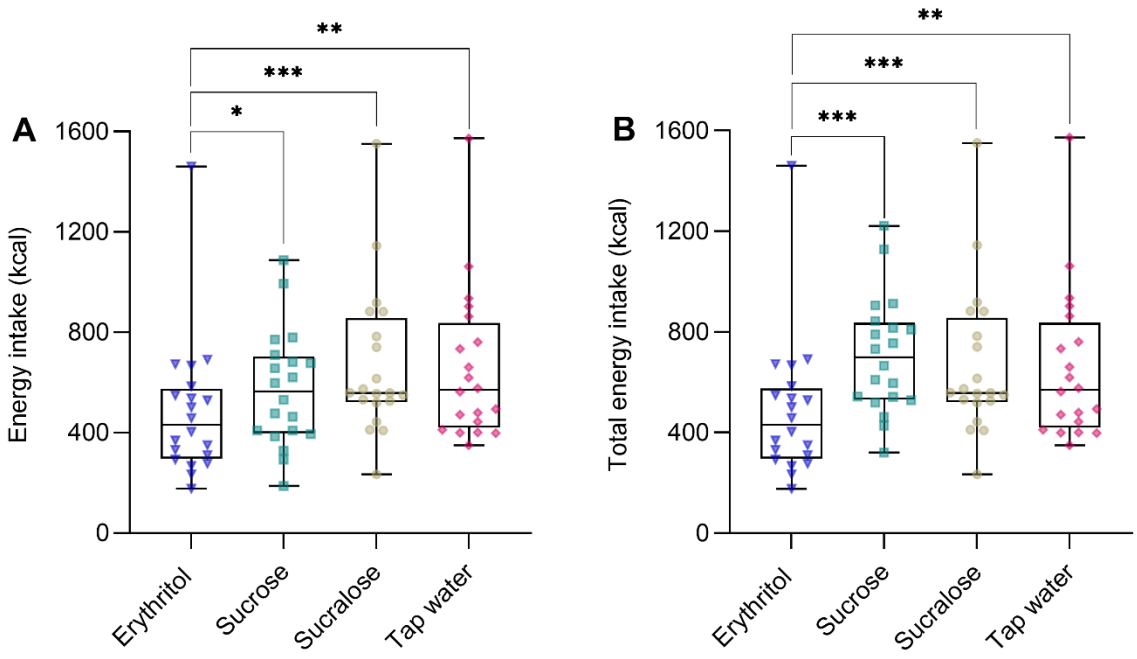

Figure 18 A) Energy intake (kcal) from the ad libitum test meal and (B) total energy intake (kcal) (preload + ad libitum test meal) after oral administration of preloads containing either 50 g erythritol, 33.5 g sucrose, 0.0558 g sucralose, or tap water. Data are shown as median and interquartile range with individual values for each substance (triangle = erythritol, square = sucrose, circle = sucralose, and rhombus = tap water), and absolute values are presented. Statistics: linear mixed models followed by planned contrasts using post-hoc Student's t-tests with Tukey correction for multiple testing in case of overall significance. *** $p_{Tukey} < 0.001$; ** $p_{Tukey} < 0.01$; * $p_{Tukey} < 0.05$. n = 20

Table 3 Effects of preloads containing either (A) 50 g erythritol, (B) 33.5 g sucrose, (C) 0.0058 g sucralose, or (D) tap water on energy intake (ad libitum test meal) and total energy intake (preload + ad libitum test meal) in 20 healthy participants[1].

Parameters	**Erythritol** ***n* = 20**	**Sucrose** ***n* = 20**	**Sucralose** ***n* = 20**	**Tap Water** ***n* = 20**	***p*-Value** **(Overall)**	***p*-Values** **(Post Hoc)**	**Effect Size**
Energy intake (kcal)	483 ± 277	573 ± 230	669 ± 297	655 ± 300	$p = 0.001$	A vs. B: $p = 0.030$	$d_z = 0.68$
						A vs. C: $p < 0.001$	$d_z = 1.08$
						A vs. D: $p = 0.003$	$d_z = 0.93$
Total energy intake (kcal)	483 ± 277	707 ± 230	669 ± 297	655 ± 300	$p < 0.001$	A vs. B: $p < 0.001$	$d_z = 1.49$
						A vs. C: $p < 0.001$	$d_z = 1.08$
						A vs. D: $p = 0.003$	$d_z = 0.93$

[1] Data are shown as mean ± SD and presented as absolute values. Statistics: linear mixed models followed by planned contrasts using post-hoc Student's *t*-tests with Tukey correction for multiple testing in case of overall significance and Cohen's d_z for paired *t*-tests (effect sizes).

GI Satiation Hormone: Plasma CCK

The main effect of preload was significant for CCK ($F(3, 64) = 3.99$, $p = 0.011$). Furthermore, the preload-by-time interaction effect was significant for CCK ($F(21, 290) = 5.76$, $p < 0.001$). Erythritol and sucrose induced a significant increase in CCK, whereas sucralose and tap water had no effect before the start of the ad libitum test meal. Planned contrast analyses showed that CCK responses were higher after oral erythritol compared to sucrose, sucralose, or tap water at −1 min (before the start of the ad libitum test meal) and at 15 min (during the ad libitum test meal) (for all: comparisons of the changes from baseline, all $p_{Holm} < 0.001$, $d_z \geq 1.51$). **Figure *19*** and **Table 4** Effects of preloads containing either 50 g erythritol, 33.5 g sucrose, 0.0058 g sucralose, or tap water on CCK, glycemic control, and appetite-related sensations in 20 healthy participants1. show the CCK secretion in response to oral erythritol, sucrose, sucralose, or tap water.

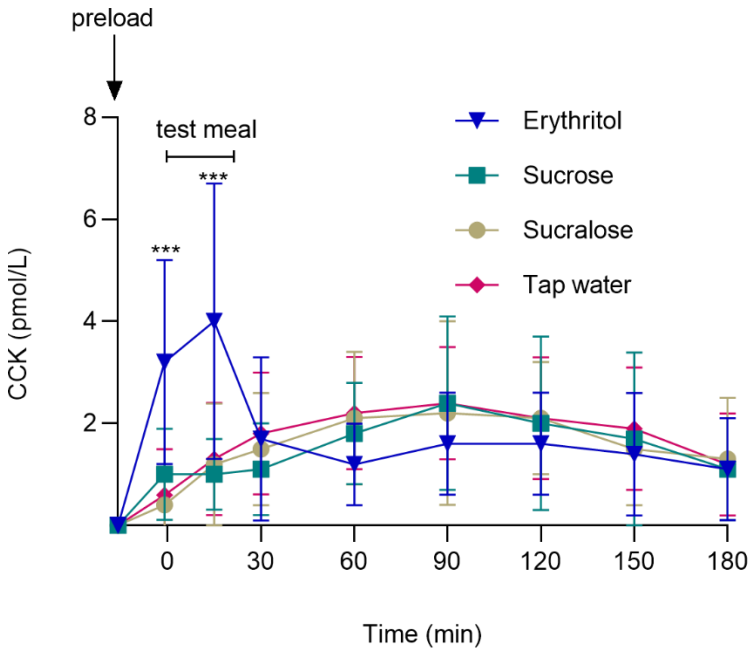

Figure 19 CCK concentrations after oral administration of either 50 g erythritol, 33.5 g sucrose, 0.0558 g sucralose, or tap water, and after the ad libitum test meal. Data are shown as mean ± SD, and baseline values are presented. Statistics: linear mixed models followed by planned contrasts with Holm correction for multiple testing. *** $p_{Holm} < 0.001$ erythritol vs. sucrose, sucralose, and tap water. CCK, cholecystokinin. n = 20.

Table 4 Effects of preloads containing either 50 g erythritol, 33.5 g sucrose, 0.0058 g sucralose, or tap water on CCK, glycemic control, and appetite-related sensations in 20 healthy participants[1].

Parameters	Time Points	Preloads			*p*-Values	
		Erythritol vs. Sucrose	Erythritol vs. Sucralose	Erythritol vs. Tap Water	Main Effect of Preload	Preload-by-Time Interaction
CCK (pmol/L)	−1 min	0.43 ± 0.06	0.62 ± 0.07	0.52 ± 0.07	$p = 0.011$	$p < 0.001$
		$p_{Holm} < 0.001$	$p_{Holm} < 0.001$	$p_{Holm} < 0.001$		
		$d_z = 1.51$	$d_z = 1.89$	$d_z = 1.71$		
	15 min	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01		
		$p_{Holm} < 0.001$	$p_{Holm} < 0.001$	$p_{Holm} < 0.001$		
		$d_z = 1.76$	$d_z = 1.46$	$d_z = 1.43$		
Glucose (mmol/L)	−1 min	−0.92 ± 0.18	0.06 ± 0.16	0.09 ± 0.18	$p = 0.003$	$p < 0.001$
		$p_{Holm} < 0.001$	$p_{Holm} = 1$	$p_{Holm} = 1$		
		$d_z = 1.16$				
	15 min	−0.18 ± 0.03	−0.05 ± 0.02	−0.04 ± 0.02		
		$p_{Holm} < 0.001$	$p_{Holm} = 0.053$	$p_{Holm} = 0.085$		
		$d_z = 1.61$				
Insulin (mIU/L)	−1 min	−0.99 ± 0.25	0.33 ± 0.24	0.22 ± 0.23	$p < 0.001$	$p < 0.001$
		$p_{Holm} < 0.01$	$p_{Holm} = 0.344$	$p_{Holm} = 0.344$		
		$d_z = 0.87$				
	15 min	−0.28 ± 0.04	−0.07 ± 0.03	−0.07 ± 0.03		
		$p_{Holm} < 0.001$	$p_{Holm} = 0.074$	$p_{Holm} = 0.074$		
		$d_z = 1.73$				
Hunger (cm)	−1 min	−0.66 ± 0.30	−0.32 ± 0.26	−0.94 ± 0.27	$p = 0.106$	$p = 0.520$
		$p_{Holm} = 0.065$	$p_{Holm} = 0.210$	$p_{Holm} = 0.003$		
				$d_z = 0.77$		
	15 min	−0.09 ± 0.04	−0.04 ± 0.04	−0.08 ± 0.04		
		$p_{Holm} = 0.094$	$p_{Holm} = 0.257$	$p_{Holm} = 0.094$		
Pfc (cm)	−1 min	−0.05 ± 0.33	−0.14 ± 0.30	−0.38 ± 0.29	$p = 0.848$	$p = 0.205$
		$p_{Holm} = 1$	$p_{Holm} = 1$	$p_{Holm} = 0.558$		
	15 min	−0.02 ± 0.05	−0.05 ± 0.04	−0.02 ± 0.04		
		$p_{Holm} = 1$	$p_{Holm} = 0.725$	$p_{Holm} = 1$		
Satiety (cm)	−1 min	0.03 ± 0.43	0.08 ± 0.38	0.34 ± 0.40	$p = 0.862$	$p = 0.912$
		$p_{Holm} = 1$	$p_{Holm} = 1$	$p_{Holm} = 1$		
	15 min	0.02 ± 0.06	0.05 ± 0.05	0.02 ± 0.06		

Parameters	Time Points	Preloads			*p*-Values	
		Erythritol vs. Sucrose	Erythritol vs. Sucralose	Erythritol vs. Tap Water	Main Effect of Preload	Preload-by-Time Interaction
		$p_{Holm} = 1$	$p_{Holm} = 1$	$p_{Holm} = 1$		
Fullness (cm)	−1 min	0.19 ± 0.29	0.19 ± 0.29	0.52 ± 0.28	$p = 0.874$	$p = 0.140$
		$p_{Holm} = 1$	$p_{Holm} = 1$	$p_{Holm} = 0.190$		
	15 min	0.03 ± 0.04	0.03 ± 0.04	0.01 ± 0.04		
		$p_{Holm} = 1$	$p_{Holm} = 1$	$p_{Holm} = 1$		

[1]Estimates from linear mixed models are shown as means ± standard error and present the changes from baseline for erythritol vs. sucrose, sucralose, or tap water at −1 min and at 15 min. Statistics: linear mixed models followed by planned contrasts with Holm correction for multiple testing and Cohen's dz for paired t-tests (effect sizes). CCK, cholecystokinin; pfc, prospective food consumption.

Associations between CCK and Energy Intake

The difference in CCK concentrations between oral erythritol and sucrose, sucralose, or tap water were not associated with the respective difference in energy intake (P = −0.212, P = −0.234, P = 0.053, respectively, all $p > 0.05$).

Glycemic Control: Plasma Glucose and Insulin

The main effect of preload was significant for glucose ($F(3, 74) = 4.98$, $p = 0.003$) and insulin ($F(3, 70) = 8.89$, $p < 0.001$). Furthermore, the preload-by-time interaction effect was significant for glucose ($F(21, 290) = 5.79$, $p < 0.001$) and insulin ($F(21, 288) = 6.29$, $p < 0.001$). Sucrose induced a significant increase in glucose and insulin concentrations, whereas erythritol, sucralose, and tap water had no effect before the start of the ad libitum test meal. Planned contrast analyses showed that glucose and insulin responses were lower after oral erythritol than after sucrose (for both: comparisons of the changes from baseline, $p_{Holm} < 0.01$, $d_z \geq 0.87$), with no difference between erythritol and sucralose or tap water at −1 min (before the start of the ad libitum test meal) and at 15 min (during the ad libitum test meal) (for all: comparisons of the changes from baseline, all $p_{Holm} > 0.05$). **Figure 20** and **Table 4** show glucose and insulin concentrations in response to oral erythritol, sucrose, sucralose, or tap water.

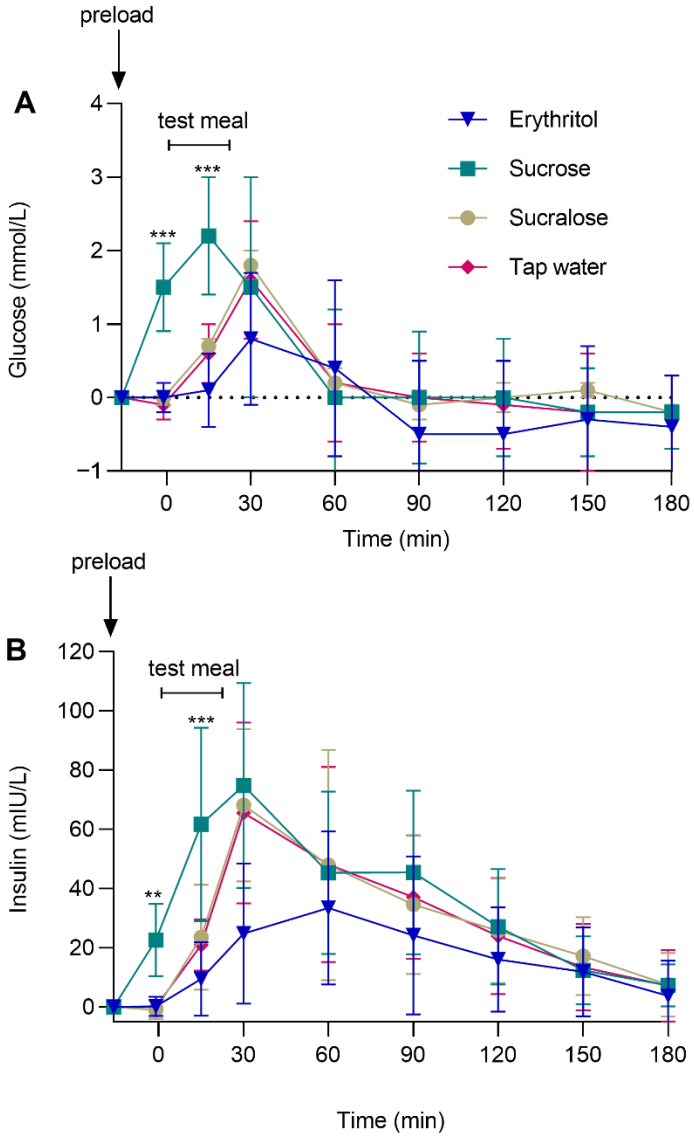

Figure 20 (A) Glucose and (B) insulin concentrations after oral administration of preloads containing either 50 g erythritol, 33.5 g sucrose, 0.0558 g sucralose, or tap water, and after the ad libitum test meal. Data are shown as mean ± SD, and baseline values are presented. Statistics: linear mixed models followed by planned contrasts with Holm correction for multiple testing. *** $p_{Holm} < 0.001$ and ** $p_{Holm} < 0.01$ for erythritol vs. sucrose. n = 20.

Appetite-Related Sensations: Hunger, Prospective Food Consumption, Satiety, and Fullness

Hunger

Neither the main effect of preload (F (3, 62) = 2.13, p = 0.106) nor the preload-by-time interaction effect (F (21, 288) = 0.96, p = 0.520) were significant. Planned contrast analyses showed that hunger was lower after oral erythritol compared to tap water at −1 min (before the start of the ad libitum test meal, p_{Holm} = 0.003, d_z = 0.77), but not at 15 min (during the ad libitum test meal, p_{Holm} = 0.094). There was no difference between erythritol and sucrose or sucralose at −1 and 15 min (for all: comparisons of the changes from baseline, all $p_{Holm} > 0.05$). **Table 4** shows appetite-related sensations in response to oral erythritol, sucrose, sucralose, or tap water.

Prospective Food Consumption, Satiety, and Fullness

Neither the main effects of preload ((F (3, 62) = 0.27, p = 0.848), (F (3, 59) = 0.25, p = 0.862), and (F (3, 58) = 0.25, p = 0.874), respectively) nor the preload-by-time interaction effects ((F (21, 290) = 1.34, p = 0.205), (F (21, 288) = 0.61, p = 0.912), and (F (21, 288) = 1.35, p = 0.140), respectively) were significant. None of the planned contrast analyses were significant.

Perceived Sweetness and Liking of the Preloads

A significant main effect of preload was found for the perceived sweetness of the preloads (F (3, 19) = 77.43, p < 0.001). Planned contrast analyses showed that the perceived sweetness of the preload was not different between oral erythritol and sucrose (p_{Tukey} = 0.665), but higher after erythritol compared to sucralose and tap water (p_{Tukey} = 0.002 and p_{Tukey} < 0.001, respectively). No significant main effect of preload was found for the perceived liking of the preloads (F (3, 19) = 1.30, p = 0.304). None of the planned contrast analyses were significant.

Perceived Liking of the Test Meal

No significant main effect of preload was found for perceived liking of the test meal (F (3, 19) = 1.45, p = 0.260). None of the planned contrast analyses were significant.

4.3.5 Discussion

In this double-blinded, four-way crossover study in healthy participants, the effects of oral erythritol on energy intake compared to sucrose, sucralose, or tap water during a subsequent *ad libitum* test meal were investigated. The results can be summarized as follows: (1) The energy intake from the *ad libitum* test meal and the total energy intake (preload + *ad libitum* test meal) were significantly lower after erythritol compared to sucrose, sucralose, or tap water. (2) Erythritol led to a significant increase in CCK compared to sucrose, sucralose, or tap water before the start of the *ad libitum* test meal. (3) Glucose and insulin concentrations were significantly lower after erythritol compared to sucrose with no significant difference between erythritol and sucralose or tap water.

The role of artificial LCS and their impact on obesity and T2DM is highly debated; the alternatives, such as low-caloric bulk sweeteners (e.g., erythritol), are more intensely researched. Overduin et al. [280] partially replaced sucrose by erythritol in a test breakfast and reported that the energy intake during the subsequent *ad libitum* test meal was similar between the two breakfasts (sucrose or sucrose + erythritol). Moreover, the release of GLP-1 and PYY including appetite-related sensations were comparable between the sucrose and sucrose + erythritol test breakfast [280]. Our results are different as we show a significantly reduced energy intake after the oral intake of erythritol alone compared to sucrose suggesting that the satiation effect of erythritol is greater than that of sucrose.

Artificial LCS are frequently used in foods and beverages. In a recent meta-analysis, which included several human studies, Lee et al. [239] analyzed the effects of unsweetened preloads and preloads sweetened with either LCS or caloric sugars on subsequent energy intake. The total energy intake after unsweetened preloads or after preloads sweetened with LCS followed by an *ad libitum* test meal was lower compared to preloads sweetened with caloric sugars. Of note, the energy intake without the calories of the preloads was greater for the unsweetened and LCS-sweetened preloads compared to the preloads with caloric sugars, with no significant differences between the unsweetened and LCS-sweetened preloads. The authors conclude that the caloric differences of the preloads rather than differences in sweetness account for the results. The preloads with caloric sugars possibly resulted in a satiation effect during the *ad libitum* test meal [239, 425]. The meta-analysis included studies with different designs:

(1) various LCS were included, including artificial and bulk LCS and caloric sugars, and (2) the test meal composition and the time between preload and test meal were variable. All these factors can influence individual effects on energy intake. It is therefore interesting to note that the results indicate similar trends as found in the present study, although not formally statistically tested, as follows: the energy intake after caloric preloads is decreased compared to LCS-sweetened (e.g., sucralose) or unsweetened preloads (water) but the total energy intake is greater due to the calories of the preload. These studies highlight a notable difference between the artificial LCS sucralose and the natural bulk sweetener erythritol. Artificial LCS and water were not able to induce satiation and reduce energy intake during *ad libitum* test meals [239]. Erythritol, on the other hand, seems to induce a satiation effect comparable to that of sucrose as shown by Overduin et al. [280] and indicated in the present study.

A possible explanation for the differences in energy intake between erythritol and sucrose or sucralose might be the secretion of GI satiation hormones. In the present study, oral erythritol resulted in a strong CCK release until time point 15 min during the *ad libitum* test meal. These results are in line with previous studies [103, 290, 406] and might partially explain the reduced energy intake. A recent review reported that CCK and its analogues have a significant effect on satiation [426]. In fact, not only CCK but also GLP-1 and PYY are linked to a reduced energy intake and, in addition, with a delay in gastric emptying [42, 59, 384, 386]. For erythritol, a reduction in gastric emptying has previously been observed [290, 406] and seems to contribute to a reduced energy intake. Sucrose can both stimulate GLP-1 and PYY release [192, 193, 280] and induce a delayed gastric emptying in humans [427]. In this trial, we only observed a small release of CCK in response to sucrose compared to erythritol before the start of the *ad libitum* test meal. In the present study, sucrose also affected satiation because the subsequent energy intake was lower compared to sucralose and tap water. In contrast, sucralose does not stimulate the release of GI satiation hormones in humans [28, 188, 194, 232] as confirmed in the present study. The observation is in line with the meta-analysis by Lee et al. [239]. The detailed mechanisms of the GI satiation hormone secretion (especially CCK and GLP-1) in response to erythritol are still unknown. One hypothesis was the stimulation of GLP-1 secretion via the activation of the sweet taste receptor located on enteroendocrine cells (EECs) in the gut as previously shown for glucose [159]. However, inhibiting the sweet taste receptor did not affect the erythritol-stimulated GLP-1 release [406]. Another possible mechanism involves the

sodium-glucose transporter-1 (SGLT-1) for glucose-induced GLP-1 release [197, 199, 201]. However, studies to date with erythritol and SGLT-1 are lacking. In addition, at least in mice, GLP-1 induced satiation requires vagal CCK receptor activation [154].

Another possible explanation for the difference in energy intake between erythritol and sucralose might be the differences in neuroepithelial circuits. A previous hypothesis suggested that the brain largely senses nutrients via the passive release of GI hormones [428]. However, Bohórquez et al. [91] found a neuroepithelial circuit where EECs synapse with vagal neurons. This gut-brain circuit enables the transduction of sugar signals in milliseconds by using the neurotransmitter glutamate [92]. They call this EEC innervation neuropod cells [91]. Recently, the same research group reported that the preference for sucrose over sucralose in mice depends on duodenal neuropod cells [429]. These neuropod cells convey signals to the vagus nerve by using two individual neural pathways. While sucralose activates the sweet taste receptor subunit T1R3 to promote the release of adenosine triphosphate (ATP), sucrose enters the neuropod cell via the SGLT-1 and stimulates the release of glutamate [429]. Thus, to discern sucrose from sucralose, glutamatergic signaling is necessary [429]. Whether these mechanisms apply to guide nutritive choices and have an impact on subsequent energy intake in humans remains to be determined. Therefore, more research is required to investigate whether these neuroepithelial mechanisms are applicable to erythritol and transferable to humans.

In the current study, glucose and insulin concentrations were affected neither after oral erythritol nor after sucralose intake before the *ad libitum* test meal, supporting results in previous studies [103, 430]. Additionally, the oral intake of erythritol over 7 weeks had no effect on glycemic control (unpublished, Meyer-Gerspach et al., 2018), nor on glucose absorption [298]. However, when sucralose was administered together with carbohydrates (typical scenario in a real-world setting), insulin sensitivity was decreased in healthy humans [256, 257]. An upregulation of SGLT-1 and glucose transporter 2 (GLUT2) (as observed in mice) might be an explanation, which results in an increased glucose absorption [246, 423]. The hypothesis has yet to be tested in humans.

The strengths of our study comprise the study design (randomized, controlled, double-blinded, cross-over design), which reduces interindividual variability as well as the comparison of erythritol to one of the most widely used sugars, sucrose, and the artificial LCS, sucralose.

Some limitations of the study require consideration. First, only acute effects of preloads on subsequent energy intake were investigated. The effect of chronic exposure needs to be investigated. Hence, the results cannot be extrapolated to chronic intake. Second, the intake of a preload in the form of a liquid drink and not in the form of a solid snack can influence satiation due to different effects on the cephalic phase of ingestion. Third, the comparison to other energy intake studies is difficult since differences in design, such as the time points between preloads and test meal intake as well as their compositions, have major impacts on satiation and energy intake. Nonetheless, the results are relevant because they show novel insights into two sweeteners and their effects on energy intake representing an every-day scenario.

In conclusion, we show that oral erythritol given before an *ad libitum* meal induces the release of the GI satiation hormone CCK and reduces subsequent energy intake compared to sucrose, sucralose, or tap water. Moreover, erythritol has no effect on glucose and insulin concentrations supporting a role as a useful sugar alternative.

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Chapter 5

General Discussion and Conclusion

5 General Discussion and Conclusion

This PhD thesis comprises the preparation, conduct and analysis of two randomized, placebo-controlled, double-blind, crossover studies called PolyAlluLac and PolyFoodIntake. The first aim of this thesis was to investigate the importance of the sweet taste receptor T1R2/T1R3 for the release of CCK, GLP-1, and PYY in response to intragastric administration of erythritol and D-allulose by assessing the effect of lactisole on these responses. Moreover, we studied the metabolic effects and safety aspects of acute intragastric administration of erythritol and D-allulose on glucose, insulin, ghrelin, blood lipids, uric acid, and hsCRP concentrations. Knowing that erythritol provides zero calories, similar to artificial LCS, but in contrast induces the release of CCK, GLP-1, and PYY and reduces ghrelin concentrations, we wanted to assess whether this GI satiation hormone release affects energy intake. To this purpose, the second aim of the present thesis was to investigate the effect of oral administration of erythritol on subsequent energy intake.

Besides summarizing, discussing and interpreting the main results of the studies, this chapter provides additional details concerning the potential underlying mechanisms involved. Furthermore, the implications of the examined substances as well as potential applications and necessary considerations will be discussed. Finally, the outlook for this field of research will be presented.

5.1 PolyAlluLac Part I

5.1.1 Main Findings

Prior studies showed that erythritol induces the release of GI satiation hormones [103, 280, 290]. The mechanism underlying the secretion of these hormones is unknown. In 2011, Gerspach et al. [159] showed that glucose stimulated the release of CCK, GLP-1, and PYY in part via activation of the sweet taste receptor T1R2/T1R3 located on EECs in the intestine in humans; lactisole, a competitive inhibitor of the T1R3 subunit, attenuated the glucose-stimulated release of GLP-1 and PYY, whereas CCK release was unaffected. We, therefore, used lactisole to investigate whether the secretion of GI satiation hormones in response to

erythritol and D-allulose is mediated via T1R2/T1R3. Moreover, we aimed to study the effect of the T1R2/T1R3 blockade on gastric emptying, appetite-related sensations, and GI symptoms.

Erythritol and D-allulose both significantly stimulated the release of CCK, GLP-1, and PYY, whereas lactisole had no effect on the erythritol- and D-allulose-induced GI satiation hormone secretion. Erythritol delayed gastric emptying rates which was not the case for D-allulose. The lack of effect of lactisole suggests that D-allulose and erythritol induce the release of GI satiation hormones via different receptor/transporter mechanisms than the sweet taste receptor in the gut.

5.1.2 Potential Mechanisms

Besides the already discussed transporters SGLT-1 or GLUT5, other mechanisms such as L- type Ca^{2+}-channels have been proposed to stimulate GLP-1 and CCK release upon glucose administration *in vitro* [200, 201]. Apart from glucose, further nutrients (amino acids and fats) are known to contribute to GI satiation hormone release [85, 152, 161, 431]. These nutrients bind i.e. to the CASR expressed in CCK secreting I-cells, which leads to CCK release [431, 432]. In addition, some studies suggest that the secretion of GLP-1 depends on synergism with other GI satiation hormones [152-154]. Just recently, Vana et al. [154] investigated whether CCK might influence the release of endogenous GLP-1 in response to a meal in mice. They found that prior CCK secretion is important for GLP-1 to suppress eating and that the intestinal free fatty acid receptor 1 plays a central role in inducing CCK release [154]. Of note, it has been shown that fat-induced GLP-1 and PYY secretion is mediated via CCK and CCK-1 receptors in humans several years ago [85, 152].

Although the PolyAlluLac study focused on a gut mechanism underlying the secretion of GI satiation hormones, it is conceivable that gut-brain neural circuits are also potentially involved. In addition to the endocrine and paracrine modes of action of GI hormone release, another form of action, releasing hormones from neuropod cells that form synapses with cells of the enteric nervous system and other cell types, has been described for CCK and PYY cells [91-93]. Indeed, recent rodent data showed that CCK-labeled neuropod cells are responsible for synapsing with the vagus nerve using neurotransmitters to transduce sugar stimuli from the gut to the brain within milliseconds [91, 429]. Interestingly, sucrose and the artificial LCS sucralose

use different neurotransmitters and receptors/transporters. Sucralose activates the sweet taste receptor subunit T1R3 to induce the secretion of ATP, sucrose enters the neuropod cell via the SGLT-1 and promotes the release of glutamate [429]. This again highlights that sweeteners are rather a heterogenous than a homogenous group and should be investigated separately. Nevertheless, whether these discussed mechanisms account for the erythritol-and D-allulose-induced GI satiation hormone secretion remains to be determined. Important to note, most of these studies have limited translatability to humans and therefore the mechanism for the secretion of GI satiation hormones in response to alternative sweeteners needs further investigation.

Another hypothesis is that duodenal osmolarity might be associated with CCK, GLP-1 and PYY secretion [433]. However, when using sweeteners in a study one can either control for i) calories (which was not necessary in the PolyAlluLac study); ii) sweetness, or iii) osmolarity. To answer the research question, whether the sweeteners under investigation are suitable as sugar alternatives, we chose sweetness: 50 g erythritol correspond to around 33.5 g sucrose typically found in sweet beverages and 25 g (the recommended maximal single dose and a little less than a typical sweet beverage) [290]. In the end, the sweetening power must remain the same, rather than osmolarity or calories. Of note, in a previous dose-ranging study, the test solution with the lowest concentration (10 g) was close to being iso-osmolar (272.7 mOsmol/L), yet clearly induced the release of CCK and GLP-1 [290].

Although we did expect that D-allulose might slowdown gastric emptying – similar to erythritol but especially in view of the observed effect on the GI satiation hormones – we were not able to confirm our hypothesis. A possible explanation for the missing effect of D-allulose on gastric emptying is discussed in the following: Strunz et al. [434] showed that the ^{13}C-octanoate breath test method could be sensitive to variation in hepatic metabolism. They suggested that accelerated $^{13}CO_2$-clearance might be due to increased beta-oxidation in the liver [434]. Interestingly, it has been reported that D-allulose regulates hepatic metabolism such as fatty acid beta-oxidation in rats [435]. Data in humans are not yet available. Based on these results, changes in the beta-oxidation in response to D-allulose might explain the missing effect on gastric emptying. However, there are also several limitations to this explanation: First, the study by Strunz et al. was conducted under hypoxic conditions and the assessment of gastric emptying was done after a test meal (^{13}C-octanoate breath test method) and not – as in the PolyAlluLac

study – after a liquid solution (^{13}C-sodium acetate breath test method); and second, more studies are necessary to understand metabolomics of D-allulose especially in humans.

5.2 PolyAlluLac Part II

5.2.1 Main Findings

The second part of the study focused on metabolic effects and safety aspects of acute intragastric administration of erythritol and D-allulose. We found that glucose and insulin concentrations were lower after D-allulose compared to tap water, but Bayesian models showed no difference for insulin in response to D-allulose compared to tap water, there was no effect after erythritol; an exploratory analysis showed that ghrelin concentrations were reduced after erythritol compared to tap water. Moreover, both alternative sweeteners had no effects on blood lipids, uric acid and hsCRP.

5.2.2 Potential Mechanisms

The mechanism behind the reduction of glucose in response to D-allulose is not known yet. An *in vitro* study showed that D-allulose inhibited the uptake of 2-deoxyglucose and fructose in Caco-2 cells [436]. In an *in vivo* animal experiment the same research group found that 58% fructose + 5% D-allulose compared to 58% fructose during a 15-week intervention period resulted in lower body weight gain, fat storage, as well as leptin in the D-allulose-containing group. This indicates that the reduced glucose concentrations are potentially due to a competition between the uptake of sugars and D-allulose but do not exclude other mechanisms [436]. However, in our study D-allulose was administered without any other nutrients, and thus, more research is needed to understand the underlying mechanism. Moreover, the lack of effect on ghrelin concentrations in response to D-allulose reflects the gastric emptying rates reported in PolyAlluLac Part I. This could be due to several potential mechanisms, as previously discussed.

5.3 PolyFoodIntake

5.3.1 Main Findings

In this study we investigated the effects of oral administration of erythritol compared to sucrose, sucralose, or tap water on energy intake, CCK, glucose and insulin, and appetite-related sensations. We found that erythritol significantly reduced (total) energy intake and induced a stronger increase in CCK before the *ad libitum* test meal compared to the other substances. Erythritol had no effect on glucose and insulin concentrations and the feelings of hunger were lower compared to tap water before the start of the *ad libitum* test meal.

5.3.2 Potential Mechanisms

Our findings raise the question whether adaptive processes towards the release of CCK may occur with a chronic intake of erythritol. Animal studies showed that continuous infusions of CCK contributed to a loss of effectiveness on energy intake and satiation of CCK in the long-term [115-118]. Moreover, a study investigating the effects of two CCK-agonists reported that these two compounds initially decreased energy intake and body weight compared to the control in rats [437]. However, there was a trend towards regained body weight over time, indicating that, similar to CCK, tolerance to the acute effects of these compounds may develop [437]. Thus, the inhibitory effect of CCK on energy intake seems to be attenuated in animal models. Whether this occurs with chronic erythritol administration in humans is currently unknown.

In addition, the study revealed that subjective appetite-related sensation ratings in response to the different preloads may not consistently predict subsequent *ad libitum* energy intake. Only hunger was reduced in response to erythritol compared to the other preloads before the start of the *ad libitum* energy intake, while no differences were observed in prospective food consumption, satiety, and fullness. These findings are consistent with those of a review by Holt et al. [438], which cautioned against drawing conclusions about the relationship between self-reported appetite scores and prospective energy intake. Nevertheless, using VASs to evaluate appetite-related sensations is a sensitive, reliable, and valid approach under controlled conditions [40, 377].

5.4 Implications, Applications, and Considerations

Up to date, both erythritol and D-allulose are efficacious as sugar alternatives and elicit a favorable safety profile in acute studies. As a next step, chronic and more long-term safety studies are necessary, especially in individuals with obesity and associated NCDs to assess whether the observed effects sustain.

As stated in the introduction, excessive sugar consumption has risen globally which is the leading cause of many health issues including obesity and T2DM. Research has been focused on finding optimal and natural sugar substitutes that mimic the satiating effects of caloric sugars without their negative effects on health outcomes. Based on our findings, especially for erythritol and its effect on subsequent energy intake, its application in real-life could be particularly beneficial for individuals who are looking to reduce their caloric intake and manage their weight as well as blood glucose concentrations, without compromising on the sweet taste. Whether our examined substances, both erythritol and D-allulose are safe and applicable sugar alternatives in the long-term needs further investigation. For D-allulose in particular, we need more well-designed human intervention studies for approval in the EU. Commercialization of this rare sugar could also be of great value in mitigating the risk of obesity and associated NCDs.

5.5 Limitations and Strengths

Some limitations of the projects in the current thesis require consideration. First, both studies were acute trials and do not allow the investigation of chronic effects of erythritol and D-allulose. Moreover, there could be some unidentified side effects that may occur under long-term treatment. Second, the PolyAlluLac study used an intragastric administration of the two alternative sweeteners, which may limit the translatability to real-life consumption and could have affected appetite-related sensations. Comparing erythritol and D-allulose to a sucrose solution would have been informative. Third, comparing the PolyFoodIntake study to other energy intake studies was challenging because differences in design, such as the time points between preloads and test meal intake as well as their compositions, have a significant impact on satiation and energy intake. Forth, we did not record the energy intake of the participants after the study visit. Whether the reduced energy intake in response to erythritol would have been compensated throughout the day needs to be assessed in the future. Finally, other studies

found differences in energy intake in response to preloads between female and male participants as well as GI hormone secretion [194, 439]. Although the PolyFoodIntake study included the equal number of male and female participants, we did not perform a separate statistical analysis given the rather small sample size. However, future studies should investigate whether there is a difference between females and males in response to oral erythritol.

Nonetheless, the results of the studies presented in this thesis are relevant because they provide novel insights into several alternative sweeteners and their effects on metabolic parameters. One strength is the study design used in both studies. The randomized, controlled, double-blinded, cross-over design reduces interindividual variability and enhances the reliability of the results obtained. Furthermore, the inclusion of D-allulose is particularly noteworthy, given that this alternative sweetener is currently under review for approval in the EU. The addition of relevant evidence to the literature on D-allulose is therefore valuable. Moreover, we did show that erythritol administered as an oral preload evokes not only a satiating effect by releasing GI satiation hormones but also reduces energy intake. The PolyFoodIntake study limits the paradigm that only nutrients containing calories are able to induce a satiating effect and reduce subsequent energy intake. In addition, we did not find a difference in liking in response to erythritol compared to the sucrose or sucralose preloads. This is particularly important in terms of acceptability of erythritol as a sugar alternative.

5.6 Conclusion and Outlook

This thesis was able to fill several relevant research gaps. In brief, the conducted studies were able to show: i) D-allulose induces the release of GI satiation hormones in healthy humans, ii) the sweet taste receptor T1R2/T1R3 in the gut is not involved in the erythritol- and D-allulose-induced secretion of CCK, GLP-1, and PYY, iii) erythritol and D-allulose display several positive metabolic properties and safety aspects in the acute setting (i.e. no increase of glucose and insulin concentrations, no effect on blood lipids, uric acid and hsCRP), iv) the non-caloric bulk sweetener erythritol reduces subsequent energy intake which is potentially due to the stronger increase of CCK compared to sucrose, sucralose, or tap water. Whether these acute effects sustain in long-term studies, needs to be investigated.

Together these findings emphasize that alternative sweeteners (low-caloric bulk sweeteners, artificial LCS, or rare sugars) are not inert and should be investigated separately rather than evaluating them as a group as they all differ in their physiological effects. Additionally, further mechanistic studies are necessary to understand the satiating effects of these alternative sweeteners. Short-term studies have shown that erythritol and D-allulose can have beneficial effects on several metabolic outcomes, but long-term controlled clinical trials are needed to confirm their efficacy in reducing risk factors for obesity and associated NCDs. Moreover, it is crucial to clarify the connection between circulating erythritol and CVD, and whether the increases in endogenous erythritol are due to excessive sugar consumption impairing glycemia or PPP dysregulation.

If long-term studies show positive results for erythritol and D-allulose, such as in relation to safety aspects or the gut microbiome, these alternative sweeteners have the potential to become promising sugar alternatives. Nevertheless, it is important to recognize that even if erythritol and D-allulose may not have any observable effects, this is still a favorable outcome in comparison to the established negative impacts associated with high and excessive sugar consumption. In this context, erythritol and D-allulose could be functioning as a simple, effective, and low-cost approach that can be used both preventively and therapeutically in the fight against the obesity pandemic and associated NCDs.

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