Stevia and its impact on health
Food or Medicine?

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Stevia and its impact on health - Food or Medicine?

1) Why do we need a substitute for sugar
2) The etnomedical story about stevia.
3) Stevia as a new natural sweetener
4) Regulatory and commercial status
5) Stevia as a new drug for the treatment of type 2 diabetes and the metabolic syndrome
6) Future perspectives
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WARNING: Sugar addiction may be harmful to your health and contribute to weight gain, diabetes, heart disease and other health problems.
Sucrose is an easily assimilated macronutrient that provides a quick source of energy to the body, provoking a rapid rise in blood glucose upon ingestion.

Although the brain represents only 2% of the body weight, it receives 15% of the cardiac output, 20% of total body oxygen consumption, and 25% of total body glucose utilization.
Type 2 diabetes a growing epidemic.

The number of individuals with diabetes is growing as an epidemic globally.

The age at diagnosis is going down, the diabetic subjects live longer resulting in increased economic burden.

Today the number of diabetics is estimated to be about 260 million and is expected to increase to more than 380 million by 2025.
Global Projections for the Diabetes Epidemic: 2003-2025

Food intake

Obesity

Causing diabetes and CVD

Moking

Exercise
Metabolic syndrome

Central adipositas

Genetic factors

insulin resistance and/or glucose intolerance

IGT → Type 2 DM

Increased blood pressure

Cardiovascular diseases

Dyslipidemia

\( \uparrow \text{TG} + \downarrow \text{HDL} \)

The First Law of Thermodynamics

Weight Gain

Calories Out

Calories In
Total Caloric Intake

↑ 275 kcal in teen boys

Children 2-17 yrs, CSFII (USDA) 1989-91 vs. 1994-95
http://www.usda.gov/cnpp/FENR%20V11N3/fenrv11n3p44.PDF
Fat Intake: Grams

↑ 5 g (45 cal) in teen boys

Children 2-17 yrs, CSFII (USDA) 1989-91 vs. 1994-95
Prevalence of Obesity Compared to Percent Calories from Fat Among US Adults
Dr. Atkins diet
Atkins diet

Whole Grain Products
- Wheat Flour, Oat Meal, Sprouted Grains, Brown rice, Wheat Bread

Vegetables & Seed Oils Sources
- Dairy Products, Nuts, legumes, Olive

Fruits Sources
- Blueberry, Avocado, Peers,

Vegetables Sources
- Salad greens, Broccoli, Cauliflower, Spinach

Protein Sources
- Sea Foods, Egg, Meat, Soya Products,
Japanese diet

Food Balance Guide

Daily number of portions

食事バランスガイド
あなたのお食事は大丈夫？
Japanese diet is low in fat, high in carbohydrates, devoid of dairy foods and rich in soy foods
Both DIET Works!
Carbohydrate Intake: Grams

↑ 57 g (228 cal) in teen boys

Children 2-17 yrs, CSFII (USDA) 1989-91 vs. 1994-95
Consumption of sugar and obesity prevalence

Beverage Intake

Children 2-17 yrs, CSFII (USDA) 1989-91 vs. 1994-95

↑41% soft drinks
↑35% fruit drinks
The Coca-Cola Conspiracy

1915
6.5 oz
8 lb/yr

1955
10 oz
13 lb/yr

1960
12 oz
16 lb/yr

1988
44 oz
57 lb/yr

1992
20 oz
26 lb/yr
High Fructose Corn Syrup (jarabe de maiz de alta fructosa (JMAF))

Current US annual Consumption of HFCS

-63 pounds per person/y
<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Sweetness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>173</td>
</tr>
<tr>
<td>invert sugar*</td>
<td>120</td>
</tr>
<tr>
<td>HFCS (42-55% fructose)</td>
<td>120</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
</tr>
<tr>
<td>Xylitol</td>
<td>100</td>
</tr>
<tr>
<td>Tagatose</td>
<td>92</td>
</tr>
<tr>
<td>Glucose</td>
<td>74</td>
</tr>
<tr>
<td>high-DE corn syrup</td>
<td>70</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>55</td>
</tr>
<tr>
<td>Mannitol</td>
<td>50</td>
</tr>
<tr>
<td>Trehalose</td>
<td>45</td>
</tr>
<tr>
<td>regular corn syrup</td>
<td>40</td>
</tr>
<tr>
<td>Galactose</td>
<td>32</td>
</tr>
<tr>
<td>Maltose</td>
<td>32</td>
</tr>
<tr>
<td>Lactose</td>
<td>15</td>
</tr>
</tbody>
</table>
High Fructose Corn Syrup is 42-55% Fructose; Sucrose is 50% Fructose

Glucose

Fructose

Sucrose
Annual per capita availability of sugar and HFCS adjusted for loss

USDA food disappearance data

Source: USDA, Economic Research Service, Sweetener Yearbook, Tables 51 and 52
*Estimated annual per capita sugar consumption calculated by adjusting sugar deliveries for domestic food and beverage use for food losses.
**Estimated annual per capita HFCS consumption calculated by adjusting HFCS deliveries for domestic food and beverage use for food losses.
Annual Per Capita Availability of Sugar and HFCS Adjusted for Loss

USDA Food Disappearance Data

- TOTAL HFCS & SUGAR
- MOST FRUCTOSE ITEMS
- SUGAR*
- HFCS**
- JUICE

Source: USDA, Economic Research Service, Sweetener Yearbook, Tables 51 and 52
*Estimated annual per capita sugar consumption calculated by adjusting sugar deliveries for domestic food and beverage use for food losses.
**Estimated annual per capita HFCS consumption calculated by adjusting HFCS deliveries for domestic food and beverage use for food losses.
Fed-state plasma triglyceride concentrations of rats from one of five diet groups (suckle controls (SCs), Rat Milk Substitute, Fructose formula, Galactose formula, SC-Fructose diet from 8 to 12 weeks) at different times throughout the postweaning period

<table>
<thead>
<tr>
<th>Diet group</th>
<th>8 Weeks</th>
<th>10 Weeks</th>
<th>11 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>118 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>163 ± 33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SC</td>
<td>125 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>161 ± 33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rat Milk Substitute</td>
<td>129 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>219 ± 33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fructose</td>
<td>92 ± 18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>144 ± 37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galactose</td>
<td>100 ± 18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>223 ± 37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Leptin (ng/ml)

Huynh M. *Obesity* (2008) **16**, 1755–1762
FIG. 4. Time course of changes in percentage palmitate (16:0) in VLDL TG after single oral boluses of fructose 0.5 g/kg (open circles); fructose 0.5 g/kg + glucose 0.5 g/kg (closed triangles); fructose 1 g/kg + glucose 1 g/kg (open triangles); and glucose 75 g, ~1 g/kg (closed circles). Data are shown as mean ± se.
FIG. 5. Δ for percentage palmitate (16:0) in VLDL TG before and after OGTT screening test (glucose, 75 g; average, 0.8 g/kg), F (fructose, 0.5 g/kg), F:G (fructose, 0.5 g/kg, glucose 0.5 g/kg), and 2X F:G (fructose, 1 g/kg, and glucose, 1 g/kg). Data are presented as mean ± sd. All fructose-containing test means were significantly different from the OGTT mean (repeated measures ANOVA, P < 0.05). One outlier was excluded from the statistical comparisons of F vs. F:G and F:G vs. 2X F:G (paired Student’s t test).
<table>
<thead>
<tr>
<th></th>
<th>Coca-Cola</th>
<th>Schlitz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Percent CHO</td>
<td>10.5% (sucrose)</td>
<td>3.6% (alcohol)</td>
</tr>
<tr>
<td>Calories from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fructose</td>
<td>75 (4.1 kcal/gm)</td>
<td></td>
</tr>
<tr>
<td>other carbs</td>
<td>75 (glucose)</td>
<td>60 (maltose)</td>
</tr>
<tr>
<td>alcohol</td>
<td></td>
<td>90 (7 kcal/gm)</td>
</tr>
<tr>
<td>1st pass GI metabolism</td>
<td>0%</td>
<td>10%</td>
</tr>
<tr>
<td>Calories reaching liver</td>
<td>90</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Calories from</td>
<td>Percent CHO</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------</td>
<td>-------------</td>
</tr>
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<tr>
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<td>10%</td>
<td></td>
</tr>
</tbody>
</table>
Fructose is a carbohydrate
Fructose is a carbohydrate

Fructose is metabolized like fat
Ethanol is a carbohydrate

\[ \text{CH}_3\text{-CH}_2\text{-OH} \]
Ethanol is a carbohydrate

CH$_3$-CH$_2$-OH

But ethanol is also a toxin
How should we define fructose?
<table>
<thead>
<tr>
<th>Chronic ethanol exposure</th>
<th>Chronic fructose exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Hematologic disorders</td>
<td>• Hypertension</td>
</tr>
<tr>
<td>• Electrolyte abnormalities</td>
<td>• Myocardial infarction</td>
</tr>
<tr>
<td>• Hypertension</td>
<td>• Dyslipidemia</td>
</tr>
<tr>
<td>• Cardiac dilatation</td>
<td>• Pancreatitis (2º dyslipidemia)</td>
</tr>
<tr>
<td>• Cardiomyopathy</td>
<td>• Pancreatitis</td>
</tr>
<tr>
<td>• Dyslipidemia</td>
<td>• Hepatic dysfunction (NASH)</td>
</tr>
<tr>
<td>• Pancreatitis</td>
<td>• Fetal insulin resistance</td>
</tr>
<tr>
<td>• Malnutrition</td>
<td>• Habituation, if not addiction</td>
</tr>
<tr>
<td>• Obesity</td>
<td></td>
</tr>
<tr>
<td>• Hepatic dysfunction (ASH)</td>
<td></td>
</tr>
<tr>
<td>• Fetal alcohol syndrome</td>
<td></td>
</tr>
<tr>
<td>• Addiction</td>
<td></td>
</tr>
</tbody>
</table>
There may be an indirect correlation as over-consumption of sugar lead to overweight and insulin resistance, that finally will initiate type 2 diabetes. Fructose may be the bad guy!
Interestingly, one of the solutions for this worldwide burden of increased lifestyle diseases as T2D and obesity, may come from Latin America!
Stevia rebaudiana Bertoni
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New non-caloric sweetener compound has been discovered in the plant *Stevia rebaudiana* Bertoni which has been used among the Guarani Indians in Paraguay and Brazil for centuries to sweeten their Mate tea in Paraguay and Brazil.
Stevia was first discovered by the Paraguayan Botanist, Moises Santiago Bertoni in 1899, who learned of its unique properties from the Paraguayan Guarani Indians.

He described the plant with these simple words:

”When one first observed the plant, nothing particular about it summons the attention, but when even a small piece of the leaf is placed in the mouth, one is amazed by its Sweetness. A mere fragment of leaf is enough to sweeten the mouth for an hour”
Uno de los trabajos más importantes de Bertoni en la etnobotánica es la presentación en los crónicos científicos de la planta medicinal guaraná. Kaa Hee, denominada por sus propiedades como adelgazante natural no calórico y regulador de la diabetes. Esta planta posee el Servisio que es una sustancia varias veces más dulce que el azúcar. La clasificación botánica pertenece a Misilé Bertoni, y el estudio químico de sus componentes a Osvaldo Rebahi. De ahí su nombre: STEVIA REBAUDIANA BERTONI!
Stevia film

Manden med Regnskovsplanten
Bliver sendt på DR

Dybt inde i Paraguays regnskov vokser en lille grøn plante kaldet Stevia, som medicinmænd har brugt i århundreder. Det er en udsædvanlig plante, der søder 400 gange mere end sukker, og som stimulerer organismens evne til at anvende den insulin, kroppen selv producerer. Stevia kan dermed muligvis revolutionere behandlingen af diabetes i verden.
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Intensive sødestoffer der har opnået godkendelse af FDA.

- Aspartame (1981)
- Acesulfame potassium (1988)
- Sucralose (1998)
- Neotame (2002)
- Alitame is pending FDA approval
- Cyclamate is pending re-approval

Ligeledes polyoler (alkohol sukker) anvendes i forskellige fødevarer.
• Mest opmærksomhed er dog i de sidste 10 år blevet tildelt stevia planten, der indeholder intensive sødestof forbindelser som steviosid og rebaudiosid A.

• Stevia planten har flere interessante perpektiver, dels i beskyttelse af vores natur og miljø, dels ved at forbedre rural økonomi i især fattige udviklingslande.
Stevia er blevet anvendt i forskellige former, dels som friske blade, tørret blade, ekstrakt og i opløsninger.

I 70’erne og 80’erne blev stevia brugt som sødestof og smagsfremmer i lande som Japan, Kina, Korea, Isral, Brasilien og Paraguay. Her anvendes forbindelsen stevisoid fra planten stevia.

Japan var det første land til at benytte stevia som bordsukker. I 1991 udgjorde Stevia ca. 41 % af al anvendt sødestof i fødevarer i Japan.
<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_1$</th>
<th>$R_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>steviol</td>
<td>$-H$</td>
<td>$-H$</td>
</tr>
<tr>
<td>steviolbiosid</td>
<td>$-H$</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
</tr>
<tr>
<td>steviosid</td>
<td>$\beta\text{Glc}$</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
</tr>
<tr>
<td>rebaudiosid A</td>
<td>$\beta\text{Glc}$</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
</tr>
<tr>
<td>rebaudiosid B</td>
<td>$-H$</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
</tr>
<tr>
<td>rebaudiosid C (dulcosid B)</td>
<td>$\beta\text{Glc}$</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
</tr>
<tr>
<td>rebaudiosid D</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
</tr>
<tr>
<td>rebaudiosid E</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
<td>$\beta\text{Glc}^2 - \beta\text{Glc}$</td>
</tr>
<tr>
<td>dulcosid A</td>
<td>$\beta\text{Glc}$</td>
<td>$\beta\text{Glc}^2 - \alpha\text{Rha}$</td>
</tr>
</tbody>
</table>
Stevia

Sødestof ↔ lægemiddel
Aspartame is a dipeptide of the amino acids: L-aspartic acid and L-phenylalanine connected with methyl alcohol
Aspartame was discovered by James M. Schlatter in 1965. He was a chemist and work for G.D. Searle & Company now owned by Pfizer.

Schlatter had synthesized aspartame in the course of producing an antiulcer drug candidate. He accidentally discovered its sweet taste when he licked his finger, which had become contaminated with aspartame, to lift up a piece of paper.
Stevia
Sweetener for the Food industry

Stevioside, Rebaudioside A

Isosteviol/steviol
Candidate for the treatment of type 2 diabetes and CVD

Stevia
<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>steviol</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>steviolbiosid</td>
<td>H</td>
<td>βGlc² – 1βGlc</td>
</tr>
<tr>
<td>steviosid</td>
<td>βGlc</td>
<td>βGlc² – 1βGlc</td>
</tr>
<tr>
<td>rebaudiosid A</td>
<td>βGlc</td>
<td>βGlc³ – 1βGlc</td>
</tr>
<tr>
<td>rebaudiosid B</td>
<td>H</td>
<td>βGlc³ – 1βGlc</td>
</tr>
<tr>
<td>rebaudiosid C (dulcosid B)</td>
<td>βGlc</td>
<td>βGlc³ – 1αRha</td>
</tr>
<tr>
<td>rebaudiosid D</td>
<td>βGlc² – 1βGlc</td>
<td>βGlc³ – 1βGlc</td>
</tr>
<tr>
<td>rebaudiosid E</td>
<td>βGlc² – 1βGlc</td>
<td>βGlc² – 1βGlc</td>
</tr>
<tr>
<td>dulcosid A</td>
<td>βGlc</td>
<td>βGlc² – 1αRha</td>
</tr>
</tbody>
</table>
Oprindelige Stevia plante kloner

5-10% 2-4%

% of total dry weight

Fig. 1. Chemical structures of stevioside and its related compounds.
Nye Stevia plante kloner

Fig. 1. Chemical structures of stevioside and its related compounds.

% of total dry weight
Kemiske strukturer af molekyler

Isosteviol

Steviol

Stevioside
Stevia og type 2 diabetes

P2 projekt
Gruppe E3-210
Medicin med Industriel Specialisering
2. semester
Aalborg Universitet
Standard meal test was served with other 0.15 g steviosid or 55 g sugar dissolved in 170 g of grape juice.

The total calorie intake for the two meal was 1114.7 kJ for steviosid and 2049 KJ for sucrose diet.
Postprandial blood glucose

A)

Plasmaglucose concentration (mmol/l)

Time (min.)

-Sucrose
- Stevioside
Postprandial bloodglucose (AUC)
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Regulatorisk godkendelse af Stevia som sødestof til fødevare.

Japan, Korea, China, Taiwan, Australia, Russia, Ukraine, Kazakhstan, Malaysia, Indonesia, Latin America

Senest:
Godkendt i Schweiz 2008
Godkendt i Frankrig i juli 2009
EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives

Food and Agriculture Organization of the United Nations

World Health Organization

World Health Organization

© World Health Organization 2007
| Steviol glycosides | ADI | ADI of 0–4 mg/kg bw expressed as steviol, based on a NOEL of 970 mg/kg bw per day from a long-term experimental study with steviolide (383 mg/kg bw per day expressed as steviol) and a safety factor of 100. The results of the new studies presented to the Committee showed no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks.

Some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides. The Committee recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI. |
|-------------------|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

ADI=acceptable daily intake

NOEL= no observed effect level
SCIENTIFIC OPINION

Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive\(^1\)

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)\(^2,3\)

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Steviol glycosides in the present evaluation are mixtures of steviol glycosides that comprise not less than 95% of stevioside and/or rebaucioside A. Stevioside as a sweetener was evaluated by the SCF in 1984, 1989 and 1999. JECFA reviewed the safety of steviol glycosides in 2000, 2005, 2006, 2007, and 2009 and established an ADI for steviol glycosides (expressed as stevial equivalents) of 4 mg/kg bw/day. The Panel considers that the results of toxicology studies on either stevioside or rebaucioside A are applicable for the safety assessment of steviol glycosides as both rebaucioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both. Considering the available toxicity data (in vitro and in vivo animal studies and some human tolerance studies), the Panel concludes that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic or associated with any reproductive/developmental toxicity. The Panel establishes an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in the rat of 2.5% stevioside in the diet. This is equal to 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that it is likely that the ADI would be exceeded at the maximum proposed use levels.

Key words
Hvad med FDA (USA) and Stevia?

- 1987 FDA forbød brugen af stevia da den ikke var godkendt som et føde tilspændingsstof.
- 1995 FDA gav tilladelse til at Stevia måtte benyttes som et diæt sødestof.
- Så i 2009 ..
Healthnotes Newswire (February 12, 2009)—

- Producenører af oprenset ekstrakt fra stevia planten kaldet rebaudioside A eller rebiana har fået tilladelse fra FDA til at liste deres produkt som general anerkendt som sikker (GRAS status) og til brug for menneske føde kategoriseret som sødestof.
Pepsico og Coca-Cola var de første til at få en FDA godkendelse af stevia sødestoffer.
Approval of Steviol Glycosides in the EU, state of play

Wim Debeuckelaere
Unit E3: Chemicals, contaminants and pesticides
Additional conditions for sweeteners

- replace sugars for the production of energy-reduced food (*), non-cariogenic food or food with no added sugars
- replace sugars where this permits an increase in the shelf-life of the food
- produce food intended for particular nutritional use
Authorisation of steviol glycosides

Applications

- 2007 – 2008 three separate independent petitions for the authorisation of steviol glycosides
  - Cargill Incorporated,
  - The European Stevia Association (EUSTAS),
  - Morita Kagaku Kogyo Co., Ltd

- European Food Safety Authority requested cross reference

- April 2009, roadmap for combined assessment was introduced on behalf of these companies.
Opinion of the European Food Safety Authority

Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive, March 2010.

- Mixture of Stevioside and/or rebaudioside A > 95%

- Not genotoxic, not carcinogenic, no reproductive/developmental toxicity

- ADI of 4 mg/kg
Next steps

- Presentation: Approved!
  - 4 July 2011
- Scrutiny by the Working Committee: Approved!
  - September
- Adoption and publication: November 2011.
PepsiCo
Sobe Life water
Coca Cola company

Dr Pepper Snapple Group (DPSG) and All Sport
Dark Chocolat 70%. Reduced sugar content - contains Rebaudioside A, a sweetener from the Stevia Plant.

Indeholder kun 4 % sukker
Stevia-Joghurt
Stevia and its impact on health - Food or Medicine?

1) Why do we need a substitute for sugar
2) The etnomedical story about stevia.
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4) Regulatory and commercial status
5) Stevia as a new drug for the treatment of type 2 diabetes and the metabolic syndrome
6) Future perspectives
Drug development

Over 120 pharmaceutical products currently in use are plant derived, and about 75% have been discovered by examining the use of plants in traditional medicine.

WHO has estimated that aprox. 80% of the World's population rely chiefly on traditional medicines for their primary health care needs - a major part of this involves the use of plant extracts or their active principles.

Maybe less side effects by using natural product as medical drug
How are drug and plants connected

Nature has produced specific complex molecules that no synthetic chemist could ever dream up.

These molecules evolved over millions of years as chemical defenses by plants, animal, and microorganisms, enabling them to survive attack by fungi, viruses, and other threats.

An estimated 50000-70000 plant species alone are thought to have medicinal qualities.
• The most widely prescribed anti-diabetic drug in the world is Metformin, an alkaloid isolated from the medicinal plants *Galega officinalis*.

• It is accepted as the first-line drug of choice for the treatment of T2D and in the United States alone, more than 40 million prescriptions were filled in 2008.
Traditional plant medicines as treatment of diabetes

• More than 1000 traditional plants have been used in the treatment of diabetes mellitus.

• Only a small number have received scientific and medical evaluation to assess their efficacy.

• Traditional treatments have mostly disappeared in western societies, but are still in use in other regions.

• They may provide valuable tools for the development of new oral hypoglycemic agents.
Stevia rebaudiana Bertoni
Kemiske strukturer af molekyler.

Left: Structure of steviol (upper panel) and stevioside (lower panel).
Right: chemical structure of isosteviol, steviol and stevioside
Islet isolation
Pancreas

• Islets of Langerhans
  – α-cells – glucagon (green)
  – β-cells – insulin (red)
  – δ-cells – somatostatin (blue)
  – PP-cells – Poly peptide
  – ε-cells - ghrelin
Kemiske strukturer af molekyler.

Left: Structure of steviol (upper panel) and stevioside (lower panel).
Right: chemical structure of isosteviol, steviol and stevioside
Glucose mM : 3.3 [ 16.7 ]

Steviol - log M : - - 9 8 7 6 5 4 3

STX 02 Steviol

Dose response

#=p<0.01

Jeppesen P B et al. Metabolism 2000
Glucose dose response

- Steviol: $10^{-6}$ M
- Control

Jeppesen P B et al. Metabolism 2000
ISLET PERIFUSION CHAMBER

- UPPER PISTON
- GEL (FLOW PROTECTION)
- ISLETS
- GEL (ISLET SUPPORT)
- LOWER PISTON

FILTER (GEL SUPPORT)
WATER-FILLED MANTLE

FLOW DIRECTION 0.1 mL / min.
Acute and reversible action
Effect of stevioside on plasma glucose

Results (IAGTT on GK rats)

Effect of stevioside on plasma insulin

Mean ± SEM; n=10 in each group

Mean ± SEM; n=10 in each group.

Effect of stevioside on the plasma glucagon

Impact of STX 01 stevioside on the blood pressure

Mean ±SEM ; n=10 in each group

Jeppesen PB et al., Metabolism 2003
Non-diabetic hypertensive subjects (n=106)

The serial changes in systolic and diastolic blood pressure in patients receiving stevioside 250 mg thrice daily or placebo for 12 months.
Effect of stevioside in type 2 diabetic patients

IAUC = P < 0.05

Stevioside reduced the postprandial blood glucose response by 18±5% (p<0.003)

Glucagon response to Stevioside in T2D patients

IAUC (Glucagon) pmol/l

- Stevioside reduced glucagon (p=0.02)
- 30% reduction

Gregersen S. et al. Metabolism 2004
Left: Structure of steviol (upper panel) and stevioside (lower panel).
Right: chemical structure of isosteviol, steviol and stevioside.
Steviol
Isosteviol
Glucose

In vitro stimulation of GSIS by isosteviol and steviol

![Graph showing insulin ng/ml levels with different concentrations of steviol, isosteviol, and glucose.](image)

- Steviol: 10^{-10}, 10^{-8}, 10^{-6}
- Isosteviol: 10^{-10}, 10^{-8}, 10^{-6}
- Glucose: 3.3 mM, 16.7 mM

P<0.05

*P<0.05
20 KKAy-mice, age 5 weeks, were divided into 2 groups and treated for 9 weeks with:

A: 20 normal C57BL-mice fed with standard chow diet

B: 10 (KKAy) standard chow diet (control)

C: 10 (KKAy) standard chow diet + 0.02 g/kg BW /day of Isosteviol (ISV)
Characterisation of the KKA\textsuperscript{y} mice

The yellow offspring KKA\textsuperscript{y} are obese and insulin resistant with hyperglycaemia, hypertriglyceridaemia and insulin resistance.
Results: Fasting plasma glucose

**P<0.01

![Graph showing fasting plasma glucose levels for different groups.](attachment:image)

Plasma glucose mM

Age 5 week
Age 14 week

Fasting plasma glucose 59 % **
Results: Fasting plasma insulin

**p<0.01

Diabetes Obes Metab.10: 939-49 2008
Results: Glucose-insulin index

Diabetes Obes Metab. 10: 939-49 2008

**p<0.01
Affymetrix GeneChip expression probe array.

More than 35,000 genes were investigated.
GeneSpring analyses

Principal Component Analysis performed using GeneSpring (Agilent Technologies)

Cluster analysis performed using GeneSpring (Agilent Technologies)
Results:

RT PCR gene expression

Ins1 (132%, p<0.025)
Results: insulin

Effects on total islet insulin protein content in KKAy mice after 9-weeks intervention with ISV

Each bar represents mean ± SEM from 3 protein purifications, each pooled from 3-4 mice (n=3 each bar).

**p< 0.01 vs. control.

Diabetes Obes Metab.10: 939-49 2008
Results: Gene expression analysis in islets

- GLUT2 mRNA levels significantly increased in ISV compared to KKAy (201%, p<0.001)

- Other genes such as Pdx1, Akt1, GLUT2, C/EBP-alpha, CPT1, IGF1, 11-Beta-HSD1, IR, Beta2, and IRS1 also showed differences in expression.

Diabetes, Obesity and Metabolism. 10 (10): 939-49, 2008.
Results:

Gene expression analysis in islets

In humans, 11 beta-HSD-1 converts cortisone into Cortisol.

Diabetes, Obesity and Metabolism. 10 (10): 939-49, 2008.
11-beta-HSD-1 is a sought target of pharmacological intervention.
BW

[Graph showing body weight (g) comparison between different groups: C57/BL, KKAy, KKAy-ISV, at 5 and 14 weeks. Bars indicate mean body weight with error bars.*** indicates statistical significance.]

Diabetes Obes Metab. 10: 939-49 2008
Result summary

- Glucose responsive
- Synergistically

Pdx1
Nkx2-2
Pax6
Beta2

ISV

Ins1

11-β-HSD-1
C/EBPalpha

GSIS

GLUT2

Beta cell differentiation
Result: Fasted plasma TG

<table>
<thead>
<tr>
<th>Age</th>
<th>C57</th>
<th>KKAy</th>
<th>KKAy-ISV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 week</td>
<td><strong>49%</strong></td>
<td><strong>p&lt;0.01</strong></td>
<td><strong>49%</strong></td>
</tr>
</tbody>
</table>
Total Cholesterol/HDL Cholesterol after 9 weeks of intervention

Unpublished
Normal Wistar rats.
20 weeks intervention
Isosteviol 0.03 g/kg

Unpublished
What is next?

- Clinical trials have been approved by
- The Danish Medicines Agency,
- The Danish Ethical Committee Submitted
- The Ministry of Interior and Health for the GMP production of the drug

Lægemiddelstyrelsen bekræfter hermed for modtagelsen af Deres brev, dateret 8. november, 2010, vedrørende ovenstående forsøg. I Deres brev var følgende dokumenter vedlagt:

- Opdateret EudraCT ansøgningsform, ikke underskrevet, inklusiv xml-fil
- Forsøgsprotokol, version 7
- Opdateret deltagerinformation

Styrelsen accepterer de indsendte ændringer og har ingen yderligere kommentarer til det vedlagte.
What is next?

- Clinical trials have been approved by
  - The Danish Medicines Agency,
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  - The Ministry of Interior and Health for the GMP production of the drug
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Total produktion of Stevia blade er ca. 100000 tons/år

Prisen for 1 kg stevia plade var ca. 5,25 kr/kg i 2006.

På grund af Coca-Cola/Cargill FDA godkendelse af stevia
Som sødestof er prisen steget til ca. 14 kr/kg i år 2009

Coca-cola/Cargill har estimeret at produktionen skal op på mindst 800000 ha for at dækket efterspørgslen
Hvor dyrt er stevia – et regneeksempel.

1 kg rebaudioside A (400-450 gange sødere end sukrose) 98 % rent: Pris 1000 kr.

Det vil svare til ca. 400 kg sukrose a 7 kr/kg = 2800 kr
Thank you for attention