Artificial sweeteners and stevia; the solution for the obesity problem?

The effect of artificial sweeteners and stevia on the energy balance

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Medical Biomics, UMCG
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Groningen, February 4, 2011
Artificial sweeteners and stevia: the solution for the obesity problem?
Summary

Background: Obesity has reached epidemic proportions globally; at least 300 million adults are clinically obese and more than 1 billion adults are overweight. Obesity is a major contributor to the global burden of chronic diseases. At the same time, there is an increased percentage of regular non-caloric artificial sweeteners (AS) users and there is also an increased number of new food products containing AS. Stevia, a rising natural sugar substitute, is promised to be a healthier and better sweetener than AS.

Aim: A lot of research about the possible effects of AS in relation to weight gain has been done. However, the information is ambiguous. Because of the ambiguous information about AS, and the unfamiliarity with stevia, we will attempt to give an overview of the scientific information currently available in relation to the energy balance.

Formulation of the problem: What is known about the contribution of AS to a positive energy balance in humans? What is known about the contribution of stevia to a positive and negative energy balance in humans, and what is known about its side-effects?

Methods: The three databases Pubmed, ScienceDirect and Scopus were used for the literature search. The combination of search terms used for AS is: (artificial sweetener OR sweetening agent OR high intensity sweetener OR sugar-free sweetener OR non-nutritive sweetener) AND (positive energy balance OR weight gain). The combination of search terms used for stevia is: (stevia OR stevioside OR steviol OR stevia rebaudiana OR rebaudioside A). Subsequently, the articles which were relevant for the research were selected. Also articles from the references of the studies were selected.

Results: AS decrease the feeling of satiety, influence the food reward system and disturb the predictive relationship between sweet taste and calories. This all might lead to a higher energy intake, which can cause a positive energy balance. Intervention studies which examined the effect on energy balance in animals showed an increase in energy intake and bodyweight. However, intervention studies in humans showed a decrease in energy intake and bodyweight. Both rebaudioside A and stevioside are completely converted into steviol by bacterial intestinal flora. Stevioside and rebaudioside A do not alter energy intake and bodyweight in rats and humans. Stevioside reduces the postprandial blood glucose and fasting glucose levels in obese and non-obese, type 2 diabetic rats. The postprandial glucose levels are lowered in lean and obese humans after consumption of a preload containing stevioside. Stevioside decreased the blood pressure in non-obese, obese, type 2 diabetic and hypertensive rats, as well as in hypertensive humans.

Conclusion: It seems to be that AS probably do not cause a positive energy balance in humans. However, based on the number of studies, the limitations of the studies, and the duration of the interventions, we can not conclude this with certainty. Steviol glycosides do not alter the energy intake and body weight. The main side-effects of stevioside might be that stevioside has a hypoglycemic effect in type 2 diabetic patients, and a hypotensive effect in hypertensive humans.

Keywords: Artificial sweeteners, stevia, energy balance
Preface

This thesis is the result of a literature study about artificial sweeteners and stevia and the effect on the energy balance. This literature study is accomplished for the specialization of the department Nutrition and Dietetics, School of Health, Hanze University Groningen and performed for the department ‘Nutrition and Metabolism’ of the University Medical Center Groningen (UMCG).

The aim of the study is to give an overview of the scientific information currently available, about the use of artificial sweeteners and stevia in relation to the energy balance.

We would like to thank our supervisor of the UMCG, dr. Marion G. Priebe, for helping us with this research study. Further we thank mrs. Martine J. Sealy.

Groningen, February 4, 2011

Kieneker L.M.
Tammenga J.W.
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Chapter 1: Introduction

Introduction
Obesity has reached epidemic proportions globally; at least 300 million adults are clinically obese and more than 1 billion adults are overweight. Obesity is a major contributor to the global burden of chronic disease and disability (WHO, 2003). At the same time, there is an increased percentage of regular non-caloric artificial sweeteners (AS) users and there is also an increased number of new food products containing AS (Yang, 2010).

A lot of research about the possible effects of AS in relation to weight gain has been done. However, the information is ambiguous. On one hand, AS contain none or little calories and may assist with weight control (Rodearmel et al., 2007). On the other hand, AS could influence processes in the body; it may, for example, stimulate appetite and thereby lead to weight gain (Anton et al., 2010). The media promote light products with AS as a solution for the obesity problem. If the use of AS does not stimulate weight loss, this is important to know.

Stevia, a rising natural sugar substitute, is promised to be a healthier and better sweetener than AS. There is not much known about stevia in the UMCG, therefore stevia is an important part of this thesis.

1.1 Formulation of the problem
Because of the ambiguous information about AS, and the unfamiliarity with stevia, we will attempt to give an overview of the scientific information currently available. We want to accomplish this by answering the following two questions:

- What is known about the contribution of artificial sweeteners to a positive energy balance in humans?
- What is known about the contribution of stevia to a positive and negative energy balance in humans, and what is known about its side-effects?

1.1.1. Explanation of the formulation of the problem
We want to examine the possible mechanisms in the body which could lead to weight gain instead of weight loss after consumption of AS. Therefore, we decided to focus on the positive energy balance. We want to focus on both the positive and negative energy balance for stevia, because we want to take into consideration all possible effects of it. We also look at the possible positive and negative side-effects of stevia.

1.2 Structure of the thesis
This thesis comprises six chapters. Chapter one is the general introduction. It contains the formulation of the problem, the aim of the study and some background information about AS and stevia. Chapter two describes the methods of the study. It explains how we selected the databases and how we accomplished our literature search. The results of the study are shown in chapter three and four. Chapter three is about AS and the effect on the energy balance and the possible mechanisms underlying this effect. In chapter four, we present the results of stevia; the safety of stevia, the effect it has on the energy balance and the possible side-effects of stevia. Chapter five contains the discussion and chapter six the conclusion of the study. A list of abbreviations is admitted in appendix I.

1.3. Background information
1.3.1 Artificial sweeteners
Several AS are discovered by scientists who tasted their samples, often by accident. For years, saccharin was only used as a medicinal product for diabetics. Since the sugar shortage in World War II and later on when a thin figure became more important, AS were used for more than medicinal purposes alone. A large increase in the number of products containing AS was noted in the last decade (Yang, 2010).
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Names
AS are known by several names, including: non-sucrose sweeteners, low-calorie sweeteners, intense sweeteners, high-intensity sweeteners, sugar-free sweeteners and sugar substitutes (Brown et al., 2010). The American Dietetic Association (ADA) uses the name non-nutritive sweeteners. This name is disputable for aspartame because this sweetener is metabolized and therefore provides energy, although the amount is marginal (Kroger et al, 2006).

Safety
At the moment 5 non-nutritive sweeteners are accepted by the Food and Drug Administration of the United States (FDA): sucralose, saccharin, acesulfame-K, neotame and aspartame. In 1958 cyclamate was generally recognized as safe. However in 1969, it was banned in the United States because high concentrations of it were possibly related to bladder cancer in rats. In Europe and in more than 100 other countries, cyclamate is approved. The Joint Commission of Experts on Food Additives (JECFA), the Food and Drug Administration (FDA) and the European Food Safety Agency (EFSA) have established acceptable daily intakes (ADI) (Mattes & Popkin, 2009).

Most of the sweeteners are excreted unchanged. Aspartame, on the other hand can be metabolized. Therefore, it is not totally non-caloric (4 kcal/g). However, the quantity of aspartame needed to produce a sweet taste is so small that the caloric contribution is negligible. Because aspartame is metabolized to phenylalanine it is forbidden in people with phenylketonuria (Yang, 2010).

Table 1.1: Potency, ADI and year of discovery of approved NNS

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Caloric value (kcal/g)</th>
<th>Potency (times sweeter than sucrose)***</th>
<th>ADI mg/kg/day</th>
<th>Year of discovery****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acesulfame-K</td>
<td>0</td>
<td>200 x</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Aspartame</td>
<td>4</td>
<td>160-220x</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Cyclamate *</td>
<td>0</td>
<td>30x</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Neotame **</td>
<td>0</td>
<td>7000-8000x</td>
<td>0-2</td>
<td>1</td>
</tr>
<tr>
<td>Saccharin</td>
<td>0</td>
<td>200-700x</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Sucralose</td>
<td>0</td>
<td>300-600 x</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

* This sweetener is not approved in the United States
** This sweetener is not approved in Europe
*** Kroger et al., 2006; ADA, 2004
**** Yang, 2010; Mattes & Popkin, 2009

1.3.2 Stevia
Plant and history
Stevia is the common name for the plant Stevia Rebaudiana Bertoni, which is a shrub of the Asteraceae family. The genus stevia comprises at least 110 species, with stevia Rebaudiana Bertoni the sweetest of all. When stevia is fully matured, it can reach 80 cm in height (Goyal et al., 2010). The dried leaves of stevia were used to sweeten tea, for medicinal purposes, or to chew the leaves as a ‘sweet treat’ (Chatsudthipong & Muanprasat, 2008; Goyal et al., 2010). Since 1887, stevia became more widely known outside South America because of the discovery of stevia by botanist Antonio Bertoni. Because of the ban on saccharin in the 1970s in Japan, stevia is used as a replacement of it. Therefore, stevia is best known for its use in Japan. In the 1970s and 1980s, stevia also began to appear in North America and Europe. Stevia is inexpensive and therefore for most consumers available (Anton et al., 2010).

Other names for stevia are honey leaf, sweet leaf of Paraguay, sweet herb, candy leaf and honey yerba (Carakostas et al., 2008).
Components of stevia
The plant contains a number of glycosides (table 1.1). The major sweet components are rebaudioside A and stevioside. Rebaudioside A is the sweetest, the most stable and is less bitter than stevioside (Goyal et al., 2010).

The structure of stevioside and rebaudioside A is almost the same, they only differ by the presence of one additional glucose moiety on rebaudioside A (Maki et al., 2008) (see figure 1.1). Rebaudioside A and stevioside are both heat stable and non-fermentable (Prakash, 2008). Rebaudioside A and stevioside might be appropriate for diabetic and phenylketonuria patients (Geuns, 2003).

<table>
<thead>
<tr>
<th>Component</th>
<th>Sweetness compared to sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dulcoside A</td>
<td>30-120 x</td>
</tr>
<tr>
<td><strong>Rebaudioside A</strong></td>
<td><strong>200-450 x</strong></td>
</tr>
<tr>
<td>Rebaudioside B</td>
<td>150-350 x</td>
</tr>
<tr>
<td>Rebaudioside C</td>
<td>30-120 x</td>
</tr>
<tr>
<td>Rebaudioside D</td>
<td>220-450 x</td>
</tr>
<tr>
<td>Rebaudioside E</td>
<td>150-300 x</td>
</tr>
<tr>
<td>Rebaudioside F</td>
<td>200 x</td>
</tr>
<tr>
<td>Rubusoside</td>
<td>114 x</td>
</tr>
<tr>
<td>Steviolbioside</td>
<td>90-125 x</td>
</tr>
<tr>
<td><strong>Stevioside</strong></td>
<td><strong>200-300 x</strong></td>
</tr>
</tbody>
</table>

(Chatsudthipong and Muanprasat, 2008, Prakash, 2008)

Table 1.2: Components of stevia

![Stevioside and rebaudioside A](image-url)
Chapter 2: Methods

Introduction
In this chapter we will describe our systematic literature search. In our study we try to answer two research questions. Therefore two search strategies were applied. One strategy for AS and one for stevia. This chapter exists of three phases. In phase one several databases and search engines were compared. Further, we describe our search terms and in- and exclusion criteria. In phase two we explain how we selected our articles. In phase three we describe how we performed the reference search.

2.1 Phase 1: the search
2.1.1. Databases and search engines
Eight databases and search engines were compared: EBSCOhost, Embase, Hub, Pubmed, ScienceDirect, Scopus, Web of Knowledge and Wiley. The advantages and disadvantages of them were compared. See Appendix II for the comparison of the databases and search engines. Three databases were chosen: Pubmed, ScienceDirect and Scopus. These three databases contain the most relevant hits for our research. Furthermore, we were already familiar with Pubmed.

2.1.2 Search terms
Artificial sweeteners
We wanted to find out if there are mechanisms which could influence the energy balance after consumption of AS. Because we did not know which mechanisms could be involved in this process, we were obliged to use a broad search strategy in the beginning. We searched with combinations of related terms and synonyms of AS, natural sugar substitutes, table sugar and energy balance. Out of these results, we selected several articles about possible mechanisms affecting the energy balance. On the basis of these articles, we searched with more specific terms related to the possible mechanisms, like satiety, taste perception and blood glucose. The articles that were found through this search were very specific and biological, and therefore too complicated for us. We decided that we wanted to focus on the effects of AS on the energy balance instead of underlying complex mechanisms. Our final combination of search terms for AS is:

(artificial sweetener OR sweetening agent OR high intensity sweetener OR sugar-free sweetener OR non-nutritive sweetener) AND (positive energy balance OR weight gain)

Stevia
In comparison to AS there has not been a lot of research done on stevia. We were interested to find out what is known about stevia. That is why we decided to keep a broad search. Our search comprises the following terms:

(stevia OR stevioside OR steviol OR stevia rebaudiana OR rebaudioside A)
The results of the searches are shown in the table below.

<table>
<thead>
<tr>
<th>Database</th>
<th>Hits AS</th>
<th>Stevia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubmed</td>
<td>652</td>
<td>179</td>
</tr>
<tr>
<td>Scopus</td>
<td>370</td>
<td>460</td>
</tr>
<tr>
<td>ScienceDirect</td>
<td>656</td>
<td>476</td>
</tr>
<tr>
<td><strong>Total hits</strong></td>
<td><strong>1678</strong></td>
<td><strong>1112</strong></td>
</tr>
</tbody>
</table>

*Table 2.1: Hits of AS and stevia in the databases*

### 2.1.3 In- and exclusion criteria
- All articles must be in English
- Articles published before 2000 were excluded
- All articles must be complete (including date and references)

### 2.2 Phase 2: the selection
In this phase, we explain how we selected our articles. We used the same procedure for both AS and stevia. After our final searches we organized our hits by using Reference Manager. We filtered the hits by removing the double titles and book chapters. Next, we read all the abstracts and selected the titles which we thought were relevant. We searched for the full text articles of the selected titles and looked whether they were complete and useable. After that we organized the articles in subcategories, like energy balance, metabolism and safety. An overview of the selection of articles about AS and stevia, including the articles of the reference search, can be found in table 2.2.

### 2.3 Phase 3: the reference search
For the reference search we looked at the references of the reviews we selected in phase two. We searched the articles which seemed to be relevant in full text, and in- or excluded them for our research. An overview of the selection of articles about AS and stevia, including the articles of the reference search, can be found in table 2.2.

<table>
<thead>
<tr>
<th>AS</th>
<th>Stevia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hits found in the databases Scopus, Pubmed and ScienceDirect</td>
<td>1678</td>
</tr>
<tr>
<td>Hits Reference Manager without double titles and book chapters</td>
<td>1538</td>
</tr>
<tr>
<td>Selected articles based on title and abstract</td>
<td>60</td>
</tr>
<tr>
<td>Full-text founded articles</td>
<td>48</td>
</tr>
<tr>
<td>Selected full-text articles</td>
<td>22</td>
</tr>
<tr>
<td>Reference search</td>
<td>7</td>
</tr>
<tr>
<td>Included reviews</td>
<td>17</td>
</tr>
<tr>
<td><strong>Total articles</strong></td>
<td><strong>29</strong></td>
</tr>
</tbody>
</table>

*Table 2.2: overview selection AS and stevia*
Chapter 3: Artificial sweeteners

Introduction
This chapter is divided into several subjects. First we describe the possible mechanisms underlying the effect on the energy balance. We look at satiety, the food reward system, functional magnetic resonance imaging studies, coupling of sweet taste and calories and the effect of aspartame on several neurotransmitters. Subsequently, we discuss the relation between AS and weight gain. What is the cause and what is the consequence? In the last part of this chapter we look at the effect of AS on the energy balance.

3.1 Possible mechanisms underlying the effect on the energy balance
Many studies have examined the effect of AS on the energy balance. Regardless whether or not AS influence the energy balance, some studies examined possible mechanisms which may lead to a positive energy balance after consumption of AS.

3.1.1 Effect of AS on satiety signals in the brain

Satiety and satiation
Satiation is the feeling of satisfaction that signals to stop eating. Satiety is the feeling of fullness that persists after eating. Both are important factors in determining total energy intake. The measurement of satiety and satiation is complicated by the fact that beyond satiation and satiety, there are numerous other influences on eating behavior; e.g. the portion size, palatability of the foods, appeal and energy density. Energy density is a characteristic of a food or drink that appears to have the most impact on satiety. Energy density is the amount of energy in food or drinks per unit weight (kJ/g or kcal/g). High-fat foods tend to have a higher energy density than high-carbohydrate and high-protein foods, while foods with the highest water content tend to have the lowest energy density. In soft drinks in which the energy content is provided by sugars, the replacement of sugar with AS produces a large reduction in energy density, to almost zero. In food in which energy is also provided by protein and fat, replacement of sugars with AS has less impact on energy density (Benelam, 2009; Mattes & Popkin, 2009).

Insulin secretion and satiety
Several hormones are secreted from the gut as reaction to the consumption of food. These hormones promote satiety signals on specific areas of the brain. The release of glucagon-like peptide-1 (GLP-1), a gut hormone which is released from endocrine cells in the gut mucosa, is dependent on the presence of nutrients in the lumen of the small intestine. GLP-1 stimulates the secretion of insulin from pancreatic β-cells (Benelam, 2009). The secretion of insulin is an important factor in the regulation of body weight. Insulin and insulin receptors are found in areas in the brain which are associated with energy homeostasis (Benton, 2005; Woods, 2005). The gut hormone cholecystokinin (CCK) also appears to be involved in satiation. The effect of CCK on satiety appears to be mediated via receptors in the vagus nerve. In addition, CCK stimulates pancreatic enzyme secretion and gall bladder contraction, and delays gastric emptying. CCK is also found in the brain, where it is involved in reward behavior by acting like a neurotransmitter (Benelam, 2009). Hall et al. (2003) investigated the effect of aspartame on satiety and the possible role for the satiety hormones CCK and GLP-1 in this effect. Healthy men and women consumed a preload consisting of 400 mg aspartame or 400 mg corn flour (control group). No release of CCK and GLP-1 was noted after the consumption of a preload consisting of aspartame.

Conclusion
Energy density is the most important factor of satiety. When sucrose in food is replaced by AS, the energy density decreases. This decrease in energy density is smaller in foods containing other caloric nutrients, than in foods which contain no other caloric nutrients. When the energy density of food decreases, the feeling of satiety also decreases. CCK and GLP-1 are gut hormones which also influence satiety. After ingestion of glucose, release of these hormones occurs and these hormones
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1.2.2 Effect of AS on food reward pathways

A possible mechanism which can influence the energy balance is the food reward system. Food reward consists of a sensory and a postingestive component. In the sensory component, the taste receptors on the tongue send gustatory information to higher gustatory areas such as the orbitofrontal cortex, insula and amygdala. The amygdala sends impulses to the brainstem for activation of among others dopamine (Yang, 2010). The mesolimbic dopamine system regulates motivation and pleasurable responses to food stimuli and intake, and the feeling of satisfaction after consumption of desirable foods (Benton, 2005; Davis & Fox, 2008; Wang et al., 2009; Yang, 2010).

The metabolic products of food (e.g. glucose) determine the postingestive component by mechanical satiety and neuronal signals. The mediator of the postingestive food reward is the hypothalamus, because the hypothalamus secretes various neuropeptides (e.g. opioid peptides) to regulate feeding behavior, energy and osmotic balance (Yang, 2010). Therefore, both opioid peptides and dopamine stimulate the intake of palatable foods (Benton, 2005).

Overeating may result from a lower reward value of foods (Mattes & Popkin, 2009). What kind of value do AS possess? Does the reward value depend on the palatability of food or rather on energy in food? Yang (2010) suggests that AS do not activate the food reward pathways in the same way as natural sweeteners. Because of a lack of caloric contribution, the postingestive component is not completely activated by the AS. Also the gustatory branch is activated differently by AS. This was concluded after a comparison study of sucrose and saccharin ingestion. Functional magnetic imaging showed a higher activation of the gustatory areas after ingestion of sucrose in comparison with saccharin. Yang (2010) concluded that sweetness decoupled from caloric content offers not complete, but partial activation of the food reward pathways.

Conclusion

After consumption of non-caloric food which contains AS, the gustatory branch, which activates the mesolimbic dopamine system, and the postingestive component, which causes secretion of neuronal signals (e.g. opioid peptides), do not work the same compared to consumption of food containing calories. Dopamine and opioid peptides both affect the feeling of satisfaction after consumption of food. When the postingestive component of the food reward pathway is not completely activated and the gustatory branch is activated differently after consumption of food containing AS instead of e.g. sucrose, a decreased secretion of dopamine and opioid peptides occurs. This leads to a decreased feeling of satisfaction, which leads to higher energy intake and therefore can cause a positive energy balance.

3.1.3 Effect of AS on the hypothalamus, amygdala and striatum

Several functional magnetic resonance imaging studies examined the difference in response of the brain after consumption of AS or caloric sweeteners. Smeets et al. (2005) investigated the effect of sweet taste and energy content on the hypothalamic response. The hypothalamus secretes various neuropeptides (e.g. opioid peptides) to regulate feeding behavior, energy and osmotic balance (Yang, 2010). Further they measured the changes in blood glucose and insulin concentrations. Healthy normal-weight men were scanned four times on separate days with functional magnetic resonance imaging. During the scanning they ingested a solution of 300 ml water (control), an aspartame solution (sweet taste), a maltodextrin solution (non-sweet carbohydrate), or a glucose solution. Only glucose ingestion resulted significantly and prolonged in a signal decrease in the upper hypothalamus. The other solutions had no such effect. Similar increases in blood glucose and insulin concentrations were found by ingestion of glucose or maltodextrin solution. However, only the glucose solution triggered an early increase in insulin concentrations. While no insulin response was triggered by aspartame. Therefore, they concluded that both sweet taste and energy content are crucial for a hypothalamic
response. Similar results were found in a study of Smeets et al. (2010). Healthy, normal-weight men were first scanned after two hours of fasting. Before the second scan, the men ingested 450 ml of orangeade, sweetened with 10% sucrose or sweetened with AS (aspartame, acesulfame-K, cyclamate and saccharin). Before ingestion of orangeade, the amygdala was activated more by orangeade sweetened with AS than by orangeade sweetened with sucrose. After ingestion of the orangeade, no differences in activation of the amygdala were found. Part of the striatum was activated before, but not after ingestion of orangeade sweetened with sucrose, while the AS did not cause any striatal activation. The amygdala has a primary role in the processing and memory of emotional reactions, like eating behavior. The striatum is activated by stimuli associated with reward, by activating dopamine receptors. Therefore they concluded that the brain responds differently to a caloric content and non-caloric content, and consumption of calories can modulate taste activation in the striatum.

Conclusion
Consumption of AS, compared to caloric sweeteners, leads to a different activation of several brain areas. Both sweet taste and calories are needed for a response of the hypothalamus. The amygdala was activated more before consumption of orangeade sweetened with AS, compared to orangeade sweetened with sucrose. This is probably due to the repeated orosensory stimulation with sweet taste. The striatum is not activated after consumption of AS, because the striatum responds to carbohydrates and not to AS.

3.1.4 Coupling of sweet taste and calories
Sweet taste has been identified as a strong elicitor of several pre-absorptive cephalic-phase reflexes related to ingestion (Swithers & Davidson, 2008). Sweet taste is also used in animals to predict the content of calories in food. Eating of artificially sweetened, non-caloric products may lead to a positive energy balance by degrading this predictive relationship. Therefore, Swithers and Davidson (2008) investigated in three experiments if non-predictive relationships between sweet tastes of AS and caloric content may lead to a positive energy balance. In experiment 1, one group of adult, male Sprague-Dawley rats received unsweetened yoghurt for three days in a week and three other days yoghurt sweetened with 0.3% saccharin (sweet non-predictive) for five weeks long. The other group adult, male Sprague-Dawley rats received yoghurt sweetened with 20% glucose (sweet predictive) for three days in a week, and three other days in a week unsweetened yoghurt for five weeks long. Because of the differences in calories, a third group was included for control, which received yoghurt sweetened with glucose for six days in a week for five weeks long (predictive control). Body weight and body adiposity were significantly higher in the non-predictive group of rats after the diet of five weeks, compared to the predictive and predictive control group. This was probably due to disturbing the predictive relationship between sweet taste and calories. Presently, in many food products the predictive relationship between sweet taste and calories is disturbed through the use of AS which mimic the sensory characteristics of sweet taste (Swithers & Davidson, 2008).

Training history
In experiment 2 of Swithers and Davidson (2008), they examined if the strength of the predictive relationship between sweet taste and calories may have an influence on the caloric compensation. Adult, male Sprague-Dawley rats were first trained for fourteen days, they received 30 g of low-fat plain yoghurt, with ad libitum standard lab chow. These rats were also classified in a predictive (seven days unsweetened yoghurt, and the other seven days glucose sweetened yoghurt) and a non-predictive (unsweetened yoghurt for seven days and saccharin sweetened yoghurt for the other seven days) group. After the fourteen days, half of the rats of each group received a premeal of 5 g of a novel sweet diet, with the viscosity of yoghurt. Next, all rats received standard chow and water for three days. The results showed that there were no significant differences in yoghurt intake between the non-predictive and predictive group during the diet of fourteen days. In the three days of chow and water, the rats in the predictive group showed caloric compensation by eating less chow on the day they received the premeal, compared to the other two days, while the rats in the non-predictive group did not compensate. Therefore, the caloric compensation was affected by training history.
Similar results were demonstrated in a study with healthy females. Appleton and Blundell (2007) investigated the effects of AS and sugars on energy intake in habitual high and low consumers of beverages containing AS. All women consumed 330 ml of a non-sweet/low-energy (water, 0 kJ), sweet/low-energy (AS drink, 21 kJ), or sweet/high-energy (naturally sweetened drink, 523 kJ) preload. Energy intake was subsequently measured using ad libitum test meal intake. In females with a low use of artificially sweetened beverages (ASB) (0 ml/d), energy intake was increased in response to sweet taste (water compared to AS), while high users (1 L/d) did not. Low and high users of AS responded similarly to energy. These results suggest that high users of ASB had learnt to dissociate sweetness from energy content, while low users of ASB had not. Low users of ASB compensate the sweet taste by eating less, compared to the high users of ASB. And therefore, previous experience with intense sweeteners may affect energy intake and appetite after their consumption.

Thermogenesis

Reflexive thermogenic response in humans and animals is caused by ingestion of food. The orosensory stimuli signal the absorption of nutrients in the gut, and mediate the form of heat production in part. In the third experiment of Swiethers and Davidson (2008), they investigated the effect of saccharin on the core body temperature. The design of this experiment was equal to experiment 2, besides that the rats were implanted with miniature radio-frequency transmitters before the experiment to measure the core body temperature. The results showed that departures from baseline body temperature were significantly higher in the predictive group when glucose sweetened yoghurt was consumed, than when unsweetened yoghurt was consumed. This was not seen in the non-predictive group. Therefore, energy density is an important determinant of the thermic effect of food. When the novel premeal was consumed in both groups, core body temperature was significantly higher in the predictive group, compared to the non-predictive group. This suggests that experience with AS may disturb this physiological mechanism (Swiethers et al., 2010).

Cephalic phase stimulation

The cephalic phase occurs even before food enters the stomach. It is caused by the thought, sight, taste or smell of food. Neuronal signals that cause the cephalic phase originate from the cerebral cortex and the appetite centers of the amygdala and hypothalamus (Swiethers et al., 2010). The brain sends signals to the body to optimize the digestion of food, the absorption and use of the energy, and nutrients the food content. Some researchers suggested that lack of activation of cephalic phase response may lead to a positive energy balance (Mattes & Popkin, 2009). According to Swiethers et al. (2010), AS decrease the ability of tastes to predict the caloric content. Because sweet tastes are no longer consistent predictors of caloric content, the stimulation of the cephalic phase responses will weaken, with the result of downgrading the energy regulation.

Conclusion

The energy of foods can often be predicted by the sweet taste of it; a predictive relationship. But by eating sweet, non-caloric products containing AS, this predictive relationship may be disturbed. This relationship may also be disturbed by previous experiences with AS consumption. Low users of AS cannot dissociate sweetness from caloric content, whereas high users can. This might lead to a higher energy intake in high users of AS. AS also affect the thermogenesis by lowering the core body temperature, because AS lower the energy density of foods. By disturbing this physiological mechanism, consumption of AS might lead to a higher energy intake. Disturbing the predictive relationship may also be caused through weakening the cephalic phase response, because the sweet taste of AS do not predict the amount of calories. Overall, disturbing the predictive relationship between sweet taste and calories may lead to a higher energy intake, which can cause a positive energy balance.

3.1.5 Effect of aspartame on neurotransmitters

Neuropeptide Y

Neuropeptide Y (NPY) is a neurotransmitter and has been associated with several physiologic processes in the brain, including the regulation of energy balance. The main effect of NPY is an
increased food intake and decreased physical activity by acting on receptors for NPY in the hypothalamus. Neurons that express NPY are stimulated by ghrelin and inhibited by among others GLP-1 and insulin (Benelam, 2009). Beck et al. (2002) investigated the effects of the chronic ingestion of aspartame on NPY concentrations, plasma hormones, food intake and body fat. For a period of fourteen weeks one group of Long-Evans rats received standard chow and water containing 0.1% aspartame solution, while the other group received standard chow with water. After fourteen weeks of diet, the group with water containing aspartame lowered body weight (fat mass), while there was no difference in energy intake or plasma insulin concentrations compared to control. NPY concentrations were significantly lower in the brain. The reason for the decrease of NPY is not clear. This effect on NPY can be applied for aspartame, and probably not for other AS. Because phenylalanine, a component of aspartame, is converted to tyrosine and has influence on the brain neurotransmitters dopamine and norepinephrine. These neurotransmitters are involved in the regulation of energy intake.

**Serotonin**

Serotonin (5-HT) is a neurotransmitter which is involved in the control of eating; it plays an important role in postprandial satiety. 5-HT is derived from tryptophan. The intake of carbohydrates is linked to synthesis of brain 5-HT. When carbohydrates are consumed, insulin is released and causes the uptake of amino acids by peripheral tissues. Simultaneously, the affinity of albumin for tryptophan is increased by insulin. So the ratio of tryptophan to the other amino acids in the blood increases. Therefore, more tryptophan is transported to the brain and it is metabolized into 5-HT which initiates satiety. However, it only occurs when protein offered is less than 5% of the energy. AS do not elicit this regulation. However, aspartame might disrupt this putative regulation because aspartame increases the amounts of phenylalanine and tyrosine levels in blood. The tryptophan/amino acid ratio changes and therefore the levels of brain tryptophan decrease and hence 5-HT synthesis decreases as well. This causes higher carbohydrate intake, which can lead to a positive energy balance (Benton, 2005).

**Conclusion**

Both NPY and 5-HT affect the regulation of energy intake. These effects are contradictory. After consumption of aspartame the level of NPY lowers in the brain, which can lead to a decreased energy intake. Also the level of 5-HT decreases, which can lead to a higher carbohydrate intake.

### 3.2 Effect of AS on the energy balance

#### 3.2.1 Relation AS and weight gain: cause or consequence?

In a study of Appleton and Conner (2001), a group of heavy users of AS was compared with a group of non-users. They compared weight, weight concerns, and eating styles. The group of heavy users of ASB was found to be associated with higher body weights, stronger concerns about body weight, and tendencies toward certain eating styles in comparison to the group of non-users. Phelan et al. (2009) compared the differences in dietary strategies and use of sugar and fat modified foods and beverages between a group of weight loss maintaining subjects (WLM) and a group of continued normal weight subjects. The WLM group reported a higher consumption of water, significantly fewer servings of sugar-sweetened soft drinks, and three times more servings of ASB compared to the NW group. The results suggest that WLM use more dietary strategies to maintain their weight loss, including an increased consumption of ASB. Benton (2005) reviewed several studies and drew a similar conclusion. The subjects expressed their weight concerns by choosing to use AS rather than limiting their total food intake. Therefore it is likely that the increased use of AS was a reaction on obesity rather than a cause. However, the increased use did also not prevent the high incidence of obesity, though it is not clear what would have happened if AS were not used. Further, the article concluded that diet is only one of many factors contributing to obesity, and AS are only one aspect of a diet.

Fowler et al. (2008) examined the relationship between the consumption of ASB and long-term weight gain. Weight, height and ASB consumption were measured among 5,158 humans from 1979 to 1988. 3,682 participants were re-examined seven years later. The consumption of > 21 ASB/week was associated with a nearly doubled risk of overweight/obesity among 1,250 normal-weight individuals at baseline. A doubled risk of obesity was also found among 2,571 individuals with baseline BMI < 30 kg/m². However, the relation between AS use and weight gain may not be causal. AS use might just be
a marker for persons already trying to lose weight, although the weight gain continued in spite of switching to AS. However, there was also a group with normal weight at baseline and a nearly doubled risk of overweight or obesity seven years later. A possible explanation for the weight gain in this group might be that AS support short-term caloric decrease. Therefore the resting metabolism could become lower and long-term weight gain could increase. Although this seems contradictory with the explanation given for the overweight group, that AS might just be a marker for persons already trying to lose weight.

**Conclusion**

AS users have a higher body weight and stronger concerns about it. Weight loss maintaining subjects use also more AS compared to normal weight subjects. However, also in normal weight humans was found an almost doubled risk of overweight or obesity, as well as in humans who were already overweight.

We can not conclude whether the increased use of AS is a reaction on obesity or a cause of obesity based on these observational studies. AS use is only one factor that could contribute to obesity. Is it likely that it will have a huge impact on obesity? An important question still needs to be answered: are AS feeding, rather than fighting, the epidemic they were meant to stop?

### 3.2.2 AS use and caloric compensation

Several articles examined the use of AS and caloric compensation. Do people who use AS compensate for the decrease in calories they ingest?

The effect of preloads with aspartame or sucrose on satiety and food intake was tested in obese and lean individuals. All participants received preloads containing aspartame (290 kcal), or sucrose (493 kcal) before lunch and dinner during three days. Participants did not compensate for the lower preceding energy intake at lunch or dinner when they consumed a preload with aspartame. They did not differ in their ratings of the two preloads in terms of sweetness, aroma, appearance, or texture (Anton et al., 2010). Similar results were found in a study of Holt et al. (2000). The study compared the effects of sugar-free beverages with sugar-rich beverages on feelings of fullness and hunger and the ad libitum consumption of a fat-rich snack. Healthy, normal-weight men received three drinks on separate mornings: sugar-free cola (7 kJ), sugar-rich cola (629 kJ), and mineral water (0 kJ). Twenty minutes after preload the subjects could snack freely on chips for the next ninety minutes. 110 minutes after preload the subjects were able to eat ad libitum from a buffet-style lunch. Further they had to report their food intake during the rest of the day. There were no significant differences among the preloads in the amount of food consumed after lunch until the end of the day. As a result, there were small but non-significant differences in total energy intake. Similar volumes of mineral water, sugar-rich and sugar-free beverages, did not differ in their capacity to suppress the consumption of a fat-rich snack. It is important to mention that it is likely that the participants were not only eating in reaction to their physiological hunger because of the availability of ad libitum chips and lunch. During another study, normal-weight, overweight and obese women had lunch once a week, six weeks long, where they were able to eat ad libitum. The lunch was served with a beverage of 360 g (orange juice, water, regular coke, diet coke, or milk) or without a beverage. Results showed that the type of beverage affected the energy intake. Intake did not differ between the non-caloric groups and the no-beverage group. Similarly, energy intake was not different among the caloric beverages groups. However, they did find a difference between the energy intake of groups consuming a caloric beverage and groups consuming a non-caloric beverage or no beverage. When a caloric beverage was consumed with the meal, the energy intake was higher. Thus no compensation was noticed in the AS group (Della Valle et al., 2005).

**Conclusion**

The studies did not show compensation at lunch or dinner after consumption of a preload containing AS. Further, when a caloric beverage was consumed with the meal, the energy intake was higher compared to consumption of non-caloric or no beverages.
3.2.3 AS and the effect on the energy balance

In a study of Swithers and Davidson (2008) two groups of Sprague-Dawley rats were given either sweetened yoghurt with glucose or with saccharin during fourteen days. Group one, the sweet predictive group, received unsweetened yoghurt for seven days and yoghurt sweetened with 20% glucose for another seven days. Group two, the sweet non-predictive group also received unsweetened yoghurt for seven days. The other seven days they were given yoghurt sweetened with 0.3% saccharin. An increase in caloric intake, an increased body weight and increased adiposity was found in the saccharin group. Similar results were found in a study of Swithers et al. (2009). Again all Sprague-Dawley rats received unflavored low-fat yoghurt for seven days. The other seven days they were given either sweetened yoghurt with 20% glucose or with 0.3% saccharin. This time there was also a third group that received yoghurt sweetened with 0.3% Acesulfame-K. Significantly greater weight gain was found in the saccharin as well as in the Acesulfame-K group compared with rats given the glucose sweetened yoghurt. Because both saccharin and Acesulfame-K affected the energy balance, the article concluded that the weight gain was not caused by the properties of saccharin only. The difference in weight gain between the glucose and AS groups had rather to do with the caloric versus non-caloric nature of the sweeteners.

Contrasting results were found in a human study of Raben et al. (2002). They examined the effect of long-term (ten weeks) intake of foods and drinks containing sucrose or AS on body weight and ad libitum food intake in overweight humans. In the sucrose group, carbohydrate intake and total energy intake increased while protein and fat intake decreased. Body weight and fat mass increased as well. Small but significant decreases in energy density and sucrose intake were found in the sweetener group. Body weight and fat mass decreased. A possible explanation for the increase in body weight and energy intake in the sucrose group is that 70% of the sucrose came from fluids. Energy from fluids is less satiating than energy from solid foods and therefore it may be easier to consume more energy. In a family-intervention study of Rodearmel et al. (2007), families with at least one child with (risk for) overweight were followed during a six-month period. The families were assigned to either an intervention group or a self-monitor group. The families in the intervention group were asked to make two lifestyle changes: to walk 2000 steps per day extra and to decrease their normal diet with 420 kJ/day, by replacing sugar with an AS, sucralose. The families in the self-monitor group were asked to record physical activity but they did not have to change their activity level or diet. Significant decreases in BMI for age were found in both groups. However, the intervention group had a significant higher percentage of children who reduced or maintained their BMI. Further, the intervention group reported significantly more steps per day than the self-monitor group. It is important to mention that the self-monitor group is not a non-intervention control-group. Self-monitoring increases consciousness of physical activity and diet and may therefore lead to behaviour change. The article concluded that sucralose seems to be an effective tool in decreasing energy intake. However, we wonder whether the larger decrease in BMI in the sucralose group was caused by replacing sugar for sucralose or because of the increase in physical activity.

In a meta-analyse of Hunty et al. (2006) a significant reduction in energy intake and body weight was found in healthy humans, when foods and drinks were used which were sweetened with aspartame instead of sucrose. However, these results are derived from short-term studies. More data from long-term studies are needed.

**Conclusion**

Animal studies found an increase in caloric intake, body weight and adiposity after use of saccharin and acesulfame-K. On the contrary, human studies found a decrease in body weight and/or fat mass. However, in one study it was questionable whether the weight loss was caused by replacing sugar for sucralose or because of the extra physical activity in the intervention group. Further, in a meta-analyse which mentioned a decrease in energy intake and body weight, were seven of the ten trials short-term trials. AS could have a positive effect on human energy balance, however it is not certain yet. More studies with only one variable for energy balance and more long-term research needs to be done.
Chapter 4: Stevia

Introduction
In this chapter, we first discuss the safety of stevia. Second, we discuss the pharmacokinetics of stevia. We describe the extent and rate of absorption, distribution, metabolism and excretion of stevia and its metabolic products. After that, we describe the effect of stevia on the energy balance. Subsequently, we look at the possible biological effects and the potential therapeutically applications of stevia, like the effect of stevia on glucose-, insulin levels and blood pressure. In the last part of this chapter we discuss some other possible health effects of stevia.

4.1 Safety of stevia
Worldwide there is no consensus about the use of stevia and its components. In some countries, most notably Japan, Brazil, Korea, Paraguay and China, stevia is allowed as a sweetener. In Israel and Thailand the leaves are approved for sale, but purified extracts are not approved (Carakostas et al., 2008). In the United States it is sold as a dietary supplement, but it is not approved as a food additive (Kroger et al., 2006).

There are several commissions involved in food and safety matters. The European Commission’s Scientific Committee on Food (SCF) and the European Food Safety Agency (EFSA) in Europe, and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as the overall organization of the United Nations.

Research on the safety of stevioside and rebaudioside A
Stevioside and rebaudioside A are the most important and used steviol glycosides. Therefore, the most research studies on the safety of stevia are completed with stevioside or rebaudioside A. Stevioside and rebaudioside A show overall similarities in the metabolic system, both are converted into steviol (Renwick & Tarka, 2008). A Comet assay by Nunes et al. (2007) showed that treatment with 4 mg/ml stevioside (purity 88.62%) in drinking water produced DNA lesions in peripheral blood, brain, liver and spleen cells in rats. According to the EFSA, the study of Nunes et al. does not provide enough evidence for the genotoxicity of stevioside, due to methodological concerns. The findings were also not seen in other studies in rats using stevioside with lower or higher purity (EFSA, 2010). Curry and Roberts (2008) investigated the toxicity of rebaudioside A in Wistar rats. In a study of four weeks, the rats received 0, 25,000, 50,000, 75,000 or 100,000 ppm rebaudioside A (purity 97%) in their diet. In the study of thirteen weeks the rats received 0, 12,500, 25,000 or 50,000 ppm rebaudioside A (purity 97%). In both studies the rats lost weight as a result of a reduced food intake because of poor palatability. Therefore, these findings were not caused by toxicity of rebaudioside A. The treatment was not associated with any symptoms of toxicity or negative effects on body weight, fertility, oestrous cycles, gestation length or sperm motility and concentration. In a toxicity study of Nikiforov and Eapen (2008), Sprague-Dawley rats were fed with 500, 1000 and 2000 mg/kg/day rebaudioside A (purity n.m.) for a period of ninety days. In the male group with the highest dose (2000 mg/kg/day) a different liver weight was found. However, there were no significant differences in mean organ weights. The study concluded that rebaudioside A is safe to use as a food additive.

Research on the safety of Steviol
Steviol is the metabolite of all steviol glycosides (e.g. rebaudioside A and stevioside). Some studies suggest that high doses of steviol could induce maternal and developmental toxicity. The EFSA Panel noted that these studies were of little relevance to the safety valuation of steviol glycosides. After oral administration, steviol is immediately absorbed in the gastrointestinal tract. However, steviol glycosides are not rapidly absorbed, because they first need to be hydrolyzed to steviol. Therefore, the plasma levels of steviol are much higher after a high dose of steviol than after a dose of steviol glycosides (EFSA, 2010).
Opinion of the SCF, JECFA and the EFSA on the safety of steviol glycosides

The SCF (Europe) reviewed the safety of steviol glycosides. The conclusion of their research in 1999 was that there were still uncertainties and also discrepancies between the existing studies concerning the toxicological effects. Therefore, in 2000 the European Commission refused to accept stevia as a novel food (Geuns et al., 2003). The JECFA (United Nations) also reviewed the data of steviol glycosides in 1999. Due to a lack of human metabolism studies, the commission was unable to recommend an ADI (Acceptable Daily Intake). In 2004 the missing data were available and the JECFA established a temporary ADI of 2 mg/kg/day for total steviol glycosides (Kroger et al., 2006). In 2009 the ADI was altered into 4 mg/kg/day (EFSA, 2010). In 2010 the EFSA (Europe) determined an ADI for steviol glycosides of 4 mg/kg/day. This was based on human studies which demonstrated that daily doses of steviol glycosides up to 1000 mg/person/day were well tolerated, also by individuals with type 2 diabetes (EFSA, 2010). So, both the EFSA and the JECFA have established an ADI of 4 mg/kg/day. However, steviol glycosides are still not accepted as a food additive in Europe. This because it is presumable that the maximum use levels suggested by the petitioners would exceed the ADI (EFSA, 2010).

Conclusion
Elaborated studies have been done on stevioside, rebaudioside A and their metabolite steviol. Overall, stevioside and rebaudioside A do not show evidence of genotoxicity and therefore the EFSA panel concluded that stevia is not carcinogenic, genotoxic, or associated with any developmental or reproductive toxicity. However, steviol glycosides are still not accepted as a food additive in Europe, because it is likely that the ADI would exceed when the glycosides are used in food products.

4.2 Pharmacokinetics of the main components of stevia
Pharmacokinetics describes the fate of a substance administered to living organisms. It is divided into several areas including the extent and rate of absorption, distribution, metabolism and excretion (Chatsudthipong & Muanprasat, 2008).

It has been shown that oral stevioside is not absorbed in the intestine. In addition, none of the digestion enzymes and gastric juice are able to break down stevioside. However, bacterial intestinal flora of pigs, rats, mice (Geuns et al., 2003) and humans (Koyama et al., 2003) are able to convert stevioside into its aglycone, steviol. Studies in healthy human volunteers showed that after three days of oral stevioside (purity n.m.) administration (750 mg/day), no stevioside was detected in the feces samples. However, free steviol was detected in the feces. The lack of stevioside in the feces proves that all stevioside was broken down by the bacterial intestinal flora. No stevioside or free steviol were detected in the urine samples (Geuns et al., 2007). Similarly, Wheeler et al. (2008) demonstrated that after healthy men received a single oral dose of 4.2 mg/kg stevioside (purity 99.6%), it was hydrolyzed to steviol in the colon before it was absorbed. Additionally, this study shows that rebaudioside A (purity 98.7%) (single oral dose of 5 mg/kg) underwent similar metabolic and elimination pathways in humans. Structurally, stevioside and rebaudioside A differ only by the presence of one additional glucose moiety on rebaudioside A (Maki et al., 2008). Therefore, both stevioside and rebaudioside A undergo similar degradation pathways (Geuns et al., 2007; Wheeler et al., 2008).

According to several analyses of blood, no stevioside or steviol are found in the blood samples of humans and animals after consuming stevioside or rebaudioside A (Geuns et al., 2003; Geuns et al., 2007). Besides excretion of steviol via the bile, also urine excretion of steviol occurs in humans and animals. In addition, steviol may undergo enterohepatic circulation where it is excreted via bile, and reabsorbed back into circulation (Chatsudthipong & Muanprasat, 2008; Wheeler et al., 2008). After degradation of stevioside or rebaudioside A to steviol by bacteria of the colon, part of the steviol is absorbed by the colon and transported to the liver by portal blood (Geuns et al., 2007). In the liver, steviol is converted into steviol glucuronide by extensive glucuronidation and is excreted with urine (Geuns et al., 2007; Wheeler et al., 2008; Jin et al., 2010). It is the only metabolite of steviol identified in human urine.
However, steviol glucuronide is not identified in rat urine. This is due to the different molecular weight thresholds for rat and human biliary excretion of organic anions (e.g. glucuronides) and is observed with many ingested substances (Carakostas et al., 2008; Jin et al., 2010).

**Conclusion**

Both rebaudioside A and stevioside are completely converted into steviol by bacterial intestinal flora in humans and animals before they can be absorbed in the intestine. Steviol is excreted with feces. In humans, a part of steviol is transported to the liver via portal blood, to convert steviol into steviol glucuronide after which it is excreted by urine. Steviol glucuronide is not identified in rat urine. This is due to the different molecular weight thresholds in human and rat biliary excretion of organic anions.

**4.3 Effect of stevioside and rebaudioside A on the energy balance**

**Effect of stevioside and rebaudioside A on energy intake in rats**

A study which examined the anti-hyperglycemic action of stevioside (purity 98.6%) noted a difference in food consumption between two groups of male Wistar rats. One group was administered stevioside by injections into peripheral tissues and the other group was administered stevioside by intracerebroventricular injections. When stevioside is injected in peripheral tissues, stevioside has the ability to activate peripheral mu opioid receptors to increase glycogen synthesis in liver and lowering plasma glucose. However, the consumption of food was not influenced by injections of stevioside into peripheral tissues. Only the intracerebroventricular injections increased the consumption of food in rats (Yang et al., 2009).

Sclafani et al. (2010) demonstrated that water containing 0.1%-0.3% stevia extract (61% rebaudioside A), did not promote overdrinking in female Sprague-Dawley rats. For six days, the rats received normal water, so they measured the water intake. Subsequently, the rats were divided in two groups. One group received water containing stevia extract, and the other group water containing saccharin for eight days. The rats in the group of water containing stevia extract did not drink more water compared with baseline. Contrarily, the other group which received water with saccharin almost tripled daily fluid intake. In a study with Wistar rats, which received rebaudioside A (purity 97%) for four weeks, there was a significant reduction in food intake compared to controls. A significant reduction of food intake was noted in the 75.000 and 100.000 ppm groups of female rats and in the 50.000, 75.000, and 100.000 ppm groups of male. However, this result was probably due to the poor palatability of the food. Apparently, the food containing rebaudioside A was not palatable, this might be due to the high sweetness of a bitter aftertaste (Curry & Roberts, 2008).

**Effect of stevioside and rebaudioside A on energy intake in humans**

Anton et al. (2010) investigated the effects of stevioside on food intake and satiety in healthy, lean and obese humans. The subjects all consumed a standard breakfast (469 kcal) in the morning and twenty minutes before lunch and dinner they consumed a preload at the three separate food test days. The preloads consisted of tea and crackers with cream cheese sweetened with stevioside (purity n.m.), aspartame, or sucrose. All subjects did not compensate by eating more at lunch or dinner after a preload containing stevioside, compared with a preload containing sucrose. The difference in the caloric intake was only due to the preloads (sucrose 493 kcal, stevioside 290 kcal). Both groups also reported similar levels of satiety. The preloads containing stevioside were rated as having a less pleasant taste, in comparison with the preloads containing sucrose. This was also noted in the study of Curry and Roberts (2008) described above. Similar results were demonstrated with rebaudioside A in type 2 diabetic man and women. Chronic consumption of 1000 mg/day rebaudioside A (purity 97%) for sixteen weeks did not alter intake of total energy significantly compared to placebo (Maki et al., 2008).

**Effect of stevioside on body weight in rats**

In a standard chow diet containing 60% fructose, one group of male Wistar rats received 0.5 mg/kg to 5.0 mg/kg stevioside (purity 98.6%) three times a day, whereas an other group did not receive stevioside. After four weeks fructose diet, the body weight of rats receiving 5.0 mg/kg stevioside was reduced significantly in comparison with the group receiving the diet without stevioside. This was
probably due to the poor palatability of the food, because of the high amount of stevioside (5.0 mg/kg) (Chang et al. 2005). Dyrskog et al. (2005) investigated the effect of stevioside (purity 91%) in obese ZDF rats. The rats were divided into several groups, each group was fed with another diet for ten weeks. Between the standard chow diet group and the standard chow diet with 0.03 g/kg stevioside group, no significant differences were found in weight development. Similar results were demonstrated by Jeppesen et al. (2003) in Wister rats and non-obese type 2 diabetic GK rats. Oral stevioside (purity 99.6%) treatment of 0.025 g/kg/day for six weeks had no significant effect on body weight of male GK and male Wistar rats.

Conclusion

Intracerebroventricular injections of stevioside increase the food consumption. However, due to the blood-brain barrier oral intake of stevioside does not increase the energy intake. Oral intake of stevioside or rebaudioside A does not alter energy intake in type 2 diabetic, obese or normal rats. Stevioside also has no effect on changes in body weight in normal, obese and type 2 diabetic rats.

In type 2 diabetic, obese and healthy humans no compensation was found after intake of a preload containing stevioside.

4.4 Biological effects and potential therapeutically applications

4.4.1 Effects of stevioside on blood glucose levels

Effect of stevioside on glucose levels in rats

The effects of stevioside on glucose levels in blood have especially been studied in type 1 and type 2 diabetic rats. Lailerd et al. (2004) determined the effect of stevioside on skeletal muscle glucose transport activity in both insulin sensitive lean and insulin resistant obese Zucker rats. The rats received 500 mg/kg stevioside (purity n.m.). After two hours, no significant difference was found in plasma glucose levels in either lean or obese Zucker rats. In a study with diabetic GK and normal Wistar rats, 0.2 g/kg stevioside (purity 96%) and D-glucose were administered as intravenous bolus injections in the anaesthetized rats. Stevioside did not cause any change in blood glucose to an intravenous glucose tolerance test (IVGT) in normal Wistar rats, compared with Wistar rats control group. In contrast, a reduction of the blood glucose response to an IVGT was noted in GK rats compared with GK rats control group (Jeppesen et al., 2002). Similar results are demonstrated in a following study of Jeppesen (Jeppesen et al. (2003)). Oral stevioside (purity 99.6%) treatment of 0.025 g/kg/day for six weeks has anti-hyperglycemic effects in the diabetic GK rat, and not in normal Wistar rats. Chen et al. (2005) studied the hypoglycemic effects in two kinds of diabetic rats. Half of the rats were induced with streptozotocin (STZ), so they would develop insulin-dependent diabetes. The other rats were fed high amounts (60%) of fructose, so they would develop non-insulin-dependent diabetes. The insulin-dependent and non-insulin-dependent diabetic rats received 0.5 mg/kg, 1.0 mg/kg and 5.0 mg/kg stevioside (purity 99%) by gastrogavage two times for one day, except for the control group. After twelve hours of fasting, the rats underwent an IVGT test. The results showed that an acute intake of stevioside (0.5 mg/kg) lowered the blood glucose levels in STZ-induced diabetic rats significantly, in comparison with the control group. Stevioside also demonstrated dose-dependent effects (0.5 mg/kg to 5.0 mg/kg) in lowering the glucose levels in both diabetic rat models. Stevioside also decreased the blood glucose levels in normal Wistar rats of the control group in a dose-dependent manner. Similarly, Chang et al. (2005) fed one group of Wistar rats with fructose-rich chow containing 60% fructose for four weeks to induce the insulin resistance. The other rats were taken as the control group, and received standard chow for four weeks. Both groups received water containing stevioside (purity 98.6%) three times daily during the chow-diets for four weeks. The results of an intraperitoneal glucose tolerance test after the four weeks of diet showed a dose-dependent decrease of plasma glucose in fructose-rich chow fed Wistar rats receiving different doses of stevioside from 0.5 mg/kg to 5.0 mg/kg. This result was not noted in the control group.

Effect of stevioside on glucose levels in humans

A study in human volunteers showed that oral stevioside has beneficial effects on the glucose metabolism in type 2 diabetic patients. After intake of a meal containing 1 g of stevioside (purity
the postprandial blood glucose levels in type 2 diabetic patients were reduced significantly, compared to 1 g corn starch (Gregersen et al., 2004). Similarly, Anton et al. (2010) investigated the effect of acute administration of stevioside on postprandial glucose levels in lean and obese individuals. Postprandial glucose levels were significantly lower after consumption of a preload containing stevioside (purity n.m.), compared to the groups which consumed a preload containing sucrose or aspartame. This difference in glucose levels after a preload containing sucrose compared to a preload containing stevioside can be explained by the reduced intake of carbohydrates in the stevioside group. The preload containing aspartame does also not contain carbohydrates, therefore, you would expect similar postprandial blood glucose levels. However, the postprandial blood glucose levels after a preload of stevioside were lower compared to a preload containing aspartame. Therefore, stevioside might lower the postprandial glucose levels. Only one study was conducted to investigate the effect on fasting plasma glucose. Geuns et al. (2007) showed that oral stevioside (purity 99%) administration of 750 mg/day for three days did not have an effect in healthy human volunteers.

Possible mechanism of the anti-hyperglycemic effect of stevioside
Stevioside may regulate blood glucose level in STZ-induced diabetic rats by decreasing PEPCK gene expression in the liver, by slowing down gluconeogenesis (Chen et al., 2005). In addition it was found that stevioside increases glycogen synthesis (Yang et al., 2009) in rats.

Conclusion
In most of the studies stevioside reduced the postprandial blood glucose levels in obese and non-obese, type 2 diabetic rats. Also the fasting glucose level was reduced after long-term consumption of stevioside in obese and non-obese, type 2 diabetic rats. However, in normal rats, no significant differences were found in fasting glucose levels and postprandial blood glucose levels. In studies with oral intake of stevioside in type 2 diabetic, lean and obese men and women, postprandial blood glucose levels were significant lower compared with the control groups. However, no significant differences in fasting plasma glucose were noted in healthy humans after three days administration of stevioside.

Overall, stevioside might have hypoglycemic effect in type 2 diabetic rats and people.

4.4.2 Effects of stevioside on insulin levels
Effect of stevioside on insulin levels in rats and mice
Chen et al. (2005) already showed by an IVGT test that oral intake of 0.5 mg/kg, 1.0 mg/kg and 5.0 mg/kg stevioside (purity 99%) can regulate blood glucose levels in STZ-induced rats. This study also demonstrated that this is not only because of the PEPCK gene expression that slows down gluconeogenesis, but also by enhancing insulin secretion. In a study with diabetic GK and normal Wistar rats, 0.2 g/kg stevioside (purity 96%) and D-glucose were administered as intravenous bolus injections in the anasthetized rats. In comparison with the control group, an increase in the insulin secretion and a suppression of the glucagon level was found in the GK rat stevioside group after an IVGT (Jeppesen et al., 2002). Similar results are demonstrated in a following study of Jeppesen (Jeppesen et al. 2003). After oral stevioside (purity 99.6%) treatment of 0.025 g/kg/day for six weeks, no significant difference was found in plasma insulin levels between the stevioside and control group. In the group of diabetic GK rats which was fed with stevioside, a higher first-phase insulin response was found compared to the group GK rats which did not receive stevioside (control group). Lailerd et al. (2004) showed that acute oral intake of 500 mg/kg stevioside (purity n.m.) did not affect insulin levels significantly in both insulin sensitive lean and insulin resistant obese Zucker rats, in comparison with the control group. However, acute oral stevioside increased whole-body insulin sensitivity and insulin-stimulated glucose transport activity in both lean and obese Zucker rats. Chang et al. (2005) fed one group of Wistar rats with fructose-rich chow containing 60% fructose for four weeks to induce the insulin resistance. The other rats were taken as the control group, and received standard chow for four weeks. The results demonstrated that the group rats which received 0.5 mg/kg to 5.0 mg/kg stevioside (purity 98.6%) orally had a significantly improved insulin sensitivity compared with the standard chow-fed controls. Also compared with the standard chow-fed controls, the fasting plasma insulin level in fructose-fed rats was markedly raised. Fujita et al. (2009) investigated whether
stevioside does acutely enhance incretin release from gut. Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretin hormones which are released from endocrine cells located in the gut mucosa and stimulate insulin secretion from pancreatic β-cells. Diabetic Wistar rats and diabetic C57/BL6 mice were orally administered with 5 mg/kg to 1 g/kg stevioside (purity n.m.). No effect on release of (GIP) and (GLP-1) was found in diabetic Wistar rats and diabetic C57/BL6 mice. GIP and GLP-1 release was only stimulated by glucose.

**Effect of stevioside on insulin levels in humans**
Anton et al. (2010) investigated the effect of acute administration of stevioside on postprandial insulin levels in lean and obese individuals. Postprandial insulin levels were significantly lower after consumption of a preload containing stevioside (purity n.m.), compared to the groups which consumed a preload containing sucrose or aspartame. This difference in insulin levels after a preload containing sucrose compared to a preload containing stevioside can be explained by the reduced intake of carbohydrates in the stevioside group. The preload containing aspartame does also not contain carbohydrates, therefore, you would expect similar insulin levels. However, the insulin levels after a preload of stevioside were lower compared to a preload containing aspartame. Therefore, stevioside might lower the insulin levels.

**Conclusion**
Consumption of stevioside might lead to an increased insulin secretion and sensitivity in type 2 diabetic rats. In rats without diabetes, stevioside only improves insulin sensitivity. The insulinotropic effect of stevioside does not seem to be mediated by incretins because stevioside does not elicit release of GIP and GLP-1 in diabetic rats. Postprandial insulin levels were significantly lower in lean and obese humans after intake of a preload containing stevioside compared to a preload containing sucrose or aspartame.

**4.4.3 Effects of rebaudioside A on blood glucose and insulin levels**

**Effect of rebaudioside A on glucose and insulin levels in vitro**
A study on isolated mouse islets demonstrated that stimulation of the insulin secretion in de islets depend on the dose of rebaudioside A (purity 95-98%) and the presence of glucose (3.3-16.7 mmol/L). The stimulatory effects of rebaudioside A (10^-16 to 10^-9 mol/L, purity n.m.) vanished in the presence of normal or low glucose. The effect of rebaudioside A is dependent on the presence of extracellular calcium. The insulinotropic effect of rebaudioside A disappeared in the absence of extracellular calcium (Abudula et al., 2004). Also Abudula et al. (2008) demonstrated that the insulinotropic effect of rebaudioside A (10^-9 to 10^-7 M, purity n.m.) requires the presence of high glucose (16.7 mM). They also found it is mediated via inhibition of ATP-sensitive K^+/-channels, which are probably induced by changes in the ATP/ADP ratio.

**Effect of rebaudioside A on glucose and insulin levels in humans**
Maki et al. (2008) examined the effects of chronic consumption of rebaudioside A in men and women with type 2 diabetes. The results of this study showed that 1000 mg/day consumption of rebaudioside A (purity 97%) for sixteen weeks did not affect glucose homeostasis in individuals with type 2 diabetes. In both the rebaudioside A and placebo group were found no differences in insulin levels from baseline to treatment.

**Conclusion**
In vitro studies demonstrate that the insulinotropic effect of rebaudioside A requires the presence of high glucose and extracellular calcium. Rebaudioside A does not alter glucose homeostasis and insulin levels in type 2 diabetic men and women.

**4.4.4 Effects of stevia on blood pressure**

**Effect of stevioside on blood pressure in rats**
Jeppesen et al. (2003) found a blood pressure lowering effect in type 2 diabetic rats receiving with stevioside (purity >99.6%). Because this effect of stevioside had only been demonstrated in non-obese
Artificial sweeteners and stevia; the solution for the obesity problem?

Dyrskog et al. (2005) investigated the effect of stevioside (purity 91%) on the metabolic syndrome in Zucker diabetic obese rats. Hypertension is a main feature of the metabolic syndrome. The rats were divided into four groups, each group was fed with another diet. The systolic blood pressure at baseline was comparable in all four groups. After two weeks of treatment, the stevioside groups had a significant decrease in systolic blood pressure. Stevioside might not only be orally effective. Lee et al. (2001) demonstrated that intravenous stevioside is an effective anti-hypertensive agent in spontaneously hypertensive rats. They administered a dosage of 25 mg/kg stevioside (purity 95%) intraperitoneal and found that the mean arterial blood pressure decreased from 186.2 to 167 mmHg.

**Effect of stevioside on blood pressure in humans**

Barriocanal et al. (2008) designed a long-term study of three months to evaluate the effects of stevioside (purity >92%) on blood pressure in humans. The subjects were divided into three groups. Group 1: subjects with type 1 diabetes, group 2: subjects with type 2 diabetes and group 3: subjects without diabetes and with normal or low-normal blood pressure. After the treatment the mean systolic and diastolic blood pressure were not significantly different compared to baseline in the stevioside group. Similar results were found in a study of Geuns et al. (2007). Oral administration of 750 mg/day stevioside (purity 97%) for three days, did not affect systolic and diastolic blood pressure of healthy volunteers. However, in a study undertaken to investigate the anti-hypertensive effect of oral crude stevioside (purity n.m.) in patients with mild essential hypertension, the systolic and diastolic blood pressure decreased significantly. However, a similar effect was found in the placebo group. Therefore, crude stevioside up to 15.0 mg/kg/day had no anti-hypertensive effect (Ferri et al., 2006).

On the contrary, Hsieh et al. (2003) found in a long-term study of two years in patients with mild essential hypertension a significant decrease in mean systolic and diastolic blood pressure. Compared with baseline, the systolic blood pressure in the stevioside (purity n.m.) group was reduced significantly from 150 to 140 mmHg and the diastolic blood pressure from 95 to 89 mmHg. The reductions were also significant in comparison with the placebo group. Similar results were found in the study of Chan et al. (2000) in hypertensive subjects. The systolic and diastolic blood pressure of the stevioside (purity n.m.) group was significantly lower compared to the placebo group. The mean reduction in systolic blood pressure was 12 mmHg and 8 mmHg in diastolic blood pressure. The conclusion of the study was that stevioside is effective in the treatment of hypertension, but the amplitude of the hypotensive effect was a bit less than other anti-hypertensive drugs.

**Effect of rebaudioside A on blood pressure in humans**

Maki et al. (2008) demonstrated that consumption of 1000 mg/day of rebaudioside A (purity 97%), did not affect blood pressure in subjects with type 2 diabetes. In a later study, Maki et al. (2008) investigated whether consumption of 1000 mg/day of rebaudioside A (purity 97%) altered blood pressure in healthy humans with normal blood pressure. They concluded that 1000 mg/day of rebaudioside A did also not affect blood pressure in healthy men and women.

**Possible mechanisms of the anti-hypertensive effect of stevioside**

It is not fully known how the mechanism, underlying the anti-hypertensive effect of stevioside in hypertensive humans works. The blood pressure lowering effect might be caused by a calcium antagonist mechanism, similar to the mechanism of verapamil (a calcium channel blocker). Lee et al. (2001) investigated whether the blood pressure lowering mechanism of stevioside was through Ca$^{2+}$ influx inhibition. The results were affirmative and showed that stevioside (purity 95 %) has a vasorelaxation effect mainly through Ca$^{2+}$ influx inhibition. Although other studies demonstrated that the hypotensive response to stevioside might also depend on prostaglandin activity.

**Conclusion**

In non-obese, obese, type 2 diabetic or hypertensive rats it was consistently found that stevioside decreases blood pressure. The results of the research studies in humans, however, were not consistent.
In both diabetic and healthy humans, administration of stevioside did not affect blood pressure. However, studies on hypertensive humans, showed a decreased blood pressure when stevioside was administered. Studies on the effect of rebaudioside A in healthy as well as in type 2 diabetic persons, found no effect on blood pressure. However the effect of rebaudioside A on blood pressure in hypertensive humans was not examined.

4.4.5 Other effects of stevia

Effect of stevia on blood lipids

Lailerd et al. (2004) demonstrated after two hours the lean and obese Zucker rats received 500 mg/kg stevioside (purity n.m.), no significant differences in free fatty acid levels were noted compared to placebo. Dyrskog et al. (2005) investigated the effect of treatment with a soy-rich diet and stevioside (purity 91%) on lipid profiles in obese Zucker diabetic fatty (ZDF) rats for ten weeks. The results showed that stevioside treatment of 0.03 g/kg/day did not have any independent effect on fasting plasma levels of free fatty acids, triglycerides, and cholesterol. In contrast, Park and Cha (2010) concluded that supplementation of stevia extract (purity of components n.m.) has an effect on lipid profiles in healthy C57BL/6J mice. The mice were divided into four groups: normal-diet, high-fat diet, high-fat diet and sucrose, and high-fat diet and stevia extract (1 ml/kg/day). After the diet of fifteen weeks, the levels of triglycerides in liver and serum were higher in the high-fat sucrose group than in the high-fat stevia extract group. Also, the serum total cholesterol levels were higher in the high-fat sucrose group than in the high-fat stevia extract group. Therefore, they concluded that stevia extract has a positive effect on triglyceride and cholesterol levels.

A study in hypercholesterolemic women demonstrated that oral intake of 3.3 g stevia extract (purity of components n.m.) for one month, has a hypolipidaemic effect. The elevated levels of serum cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein significantly reduced compared to baseline, while there was an increase in high density lipoprotein (Sharma et al., 2009). Contrarily, Silva et al. (2006) showed that after ninety days of oral administration of 100 mg/day stevioside (purity 80%, 20% rebaudioside A) in hyperlipidemic men and women, no significant differences in lipid levels were found between placebo and stevioside group. Remarkably, total cholesterol and low density lipoprotein levels were significantly decreased in both groups. Maki et al. (2008) showed that oral consumption of 1000 mg/day rebaudioside A (purity 97%) for 16 weeks in type 2 diabetic men and women did also not elicit any significant changes in fasting lipids between rebaudioside A and placebo groups.

Conclusion

Stevioside had no effect on levels of free fatty acids, triglycerides, and cholesterol in short- and long-term studies with obese and diabetic Zucker rats. However, long-term ingestion of stevioside had a positive effect on the triglyceride and cholesterol levels in mice on a high-fat diet. Results of studies in humans are inconsistent. Stevioside had no effect on lipid levels in hyperlipidemic humans and rebaudioside A had no effect on fasting lipids in type 2 diabetic women, while stevia extract had an hypolipidaemic effect in hypercholesterolemic women.

Anti-inflammatory effect of stevioside in vitro

Stevioside may also have an anti-inflammatory effect. Boonkaewwan et al. (2006) demonstrated this by incubating human acute monocytic leukemia cell lines (THP-1 cell lines) with different concentrations of stevioside (purity 98%) in the presence or absence of LPS (1 µg/mL). LPS elicit strong immune responses in animals. Also some THP-1 cell lines were induced with 20 µg/mL TLR4, to activate the immune system. They concluded that stevioside (1 mM) significantly decreases the production of IL-β and TNF-α and decreases the production of nitric oxide slightly in THP-1 cells stimulated with LPS. Therefore, stevioside possesses an anti-inflammatory effect.
Effect of stevioside on the immune system
An immunomodulator is a substance which has an effect on the immune system. According to Sehar et al. (2008), stevioside possesses immunomodulatory properties. They concluded this after orally administering different doses of stevioside (6.25, 12.5 and 25 mg/kg) to balb/c mice for fourteen days. Half of the mice received cyclophosphamide (200 mg/kg) to induce immunosuppression. After the fourteen days there was a significant increase in B- and T-cell mediated humoral, and delayed type hypersensitivity (DTH) response. The increase of B- and T-cell mediated humoral response elicits excretion of antibodies and the DTH response elicits a stimulatory effect on lymphocytes.

Inhibitory effect of stevioside on cancer
Glycyrrhizin is an anti-tumor promoter in chemical carcinogenesis. Konoshima et al. (2002) found that stevioside has a stronger inhibitory effect than glycyrrhizin. Stevioside (purity n.m.) was given in drinking water (2.5 mg/100 ml) one week before, to one week after an initiation treatment with peroxynitrite. This is a nitrating agent and oxidant and can therefore damage molecules in cells. One week after the initiation treatment, the mice were given a tumor promoting treatment with TPA in acetone twice a week. Stevioside inhibited both the initial stage induced by peroxynitrite and the promotion stage induced by TPA. The conclusion of the study is that stevioside might be valuable as a chemopreventive agent.

Effect of stevia on dental health
Stevia might also have potential to be beneficial in dental care. Blauth de Slavutzky (2010) designed a study to investigate the effect of rinsing with a 10% solution of stevia (purity of components n.m.) on the formation and the amount of dental plaques in comparison with rinsing with a sucrose solution. The stevia group used the stevia solution during five days, four times a day and the sucrose group used the sucrose solution for the same time, without further oral hygiene. All subjects in the stevia group had less plaque than the subjects in the sucrose group. Two different indexes were used to measure the amount of plaque. The indexes showed between 40% and 80% less plaque compared to baseline. Because stevia contains xantines, tannin and flavonoids it has anti-plaque activity. It is also shown that tannin has anti-cariogenic and anti-bacterial effects.

Effect of stevioside on atherosclerosis
Geeraert et al. (2010) investigated the effect of stevioside on inflammation, oxidative stress, and insulin resistance related to atherosclerosis. Obese insulin-resistant mice were treated with 10 mg/kg/day stevioside (purity 99.9 %) or placebo for twelve weeks. Stevioside improved antioxidant defence in the vascular wall and adipose tissue and it improved insulin signalling. This led to inhibition of atherosclerotic plaque development and also induced plaque stabilization. Stevioside could therefore be helpful in the treatment of atherosclerosis.
Chapter 5: Discussion

In this chapter we discuss the results of our study. We describe the disputable points of some studies we used. Further we discuss the limitations of our study.

The main finding of our AS search is that consumption of AS probably does not cause a positive energy balance in humans. The main finding of our stevia search is that consumption of stevia and its glycosides probably do not affect the energy balance in rats and humans.

One major factor that makes it difficult to compare the results of the studies is the use of different components of stevia, and the purity of these components used in the studies. Some studies also did not mention the purity of the used component of stevia. In studies with AS, the amount of the used AS is often missing and also which AS is used. Some studies were accomplished with stevioside, rebaudioside A or stevia extract, all with various purities. If the study is completed with e.g. stevioside, we can not draw the same conclusion for rebaudioside A or stevia extract. This partly applies for AS as well. If the study is completed with aspartame we can not draw the same conclusion for other AS, because aspartame is metabolized in the body. In addition, the effect of the compounds was studied in different patient groups. For example, Sharma et al. (2009) examined the hypolipidaemic effect of stevia extract in hypercholesterolemic women, while Silva et al. (2006) examined the hypolipidaemic effect of stevioside in hyperlipidemic patients, and Maki et al. (2008) the hypolipidaemic effect of rebaudioside A in type 2 diabetic humans. Since the subjects and the used components of stevia differ, it is difficult to draw a clear conclusion.

In several studies, no compensation was found after intake of a preload containing AS or steviol glycosides. However, these studies were short-term studies. We can not presume that long-term consumption of AS or steviol glycosides will have similar results.

The decreasing effect on energy intake and bodyweight might only be a short-term effect. Fowler et al. (2008) examined the relationship between the consumption of ASB and long-term weight gain. Also the normal-weight persons increased weight in seven years. A possible explanation might be that the resting metabolism became lower and therefore an increase in weight gain occurred after long-term consumption of AS.

Some studies reported a higher energy intake in the sucrose group, compared to the AS group (Raben et al., 2002; Della Valle et al., 2005; Anton, et al., 2010). This is plausible because AS do not contain calories. It might be suggestive to have a control group, which receive an unsweetened diet, to compare the energy intake in this control group with the AS group. This because the caloric content of the diet is similar in this way. However, this might be not possible, because the palatability of the diet will decrease if it is completely unsweetened, and therefore the energy intake might logically be expected to decrease.

An other point that may be a weakness in some studies is that the participants could eat ad libitum (Holt et al., 2000; Raben et al. 2002; Della Valle et al., 2005; Appleton and Blundell 2007). Like Holt et al. (2000) already mentioned, it is likely that the participants did not only eat in reaction to their physiological hunger or in reaction to stevia or AS, but also because of the availability of ad libitum food.

Rodearmel et al. (2007) examined the effect of replacing sucrose with AS. One group went through two lifestyle changes; extra physical activity and replacing sugar with AS. The other group was only asked to record physical activity. Both groups had a significant decrease in BMI for age. However, the group which went through two lifestyle changes had a significant higher percentage of children who reduced or maintained their BMI. The group which was asked to record their physical activity, did not have a higher activity level compared to the other group. The article concluded that sucralose seems to be effective in decreasing energy intake. However, it is questionable whether the weight loss was caused by replacing sugar for sucralose or because of the extra physical activity in the intervention group, given that both groups reduced in BMI. Self-monitoring in the second group also increases consciousness of physical activity and diet and may therefore lead to behavior change. In fact, this
study contains two interventions, and therefore we can not conclude if the higher decrease in BMI in the lifestyle intervention group was caused by the extra physical activity or by the replacement of sucrose with AS.

Several articles examined the use of AS and caloric compensation. The main finding was that there was no compensation after receiving a premeal containing AS. However, Appleton and Blundell (2007) investigated the effect of AS and sugars on energy intake in habitual high and low consumers of ASB. They concluded that high users of ASB had learnt to dissociate sweetness from energy content, while low users of ASB had not. The studies which examined the use of AS and caloric compensation did not mention if the study was completed with high- or low-users of AS. Therefore, it is questionable if there is really no compensation in high- and/or low-users of AS.

An explanation for the weight loss in the safety studies of stevia is the palatability of the food. Curry and Roberts (2008) investigated the toxicity of rebaudioside A in Wistar rats. They found a significant reduction in food intake and weight. These results were due to the poor palatability of the food. This was also probably the reason for the weight loss in Wistar rats who received 5.0 mg/kg stevioside in the study of Chang et al. (2005). In the study of Anton et al. (2010) men and women rated the preloads containing stevioside as having a less pleasant taste, in comparison with the preloads containing sucrose.

Only in type 2 diabetic rats and humans was found a blood glucose lowering effect after consumption of stevioside. This might be due to the fact that type 2 diabetic rats and humans are more sensitive for variations in glucose levels. While healthy humans are less sensitive for it, because the glucose homeostasis is more stable. This might work the same for hypertensive rats and humans.

Rebaudioside A and stevioside are almost similar when you look at the structural formula and they both are absorbed and excreted in similar ways. However, the results found in the studies are most of the time very different. Rebaudioside seems to have less effect than stevioside has. Is this because there are less studies of rebaudioside A or is it rebaudioside A that functions differently?

A study of Geuns et al. (2007) showed that oral stevioside (purity 99%) administration of 750 mg/day for three days, did not affect fasting plasma glucose in healthy human volunteers. This might be a logical result since this is a acute study of a chronic system in the body. Three days administration of stevioside might not be long enough to alter the fasting glucose levels.

Further, for some subjects we only had one article. For example the effect of stevioside on dental health or atherosclerosis. Therefore we can not conclude anything with certainty out of these results. Therefore, no discussion is possible.

Limitations of our study

One important limitation of this study is that we could not include some articles, because they were not available as free full text. We possibly also exclude several relevant articles of which we thought were not useful after reading the abstracts. Some articles were too chemical or biological and we possibly missed some relevant information out of these articles because we did not understand it.

An additional limitation that may weaken our thesis is that we decided to exclude articles before 2000. Therefore, we possibly exclude some relevant studies.

Another point is that stevia and its components are an upcoming topic in the science, and therefore there are not enough long-term studies which investigated the effects of consumption of stevia. Perhaps there are more articles published about stevia in other languages, like Japanese. In Japan, stevia is used for decades and therefore there might be done more research compared to English studies.
Chapter 6: Conclusion

6.1 Artificial sweeteners
AS might influence several mechanisms in a way that they can cause a higher energy intake, and subsequently can lead to a positive energy balance. Consumption of AS probably lowers the satiety, by decreasing the energy density of food. AS do not release GLP-1 and CCK, and therefore also do not stimulate the feeling of satiety. This decreased feeling of satiety, can lead to a higher energy intake, which can cause a positive energy balance.

Consumption of AS may also influence the food reward system. AS do not activate the postingestive en sensory component completely, which leads to a decreased feeling of satisfaction, which subsequently leads to a higher energy intake and therefore can cause a positive energy balance.
The predictive relationship between sweet taste and calories is also a possible mechanism which can be disturbed by eating sweet, non-caloric products containing AS. Disturbing this predictive relationship may lead to a higher energy intake, which can cause a positive energy balance. The predictive relationship between sweet taste and calories may be disturbed by previous experiences with AS consumption, and through weakening the cephalic phase response. AS also affect the thermogenesis, because AS lower the energy density of foods.
The effects of aspartame on neurotransmitters NPY and 5-HT are similar. Both the levels of NPY and 5-HT in the brain decrease after consumption of aspartame. However the results of this effects are different. A decrease in NPY leads to a decrease in energy intake, while a decrease in 5-HT leads to a higher carbohydrate intake.

Overall, the results of the mechanistic studies concluded that consumption of AS might lead to a higher energy intake, and subsequently to a positive energy balance.

Based on the observational studies, we can conclude that there is a relationship between the use of AS and high body weight, or body weight concerns. However, from these studies we can not conclude if the use of AS is a reaction on obesity or a cause of obesity.
Based on the intervention studies in animals, we can conclude that the consumption of AS might lead to an increase in caloric intake, body weight and adiposity. On the contrary, human intervention studies found a decrease in energy intake, body weight and/or fat mass. No compensation during lunch or dinner occurs, after men and women consumed a preload containing AS. Therefore, they decrease their energy intake.

Overall, it seems to be that AS do not cause a positive energy balance in humans. However, based on the number of studies, the limitations of the studies described in the discussion, and the duration of the interventions, we can not conclude this with certainty.
6.2 Stevia

Steviol glycosides are found to be safe by the EFSA but are still not accepted for use as a food additive. This because it is likely that the ADI (4 mg/kg/day) would exceed when the glycosides are used in food products.

Both rebaudioside A and stevioside are completely converted into steviol by bacterial intestinal flora in animals and humans before they can be absorbed in the intestine. Oral intake of stevioside and rebaudioside A does not alter the energy intake in type 2 diabetic, obese or normal rats, and in type 2 diabetic, obese or healthy humans. Intracerebroventricular injections of stevioside increase the food consumption. However, due to the blood-brain barrier oral intake of stevioside does not increase the energy intake. Both glycosides do also not affect body weight in type 2 diabetic, obese or normal rats, and rebaudioside A does not affect body weight in type 2 diabetic men and women. Thus it seems to be that steviol glycosides do not have an effect on the energy balance.

Steviol glycosides have several side-effects. In most studies, stevioside reduces the postprandial blood glucose and fasting glucose levels in obese and non-obese, type 2 diabetic rats. In normal rats, stevioside does not have a significant effect on postprandial blood glucose and fasting glucose levels. Consumption of stevioside lowers postprandial glucose levels in type 2 diabetic, obese, and lean humans. However, stevioside has no significant effect on fasting plasma glucose in healthy humans. Overall, stevioside might have a hypoglycemic effect in type 2 diabetic rats and humans. Consumption of stevioside might also lead to an increased insulin secretion and sensitivity in type 2 diabetic rats. In rats without diabetes, stevioside only improves insulin sensitivity. Contrarily, postprandial insulin levels were significantly lower in lean and obese humans after intake of a preload containing stevioside compared to a preload containing sucrose or aspartame. The insulinotrophic effect of rebaudioside A in vitro, requires the presence of high glucose and extracellular calcium. Rebaudioside A does not alter glucose homeostasis and insulin levels in type 2 diabetic men and women.

Stevioside has a decreasing effect on blood pressure in non-obese, obese, type 2 diabetic and hypertensive rats, as well as in hypertensive humans. The effect of stevioside and rebaudioside A on levels of free fatty acids, triglycerides and cholesterol in humans is not clear. Further, stevioside may have anti-inflammatory and immunomodulatory properties. It may also be a valuable chemopreventive agent. Finally, stevioside may have potential to be beneficial in dental care and in the treatment of atherosclerosis.

Overall, steviol glycosides probably do not alter the energy intake and body weight. The main side-effects of stevioside might be that stevioside has a hypoglycemic effect in type 2 diabetic patients, and a hypotensive effect in hypertensive humans.

Recommendations for further research

More studies on the relation between AS and a positive energy balance are needed. Especially long-term studies, and with only one variable for energy balance. This also applies for stevia in relation with energy balance. Further research is needed on the subject of stevia, to examine whether the ADI can be raised and therefore steviol glycosides can be accepted as a food additive.
References


Carakostas, M.C., Curry, L.L., Boileau, A.C., Brusick, D.J. (2008). Overview: The history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. *Food and Chemical Toxicology, 46*, 1-10.


Artificial sweeteners and stevia: the solution for the obesity problem?


Book

## Appendix I: Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>5-Hydroxytryptamine (serotonin)</td>
</tr>
<tr>
<td>ADA</td>
<td>American Dietetic Association</td>
</tr>
<tr>
<td>ADI</td>
<td>Acceptable Daily Intake</td>
</tr>
<tr>
<td>AS</td>
<td>Artificial sweetener(s)</td>
</tr>
<tr>
<td>ASB</td>
<td>Artificially sweetened beverage(s)</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>DTH</td>
<td>Delayed Type Hypersensitivity</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Agency</td>
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<tr>
<td>FAO</td>
<td>Food Agriculture Organization</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>GIP</td>
<td>Glucose-dependent insulinotropic polypeptide</td>
</tr>
<tr>
<td>GK rats</td>
<td>Goto-Kakazaki rats</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin-dependent diabetes mellitus</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1 beta</td>
</tr>
<tr>
<td>IVGT</td>
<td>Intravenous Glucose Tolerance</td>
</tr>
<tr>
<td>JECFA</td>
<td>Joint FAO/WHO Expert Committee on Food Additives</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non-insulin-dependent diabetes mellitus</td>
</tr>
<tr>
<td>N.m.</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>PEPCK</td>
<td>Phosphoenolpyruvate Carboxykinase</td>
</tr>
<tr>
<td>SCF</td>
<td>Scientific Committee on Food (European Commission)</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>THP-1 cells</td>
<td>Human acute monocytic leukemia cell line</td>
</tr>
<tr>
<td>TLR4</td>
<td>Toll-like receptor 4</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TPA</td>
<td>Tumor Promoting Activity</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>UMCG</td>
<td>University Medical Center Groningen</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ZDF rat</td>
<td>Zucker diabetic fatty rat</td>
</tr>
</tbody>
</table>
# Appendix II: Comparison databases

<table>
<thead>
<tr>
<th>Database/search engine</th>
<th>Description</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBSCOhost</td>
<td>Includes many databases, like CINAHL and MEDLINE.</td>
<td>A lot of hits for stevia.</td>
<td>The hits found for stevia were not relevant for our research. They were too technical and specific. The amount of hits found for artificial sweeteners was too small.</td>
</tr>
<tr>
<td>Embase</td>
<td>Embase is a trademark of Elsevier B.V.. Embase holds over 24 million indexed records from more than 7500 active, authoritative journals, including MEDLINE.</td>
<td></td>
<td>Scopus and ScienceDirect already include Embase.</td>
</tr>
<tr>
<td>Pubmed</td>
<td>Pubmed comprises more than 20 million citations for biomedical literature from MEDLINE, life science journals and online books citations.</td>
<td>Uses MeSH Terms and we already know the database.</td>
<td>There were found less hits for stevia in Pubmed, compared with other databases.</td>
</tr>
<tr>
<td>ScienceDirect</td>
<td>Portal to full text scientific articles, books, Ebooks and references, from over 2500 magazines, published by Elsevier and others, on the area of science, technology and medicine.</td>
<td>A lot of relevant hits for stevia and artificial sweeteners.</td>
<td>ScienceDirect is not easy to work with when a combined search must be done.</td>
</tr>
<tr>
<td>Scopus</td>
<td>Uses MEDLINE and other databases, gives number of citations, also contains scientific literature of different areas. Scopus also uses official expenses of Elsevier, and Nature Publishing Group.</td>
<td>A lot of relevant hits for stevia and artificial sweeteners.</td>
<td>Scopus is not easy to work with when a combined search must be done.</td>
</tr>
<tr>
<td>Web of Knowledge</td>
<td>Includes Web of Science, Biological Abstracts, Inspec, MEDLINE and Journal Citation Reports.</td>
<td></td>
<td>Small amount of hits.</td>
</tr>
<tr>
<td>Wiley</td>
<td>Covers life, health and physical sciences, social science, and the humanities. Includes Cochrane Database of Systematic Reviews.</td>
<td>A lot of hits.</td>
<td>The articles were too technical. And not many relevant hits.</td>
</tr>
</tbody>
</table>