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The Level of Sucrose Intake and the Effect of its High Concentrations on the Activity of the Digestive Enzymes

Aminet Tsikunib, Svetlana Kaytmesova, Angelina Shakhanova and Yulia Dyachenko*

Adyghe State University (AGU), Maikop - 385000, Street Pervomayskaya, 208, Russian Federation; gic-info@yandex.ru, niikpagu@rambler.ru

Abstract

The monitoring of nutrition among different population groups shows that one of the essential dietary infringements is an excessive intake of sucrose, which is substantially higher than the physiological norms and the evolutionarily established traditional levels. A multidirectional effector effect of sucrose on the enzymes was determined as a result of the studies on the impact of the sucrose's high concentrations on the activity of the digestive enzymes in vitro. These included studying the effect of the salivary ά-amylase through the amyloclastic method, the effect of the pancreatic lipase through titrimetry, and the effect of the sucrase-isomaltase complex through the glucose level dynamics in simulated environments with the specific concentrations of sucrose under the action of an enzyme. The high concentrations of sucrose reduce the activity of the ά-amylase and the intensity of the starch digestion; activate the pancreatic lipase increasing the intensity of the intestinal fat digestion; lead to the induction of the activity and amount of the intestinal sucrase-isomaltase complex increasing the rate of the sucrose splitting, its hyperglycemic effect, and the risk of the impaired glucose tolerance.

Keywords: ά-Amylase, Excessive Intake of Sucrose, Pancreatic Lipase, Sucrase-Isomaltase Complex

1. Introduction

Nutrition of a modern human is characterized by the sharp (in terms of evolution) and intense (in terms of quantity) change of the nutrient status mainly caused by the excessive intake of saturated fats and fast-digestible carbohydrates, especially of sucrose[1,2,4]. Numerous studies have established that the excessive intake of sucrose can cause alimentary obesity, diabetes, cardiovascular diseases, atherosclerosis, dental caries in children, etc[3,5,6,10,11,16]. The majority of researchers associate the physiological mechanisms of the excessive sucrose intake impact with its high glycemic effect that changes the hormonal profile of the body[4,9]. There are virtually no data considering sucrose as an independent chemical compound capable of directly affecting the metabolic processes in the body and, first of all, the digestion process, which is the initial stage of the nutrients assimilation in organism, and the adaptation to various endogenous factors, especially to the changes in the nutrition's structure and quality.

The purpose of our study is to investigate the level of sucrose intake by various population groups of the Republic of Adygea, and to identify the features of the sucrose impact on the activity of the hydrolytic enzymes of the gastrointestinal tract, as well as the processes of the macronutrients digestion.

2. Methodology of the Study

Sucrose intake by various population groups of the Republic of Adygea was monitored by questioning according to the "Guidelines for Studying the Actual Nutrition and Health Status of the Population in Relation to the Nature of Nutrition"[7,8]. The studies conducted during 2010–2014 were attended by preschool children (n = 45) at the age of 4 ± 1.2 years; among them, 20 children went to kindergarten, and 25 children did not go to kindergarten; schoolchildren of different age categories (n = 133); students (n = 82) at the age of 19 ± 3 years; and adults

*Author for correspondence

(n = 45); among them, 21 persons were of working age, and 9 persons were over the working age. In order to determine the sucrose content in the traditional nutrition of the Circassians, the authors studied the chemical composition of 17 reconstructed rations. In total, 307 daily rations were analyzed by the following indicators: common carbohydrates, sucrose, and caloric content. The nutrients content in the rations was calculated using the "Table of the Chemical Composition and Energy Value of the Food Products"[15], and estimated in compliance with the recommendations of the Research Institute of Nutrition of the Russian Academy of Medical Sciences[12].

The sucrose effect on the activity of the hydrolytic enzymes in the gastrointestinal tract, of such as ά-amylase (N.F.3.2.1.1), pancreatic lipase (triacylglycerol ester hydrolase, CF 3.1.1.3), and sucrase-isomaltase complex (β-D-fructofuranoside fructohydrolase, CF 3.2.1.48), was studied in vitro. The ά-amylase activity was determined by the amyloclastic method[13], which is based on the starch degradation under the enzyme impact at a maximum saliva dilution. The simulated environment containing 0.1% of starch, and 0%, 0.75%, 1.5%, 3.0%, and 4.5% of sucrose, accordingly, were studied. The ά-amylase activity was expressed through the number of milliliters of the 0.1% starch solution, which degrades if exposed to 1 ml of saliva at the temperature of 38˚C within 30 minutes. Normally, the activity of the salivary amylase is 160–320 units of activity. The level of quantification is 20 units of activity.

The lipase activity was inferred by the amount of fatty acids, which were produced by the milk fat hydrolysis for a given time interval, and under the effect of the bovine pancreas glycerol extract. The pancreas extracted directly after the bull clave slaughtering was frozen, delivered to the laboratory, cleared of fat, comminuted, and carefully powdered by a pestle together with glycerol in the ratio of 1:3. The resulting extract was filtered through 2–3 layers of gauze fabric. The glycerol extract in the amount of 0.5 ml was added to 5 ml of the sucrose containing lactic-acetate mixture similar to the previous experiment, then placed into a thermostat at the temperature of 37°C, and after 0', 20', 40', and 60' titrated against the 0.1N NaOH solution.

3. Results

The studies uncovering the effect of the excessive sucrose intake on the SIC activity were carried out in two stages. The stage I involved preparation of the SIC extract from the isolated avian small intestine. The stage 2 involved execution of the studies uncovering the effect of various sucrose concentrations on the enzyme activity in vitro. The study object selection is substantiated by the fact that, firstly, the SIC is generated in bird intestine[17], secondly, birds' ration, as in majority of animals, has no added sucrose, which provides for obtaining a primary response to the prolonged excessive intake of the added sucrose, and, thirdly, by the cost effectiveness of the experiment implementation.

The stage 1 of the experiment was conducted in a private farmstead of the PhD student Kaitmesova, S.R. The chickens of the domestic silver breed at the age of 2 months (n = 28) were divided into two groups using the random sampling technique, and put into different aviaries. During one month, the chickens were fed with grain accompanied by ample drinking. At the same time, the test group of chickens was receiving water with sugar at a concentration of 10 g per 100 ml. The observation of the experimental animals showed that by the end of the experiment, the test group was less active: the chickens were sleepy, sluggish, and huddled themselves up in a corner. Moreover, the chickens from the test group drank more water (by an average of 30–50 ml) than those from the control group, which drank an average of 150 ± 15 ml. On the last day of the experiment, the chickens were not fed and decapitated after 12 hours. Their abdominal cavity was opened, the small intestine was extracted (without duodenum), washed with 20 ml of the chilled Ringer's solution (pH 7.4), and placed in an ice bath. All the subsequent manipulations were carried out at the temperature close to 0˚C. The samples of the small intestine were delivered to the laboratory in a coolbox. Later, in order to prepare the SIC extract, the middle part of the small intestine was cut into sections of the same length (15 cm), since it featured the highest enzyme activity according to the literature[14,17]. The mucous membrane of the small intestine was extraverted, incubated in 10 ml of the Ringer's solution (pH 7.4) at the temperature of 37˚C for 45 minutes with constant stirring and at the rate of 120–140 rpm. The SIC extracts prepared by incubating the avian intestines of the test and control groups were combined to obtain SIC_{test} and $SIC_{control.}$

At stage 2, the studies were conducted to identify the effect of various sucrose concentrations on the SIC activity. For this purpose, the SIC extract was added to the modal media containing 0.75%, 1.5%, 3.0%, and 4.5% of sucrose in the ratio of 5:1, and incubated for 30'. The depth of the sucrose hydrolysis was estimated by the glucose concentration in a simulated environment. The glucose

value was determined through the glucose oxidase test using the recipe provided in the "Fotogliukoza" test-kit. The optical density was measured with a spectrophotometer UNIKO. The linear range for determining the glycose concentration was within the range of 2– 20 mmol/l.

4. Discussion

The study of the sucrose intake by different population groups of the Republic of Adygea demonstrates its incompliance with the physiological standards (Table 1).

As can be seen from Table 1, the carbohydrates intake by preschool children attending preschool institutions

Table 1. The levels of sucrose intake by various population groups of the Republic of Adygea (2010-2014)

The studied contingent	The content within the studied rations		
	Common carbohydrates, g	Sucrose, g	Calories, kcal
Children attending a kindergarten	271.5±44.2	47.5±13.4	1820±210
Children not attending a kindergarten (from wealthy families)	176.4 ±65.7	65.2 ±8.3	1895±105
Children not attending a kindergarten (from low-income families)	186.2±32.6	40.8±9.5	1213±267
Primary schoolchildren	223.2±36.0	58.0±6.3	1739±142
Adolescents:			
girls	248.4±28.5	76.0±10.8	1883±125
boys	299.0±23.9	75.9±13.4	1965±358
High school students:			
girls	226.2±68.0	68.4±14.0	1703±427
boys	345.4±49.5	97.2±12.8	2270±585
University students:			
girls	288.8±98.0	98.0±10.8	2090±817
boys	315.0±75.5	104.6±12.9	2299±927
Persons capable of working and elderly people:			
women (30-53 years)	271.7±28.5	65.4±5.4	1983±125.2
men (30-59 years)	282.9±24.8	70.7±15.9	2358±333.5
women (over 55 years)	241.0±28.5	50.3±12.8	1734±222.3
men (over 60 years)	261.9±64.5	54.5±18.2	1758±272.2

amounts to 271.5 ± 44.2 g per day, sucrose accounts for 10.4% of the daily caloric content, instead of the carbohydrates fraction content/rations caloric content ratio, which is physiologically recommended in accordance with the formula: CC_{kcal} : CC_g : Suc_g = *100 : 14.5 : 2.5* wherein the following ratio is obtained =100 : 14.9 : 2.6. An analysis of the rations of children not attending kindergartens (n=25) shows that the nutrition structure, i.e. the set of food products and their quantity, is essentially different in individual children. According to the parental survey, it essentially depends on the family's income. On this basis, children not attending a kindergarten were divided into two groups: children from wealthy families (n = 15, the children do not attend a kindergarten since their parents have a mainly financial opportunity to ensure, as they consider, more favorable conditions, including those on nutrition, than in a kindergarten), and children from low-income families (n = 10, the children do not attend a kindergarten since their parents are in the queue to a kindergarten, and their mothers are forced to stay at home, which also reduces the welfare of the families). The rations of children from wealthy families contain more meat, dairy products, fruits, but also a lot of sweets: candies, chocolates, cakes, pies, i.e. a "nourishing" and often "sweet" food ration (within the understanding of most parents, these are similar concepts) is formed. Undoubtedly, the structure of food intake had an impact on the food energy value and the content of carbohydrates, including sugar: children from low-income families have the ratio of CC_{kcal}: CC_g : Suc_g = 100 : 15.3 : 3.4, and children from wealthy families have the ratio of CC_{kcal}: CC_g : Suc_g = 100 : 9.3 : 3.3. The intake of carbohydrates, including sucrose, with regard to the calorie content in primary schoolchildren corresponds to the following formula = 100:12.8:3.3; in adolescents: girls = 100:13.1:4.0 , and boys = 100:15.2:3.9; in high school students: girls = 100:13.3:4.0, and boys = 100:15.2:4.3; in students: girls = 100:13.8:4.9, and boys = 100:14.5:4.8; in persons capable of working: women = 100:13.7:3.3, and men = 100:12.0:3.0 (at the rate of 100:14.6:2.5); in the elderly people: women = 100:13.9:2.9 (at the rate of 100:14.4:2.5), and men = 100:14.9:3.1 (at the rate of 100:14.6:2.5). The most significant deviations from the physiological standards of sugar intake were detected in high school students (the excess of 6.7 times), and university students (the excess of 9.6 times). On average, the sugar content in the food rations of the examined persons amounted to 73.1 ± 18.6 g. Apparently, the true level of sugar intake is higher (usually the respondents

either hardly remember the sweets eaten between meals, or, especially women, understate the amount of the sweets eaten). This is confirmed by the fact that, according to the official statistics in 2007–2008, the sucrose intake by the population of the Republic of Adygea made an average of 143 g/day per each person (also including children) (Russian Statistical Yearbook, 2011)[12]. At the same time, an analysis of the reconstructed rations showed that the sucrose content in the traditional diet of the Circassians made an average of 11.6 ± 5.6 g, while the sugar to the ration's caloric content ratio was 100_{kcal}: 0.7 g, i.e. per each 100 calories, the sugar intake was 3.7…6.9 times lower than at present.

The reasons for the abrupt increase in the sugar intake by various population groups are the dietary structure and quality infringements, which are reduced to the decrease in the intake of the full-value food categories: milk and dairy products, meat and meat products, fish and fish products, fruits and vegetables, and to the increase in the intake of bread and confectionary products; the increase of available sugar and confectionary products; the low content of national cuisine dishes in the ration; fascination of the youth with tonic and energy drinks that are characterized by the high content of sugar; the low level of knowledge among different population groups in the area of healthy nutrition, and the underestimated role of nutrition (especially by the young people) in ensuring the physical and mental efficiency; the insufficient level of control over the implementation of the youth's (high school and university students) individual rations from the side of their parents.

Due to the sugar content in the food rations of the majority of people in the amounts far exceeding the physiological standards, the further studies were focused on investigating the effect of the excessive sucrose intake on the metabolic processes in an organism.

The relevance of studying the sucrose effect on the activity of α-Amylase (α-A) and Pancreatic Lipase (PL) is substantiated by the data of the long-standing monitoring of food rations among various population groups that showed a high intake level of the food products simultaneously containing large amounts of sucrose as well as of starch and neutral fat.

The studies have shown that sucrose reduces the ά-amylase activity (Table 2).

At a concentration of 0.75% in a simulated environment, sucrose has no reliable effect on the enzyme activity, and at a concentration of 1.5%, the enzyme activity decreases with the intensity of 25.8%; at a concentration of 3.0%, the decrease intensity makes 31.1%; and at a concentration of 4.5%, the enzyme activity decreases with the intensity of 47.7%, i.e. sucrose acts as a negative modulator of the ά-amylase activity. Given that the ά-amylase of saliva and the pancreatic amylase have a similar substrate specificity and mechanism of action, the data obtained with the involvement of the ά-amylase of saliva can be extrapolated to pancreatic amylase and conclude on that when the food products simultaneously contain starch and sucrose, the intensity of starch digestion in the intestines is reduced.

Table 3 presents the data obtained by studying the sucrose effect on the pancreatic lipase reactivity.

The obtained data demonstrate that in the conditions of control, pancreatic lipase reaches the maximum

Table 2. The changes in the ά-amylase activity under the influence of sucrose

Sample code	Concentration of sucrose in a simulated environment, %	The ά-amylase activity after 30 minutes of exposure, units of activity
ά-A control	0	302±53.3
ά-A experiment No.1	0.75	297±60.5
ά-A experiment No.2	1.5	224±75.0*
ά-A experiment No.3	3.0	208±107.3*
ά-A experiment No.4	4.5	144±35.8**

*$p < 0.05$ **$p < 0.01$ - *significance of differences with control*

Table 3. The sucrose effect on the pancreatic lipase activity

Sample code	Concentration of sucrose in a simulated environment, %	Duration of the pancreatic lipase effect on a substrate, min.			
		Pancreatic lipase activity, ml of NaOH			
		0'	20'	40'	60'
PL control	0	0.4±0.10	1.3±0.15	1.9±0.25	1.8±0.13
PL experiment No.1	0.75		1.8±0.20*	2.0±0.20	1.8±0.18
PL experiment No.2	1.5		2.6±0.10**	2.4±0.15*	2.1±0.23*
PL experiment No.3	3.0		2.3±0.15*	2.2±0.20	2.0±0.37

*$p < 0.05$ and **$p < 0.01$ - *significance of differences with control*

degree of activity equal to 1.9 ± 0.25 ml of NaOH during 40 minutes of exposure. Further, the enzyme activity is reduced by a number of reasons, and primarily due to the accumulation of fatty acids (the products of the neutral fat hydrolysis), which on the one hand, leads to the pH decrease in a simulated environment (normally, lipase is active when the pH level is close to the neutral environment), and on the other hand, inhibits the lipase activity by the feedback principle. Herewith, sucrose addition leads to the increase of both rate and depth of the neutral fat hydrolysis. Besides, the sucrose effect occurs in all the studied concentrations of sucrose, but with different intensity. The maximum increase of the rate (2.0 times) and depth of the neutral fat hydrolysis (1.4 times) can be achieved at the 1.5% sucrose concentration in a simulated environment. Under the larger increase of the sucrose concentration, no further growth of either rate or depth of the neutral fat hydrolysis is observed. In general, sucrose acts as the lipase activator, wherein the activating effect starts from the low concentrations and significantly increases together with the sucrose concentration growth, but to a certain value limit, above which the lipase demonstrates no more gaining, but remains at a high level of activity. The obtained data show that at simultaneous content of neutral fat and sucrose in the food products, the intensity of the intestinal fat digestion is substantially increased, i.e. fat is better digested by the body in presence of sucrose.

The change in the chemical compound concentration in the body primarily effects on the activity of an enzyme executing its metabolism. The sucrose is digested in the small intestine under the action of the Sucrase-Isomaltase Complex (SIC). This enzyme also hydrolyzes maltose, maltotriose, and isomaltose. SIC most actively splits maltose among all the specified substrates, but it is mainly focused on sucrose. Besides, the sugar subunit of SIC is the only intestinal enzyme that performs sucrose hydrolysis[14].

The study of the effect of different sucrose concentrations on the activity of SIC extracted from isolated avian intestine of chickens that during one month were fed with sucrose (SIC_{test}), and on the activity of SIC extracted from isolated avian intestine of chickens that were not fed with the sucrose ($SIC_{control}$) have shown the following results (Table 4).

As can be seen from the data obtained, with the increase of the sucrose concentration from 0.75% to 1.5%, the SIC_{test} activity increases by 1.4 times; and at the 1.5% concentration, the maximum rate (V_{max}) of generating

Table 4. The effect of different sucrose concentrations on the SIC activity

Enzyme	Sucrose concentration, %				
	0.0	0.75	1.5	3.0	4.5
	Glucose content in a simulated environment, mmol/l				
SIC_{test}	0	7.7±3.5*	10.6±2.6*	10.3±3.4*	10.5±6.6*
$SIC_{control}$	0	5.8±1.6	6.5±1.4	6.4±2.8	6.5±3.5

**significance of differences $p< 0.05$ between $SIC_{control}$ and SIC_{test}*

the hydrolysis product (glucose) is observed. The further increase of the sucrose concentration up to 3.0% and then up to 4.5% does not result in the growth of the product generation rate. The $SIC_{control}$ activity also enhances at the increase of the sucrose concentration up to 1.5%, but insignificantly (by 1.2 times in total). The V_{max} value achieved by the $SIC_{control}$ is 1.6 times lower than the V_{max} value achieved by the SIC_{test}. The SIC_{test} activity at the 0.75% concentration of sucrose is also 1.3 times higher than the $SIC_{control}$ activity: the glucose concentration in a simulated environment under the $SIC_{control}$ impact amounted to 5.8 ± 1.4 mmol/l, and under the SIC_{test} impact—7.7 ± 3.4 mmol/l. Such difference can be explained not only by the difference between the SIC_{test} and $SIC_{control}$ activities, but also by the difference in their amount: the volatiles, whose lifelong ration has virtually no sucrose, feature a low SIC level produced by the intestinal cells, and vice versa, the volatiles, whose ration was supplemented with sucrose throughout the entire experiment, demonstrated a higher rate of the intestinal SIC generation with an enhanced activity.

5. Conclusions

The studies have shown that one of the essential dietary infringements of the nutrition among various population groups, especially among the youth, is the excessive sucrose intake, which is significantly higher than the physiological standards and the evolutionarily established traditional levels of intake. We found out that high concentrations of sucrose impose a multidirectional effector effect on the activity of individual GIT enzymes: it inhibits amylase reducing the intensity of the starch digestion; it activates lipase increasing the intensity of the fat digestion by the body; it leads to the induction of the activity and amount of the intestinal sucrase-isomaltase complex enhancing the hyperglycemic effect of sucrose. The

obtained data enlarge the concept of the cause-effect relations between the excessive sucrose intake and the risk of such socially significant diseases as obesity and insulin-dependent diabetes mellitus.

Based on the results obtained, practical guidelines were developed for the manufactures of food products concerning the implementation of the data on the sucrose effect on the efficiency of the starch and neutral fat digestion within the development of food products of the functional, therapeutic, and preventive purposes. Besides, an algorithm for optimizing the sucrose intake level was developed in the form of an individual program.

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