

## Native fructose extracted from apple improves glucose tolerance in mice

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Fructose is one of the most abundant monosaccharide in nature. It is also the sweetest naturally occurring carbohydrate. Since decades, fructose used for food preparations is not provided by fruit or vegetable but by a chemical process of starch or inulin conversion. We processed a new method of fructose extraction from apple and investigated the acute and long term effect of this carbohydrate on glucose metabolism in C57Bl6/j mice. By using the glycemic index (GI), we have shown that one of the sugars obtained from apple, FructiLight, has a very low impact on glycemic and insulin response during acute treatment compared to other sugars. This carbohydrate, essentially constituted by fructose, has also beneficial properties when administrated for long term treatment. Indeed, as two other sugars extracted from apple (FructiSweetApple and FructiSweet67), FructiLight exposure during 21 week in beverage has promoted an enhancement of glucose tolerance compared to glucose treatment without affecting food intake and weight. All these results indicate that apple-extracted sugars and more precisely fructose from these fruits could be a promising way to produce new food and sweet beverages.

**Key words:** Fructose, Glucose tolerance, Mice.

During the last decades, there have been advancement in the knowledge of the impact of alimentation-carbohydrates on human health. Dietary carbohydrates

consist of polysaccharides, disaccharides and monosaccharides and account for 45 to 70 % of energy intake in developed countries (3). Disaccharides include sucrose (found in sugar cane, sugar beets, honey...), which is the result of the association between one molecule of glucose

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and one molecule of fructose; lactose (found in milk) with one molecule of glucose and one molecule of galactose; and maltose (found in malt) with two molecules of glucose (10). Monosaccharides include glucose, fructose, galactose (10) and sorbitol is the alcohol of glucose (10). Fructose (or levulose) is a simple reducing sugar found at relatively high concentration in many fruits such as apples, pears, peaches and watermelon or in vegetables such as carrots, broccoli and onions. It is also present in other foods consumed by humans such as honey. There are different ways to obtain fructose. Indeed, this hexose can be obtained by sucrose digestion extracted from vegetables or fruits (19) or it can also be derived from glucose isomerisation to give high fructose corn syrup (HFCS) largely used in food industry (1). The main difference between fructose from HFCS and from fruits or vegetables lies in the primary matter used and mostly in the process to obtain fructose. Indeed, fructose used by industry is usually synthesized from starch, beet or sugar cane. A complex process consisting in degradation of sucrose into glucose plus fructose and isomerisation of glucose in fructose by chemical or biochemical process is used (1). Conversely, the high amount of fructose present in fruit such as apples or pears allows to extract fructose directly (2). Lot of work deals with HFCS impact on health but little is known about differences between HFCS and natural fructose on glucose homeostasis.

To compare the effect of different sugars on glucose metabolism, glycemic index (GI) had been introduced by Jenkins (12). It is a way to classify carbohydrate-containing foods according to how they are digested and absorbed during postprandial period. This measure of the carbohydrates quality is based on their

direct effect on blood glucose levels 2 hours after the meal. As a reference point, glucose is used with GI equals 100 in comparison with others sugars or nutrients (23).

In this study, we compared the effect of short and long term treatment by glucose, HFCS and fructose extracted from apples on glucose homeostasis.

## Material and Methods

*Animals.*— Animals were handled with the principles and guidelines established by the National Institute of Medical Research. Male C57Bl6/j mice were obtained from Charles River Laboratory (L'Arbresle, France). Mice were housed conventionally in a constant temperature (20 °C–22 °C) and humidity (50–60 %) animal room, with a 12/12 hr light/dark cycle and free access to food and water. During chronic treatment, mice were monitored every week by measuring body weight and blood parameters (glucose) until 21 week of treatment.

*Products.*— D-glucose and D-fructose were purchased from Sigma Aldrich (St Quentin Fallavier, France). Fructose 95% (Fructose Control) was obtained mixing 95% of fructose and 5% of glucose. HFCS were manufactured by CRITT laboratory (Toulouse, France) mixing D-Glucose and D-Fructose from starch (Glucose-[Fructose]<sub>n</sub>-Fructose). HPLC-analysis of this mixture shows the presence of maltooligosides, a dimere of glucose units provided by inulin degradation. Sugar extraction from apple juice has been assessed by CRITT laboratory (Toulouse, France). Three products were extracted from apple juice. The first we named FructiSweetApple and HPLC analysis shows that it contains 7.4 % sucrose, 60.8

% fructose, 28.6 % glucose and 3.2 % sorbitol (Table I). Sucrose hydrolysis from this fraction provides the second sugar named FructiSweet67. HPLC analysis of FructiSweet67 shows the presence of sucrose (0.6%), fructose (64.7%), glucose (31.2 %) and sorbitol (3.5 %) (Table I). In a last step, glucose was removed from this fraction giving the FructiLight. This extract is composed only by fructose (95.9 %), sorbitol (3.2 %) and traces of glucose (0.9 %) (Table I).

*Sugar tolerance test.*— Glucose, HFCS, Fructose 95%, FructiSweetApple, FructiSweet67 and FructiLight were orally (3g/kg) administrated to 12h-fasted mice. As previously described (8), blood was collected from the tip of the tail vein and glucose levels were monitored with a glucometer (Roche Diagnostic, Rotkreuze, Switzerland) at 0, 15, 30, 45, 60, 90 and 120 minutes after oral sugar load.

Serum insulin concentrations were measured 15 min before and 30 min after the oral sugar load using an ultra-sensitive mouse insulin ELISA (Mercodia, Uppsala, Sweden).

*Assessment of food and water intake.*— In order to determinate the effect of the different sugars tested, we assessed food

and water intake during 18 h after an oral sugar load on 12 weeks-old mice. Animals were food deprived for 6 h preceding the behavioural testing, with ad libitum access to water. Glucose, FructiSweetApple, FructiSweet67 and FructiLight (3g/kg) were orally administrated and food and water intake has been measure by Oxylet Food and Drink recording system (Panlab, S.L, Barcelona, Spain).

*Determination of body composition.*— Fat and lean mass was measured using dual-energy X-ray absorptiometry (Echo MRI-100TM, Echo Medical System, Houston, Texas) in accordance with the manufacturer's instructions.

*Statistical analysis.*— Data are presented as means  $\pm$  SEM. Analysis of differences between groups was performed with Student's *t* test an  $P < 0.05$  was considered to be significant.

## Results

*Glycemic index of apple extracted sugars.*— In order to evaluate the impact of the different sugars on glucose homeostasis in mouse, we performed a *per os* tolerance test. As expected, oral glucose (3g/kg)

Table I. Analysis of sucrose, glucose, fructose and sorbitol content in synthetic (glucose and fructose control) and corn (HFCS) - or apple (FructiSweetApple, FructiSweet67 and FructiLight) - extracted sugars.

	Sucrose	Glucose	Fructose	Sorbitol
<i>Synthetic</i>				
Glucose	0%	100%	0%	0%
Fructose Control	0%	5%	95%	0%
HFCS	0%	52.3%	43.5%	0%
<i>Apple extracted</i>				
FructiSweetApple	7.4%	28.6%	60.8%	3.2%
FructiSweet67	0.6%	31.2%	64.7%	3.5%
FructiLight	0%	0.9%	95.9%	3.2%

administration increased plasmatic glucose amounts by 3-times (Fig. 1A). To compare the effect of other kind of sugars with glucose, glycemic index (GI) has been calculated using area under curve obtained during tolerance test. As shown in Fig. 1B, we can separate two groups of carbohydrate compound. The first one is composed by high-to-middle GI compounds such as HFCS, FructiSweetApple and FructiSweet67. Indeed, during tolerance test, these sugars raised glycaemia with a GI superior to 50 (Fig. 1A and 1B). On the other hand, control fructose and FructiLight administration have a slight effect on glycaemia, conferring to these compounds a low GI (<50). Interestingly, FructiLight's GI is lower than control fructose ( $15.3 \pm 2.4$  vs.  $38.4 \pm 6.1$ ) thus classifying FructiLight as a very low GI sugar. During tolerance test experiments, plasmatic insulin had also been assessed before 30 and 60 minutes after the sugars administration. Results presented in Fig. 1C are in accordance with GI results since glucose considerably increased plasmatic insulin whereas other compounds such as HFCS, FructiSweetApple, FructiSweet67 and control fructose have moderate effects on this parameter. In the other hand, FructiLight administration did not significantly modify insulin levels confirming the very low GI propriety of this sugar extract. In order to know if the tested compounds could have delayed-time effects on insulinemia, we measured plasma insulin concentration 60 minutes after administration. The results presented in Fig. 1C show no difference between the tested carbohydrates.

*Chronic treatment by apple-extracted carbohydrates enhances glucose tolerance without modifying other parameters.*— The aim of these long-term experiments was to

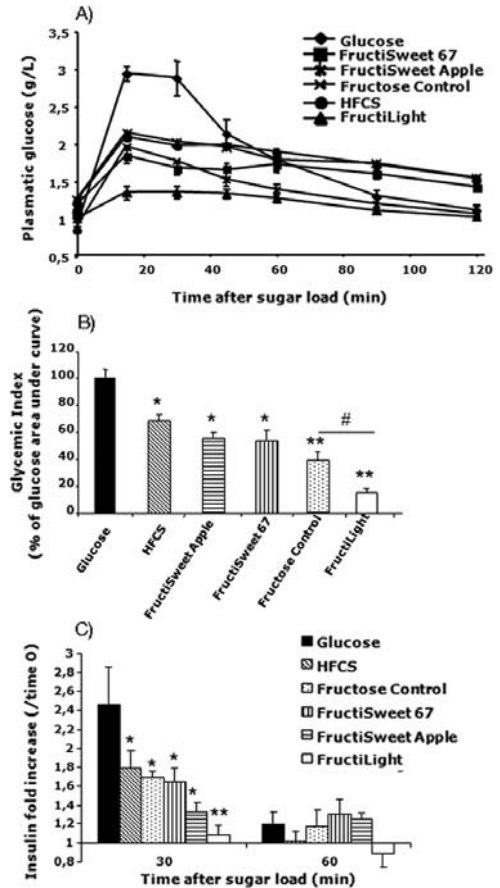


Fig. 1. A. Oral sugar tolerance test (OSTT). Overnight fasted (12h) mice were filled-up by solutions containing 3g per kg of body weight of the indicated sugar and blood glucose has been measured with glucometer before and 15, 30, 45, 60, 90 and 120 minutes after sugar load. B. Glycemic Index (GI). GI is calculated through comparison of area under curve (AUC) obtained during OSTT. Glucose AUC is the standard value (100%) and the others AUC values are compared to glucose. C. Plasma insulin variation during OSTT. Blood samples have been taken from tail vein before and 30 and 60 min after the oral sugar load. Insulin plasma levels were quantified by ELISA technique. Values are mean  $\pm$  SEM of 7 animals in each group. \*p < 0.05, \*\*p < 0.005 compared to glucose; #p < 0.05 compared to fructose control.

determine whether apple-extracted sugars exposition could modify metabolic parameters such as weight, fat mass, lean mass, glucose tolerance or plasmatic insulin levels compared to glucose. Prior to chronic treatment, we evaluated the effect of each sugar on food and water intake during short-term experiments. No difference between groups had been noticed when food and water intake was assessed 17 hours after a bolus of each sugar (3g/kg) (data not shown). For chronic treatment, each compound was administrated in beverage (3g/kg) and animals had *ad libitum* access to water. After 21 weeks of treatment, no difference in weight or in body composition had been noted (data not shown). The second part of these experiments was to investigate the effects of apple-extracted sugars long-term treatment on glucose homeostasis. For that purpose, we performed oral glucose tolerance tests at the beginning (1 week) and at the end (21 weeks) of the treatment (Fig. 2). The results presented in Fig. 2A demonstrate that 21-weeks glucose treatment in mice through an increase of OGTT area under curve whereas apple-extracted sugars (FructiSweetApple, FructiSweet67 and FructiLight) did not significantly modify this parameter. Moreover, after 21 weeks of treatment, FructiLight leads to a significant increase of glucose tolerance compared to glucose treatment. During OGTT, we also performed mice insulin secretion capacity through plasmatic measurement before and after the oral glucose load. Results did not show any alteration or improvement of insulin secretory capacity between the four groups of mice (Fig. 2B).

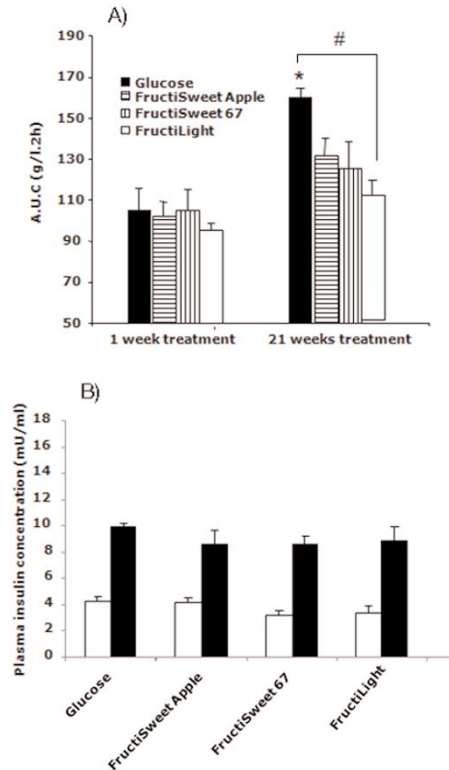


Fig. 2. Oral glucose tolerance test (OGTT) after 21 weeks of sugar treatment.

Glucose tolerance had been determined by oral glucose tolerance test (OGTT 3g/kg) after 1 week and 21 weeks of treatment by sugars in beverage. Area under curve (AUC) was calculated and the results are means  $\pm$  SEM of 7 animals per group. \* $p < 0.05$ , compared to week 1; # $p < 0.05$  compared to glucose. B. Plasma insulin determination before and after OGTT. Blood samples have been taken before (15 min, white bars) and after (30 min, black bars) oral glucose load (3g/kg). Insulin had been determined by ELISA. Results are means  $\pm$  SEM of 7 animals.

## Discussion

The ingestion of sucrose, HFCS and other sugars accounts for 25% of energy consumption in the United States and Fructose accounts for about 10% (2). Such intake may be even higher in chil-

dren and adolescents, mainly due to soft drink consumption. Several studies have highlighted the need to improve the quality of these sugars used in food (6, 9). So far, chemical process involving starch hydrolysis present in corn or wheat leads to production of fructose and glucose. Then, other steps of purification and chemical or biochemical manipulations can drive to get commercial syrups composed by various concentrations of fructose and glucose used in the composition of aliments. If the deleterious effect of these "synthetic" syrups on health has been clearly demonstrated, opposite results have been published about fructose consumption by humans or rodents on glucose homeostasis (3, 9, 13, 20). It has been shown that intense fructose consumption could produce deleterious effects on health such as glucose homeostasis disruption or weight gain (5, 6, 10, 14, 22) whereas others studies demonstrated that a slight fructose intake could lead to protective effects on glucose metabolism (16, 17, 21). Moreover, almost all these studies used corn or wheat starch as source of fructose. In our study, we showed for the first time that synthetic and natural (extracted from fruit) fructoses have different impact on glucose homeostasis in mice. During oral sugar load, we confirmed that starch-extracted solution such as HFCS as well as two sugars obtained from apple extraction, FructiSweetApple and FructiSweet67 exhibit a high GI. Conversely, FructiLight presents low GI during oral load (two fold-reduced when compared to control fructose). Insulin levels measured during these experiments before and after the sugar load showed a tight correlation between GI and insulin secretion. Eventhough different studies have shown that acute fructose administration is able to enhance glu-

cose homeostasis (16, 21), this result seems to be due to the presence or not of glucose in the tested sugars. FructiLight contains less than 1% of glucose whereas others compounds tested are composed by 5 to 100% glucose. According to HAE-COCK *et al.* (11), low-dose administration of fructose could improve the glycemic response to starchy food in humans. In our study, we cannot discriminate the sole effect of apple-extracted fructose and the impact of the absence of glucose in FructiLight. However, the difference of GI and insulinemia observed between FructiLight and fructose could indicate a combined effect. This result could also be due to the origin and method used to extract fructose to make FructiLight. Indeed, fructose contained in HFCS or in control is chemically obtained from glucose conversion whereas FructiLight is obtained from apple by using a different process.

Previous long term studies of fructose impact on health have shown contradictory results on glucose homeostasis. If low doses of fructose have been demonstrated to be protective for health (3, 24), high concentration seems to be deleterious (5). To know the effects of long term treatment with apple extracted fructose in beverage (3g/kg) on energy metabolism no variation of weight and body fat mass content have been observed (not shown). The discrepancy with previous data showing an increase of fat mass (14) could be explained by the dose used since the sugar concentration used is 15 fold less important than in Jurgens *et al.* data. Without modifying insulin secretion, food or water intake and weight, the different sugars extracted from apple have enhanced glucose tolerance compared to glucose treatment and allows us to conclude to a non-deleterious effect of apple extracted sugar such as FructiLight on glucose homeosta-

sis during long term exposure. Even though more experiments have to be performed to investigate the efficacy in human, it is a promising way to replace synthetic fructose in food by such a natural compound.

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