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IMPACT OF ARTIFICIAL SWEETENERS ON GLUCOSE UPTAKE IN 3T3-L1 ADIPOCYTES

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Abstract

Background: Escalating rates of obesity and public health messages to reduce excessive sugar intake have fueled the consumption of artificial sweeteners (AS) in a wide range of products from breakfast cereals to beverages. However, several observational and laboratory studies link AS consumption with increased appetite, food intake, weight gain and glucose intolerance(1-5). There are very few probing their effect on adipocytes. Therefore, we want to study the effect of different AS on glucose uptake in adipocytes. **Aims and objectives:** To study the effect of saccharin, sucralose and cyclamate on glucose uptake in 3T3 cell lines. **Materials and Methods:** 3T3 cells from ATCC were grown in DMEM. A day prior to the experiment, they were incubated with varying concentration (untreated, 1nM, 1μM or 1mM) of one of the AS for basal uptake and another set of wells were treated with AS and 10nM insulin for stimulated uptake. After 24h incubation, glucose uptake was studied with radiolabeled 2- deoxy glucose by liquid scintillation counter and expressed per mg of protein. **Results and Conclusion:** 2-way Anova was done. Cyclamate and saccharin showed an increase in uptake with increase in concentration. But sucralose shows a significant decrease in uptake with increase in concentration. All AS do not have similar effect on glucose uptake by cells. Sucralose decreases the glucose uptake by cells and may not be the AS of choice in people who already have decreased glucose uptake. AS are not metabolically inert, and it will be necessary to choose the right AS based on the metabolic status of the individual.

Keywords: Artificial sweeteners, glucose uptake, 3T3 adipocytes, Sucralose, Cyclamate, Saccharin.

Introduction

Excessive consumption of sugar-rich foods is one of the most important factors leading to the global pandemic of obesity. The prevalence of overweight and obesity is estimated to have almost tripled over the past five decades. The number of overweight individuals reached over 1.9 billion in 2016. According to the World Health Organization (WHO), daily energy intake from added sugars should not exceed 5–10%. Unfortunately, statistics indicate that, in many countries, sugar consumption is significantly higher[6]. Increased obesity related mortality has

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resulted in a surge of weight loss diets and products, and various fitness routines. It is widely understood that of the many contributing factors, a high sugar/high fat diet is partly to be blamed for the increasing obesity and related health issues such as type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), hypertension, and certain cancers. As a result of the many negative health conditions associated with the intake of excessive sugar, there has been an upsurge in the consumption of non-nutritive sweeteners (NNS) as an alternative. Consumption of NNS-containing foods has increased among people of all ages, with 28% of the total population reporting intake. This trend is highly prevalent among children, especially when it comes to beverage intake[7]. However, several common high-intensity sweeteners (HIS), e.g. aspartame, sucralose and saccharin have been the subjects of enduring controversy due to observational and laboratory studies indicating an association of HIS consumption with increased appetite, food intake, weight gain and glucose intolerance[2]. The scientific statement issued by American Heart Association (AHA) and American Diabetes Association (ADA) concludes that there are insufficient data to determine conclusively whether the use of NNS to displace caloric sweeteners in beverages and foods reduces added sugars or carbohydrate intakes, or benefits appetite, energy balance, body weight, or cardio-metabolic risk factors[8]. There is no clear evidence whether AS consumption is bad or has no risks. Therefore, we wanted to study the effect of some of the commonly used AS- saccharin, sucralose and cyclamate on glucose uptake in 3T3 adipocytes.

Aim: To study the effect of varying concentrations of saccharin, sucralose and cyclamate on glucose uptake by 3T3 cells.

Materials and Methods

Cell Culture

The study was conducted on 3T3 adipocytes (mouse origin) from ATCC (American Type Culture Collection). They were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 4mM L-glutamine adjusted to contain 1.5g/L NaBicarbonate, 4.5g/L glucose and 1mM NaPyruvate and 10% FBS. After the cells reached confluence, freshly prepared differentiation mix (Insulin, dexamethasone, Biotin and Isobutyl-1-methyl-xanthine) was left on the cells for 3 days before changing to post-differentiation medium (Insulin) for a further 3 days. Medium is replenished once during these 3 days. A day prior to experiment, 12 wells were incubated with varying concentration (untreated, 1nM, 1μM or 1mM) of one of the AS for basal uptake and another 12 wells were treated with AS and 10nM insulin for stimulated uptake.

Glucose Uptake

All experiments were performed in triplicates. After 24h incubation with AS, 3T3 adipocytes were incubated with deoxyglucose in no-glucose DMEM media for 10 minutes. After 10 minutes, 0.1μCi/ mL 2-deoxy[3H] glucose (PerkinElmer Life Sciences) was added alone for basal uptake and with 10nMinsulin for stimulated uptake. 10 minutes later, 3T3 adipocytes were placed on ice, washed with ice cold phosphate buffered saline and lysed in 0.1% sodium dodecyl sulfate. It was then transferred to vials with scintillation cocktail for counting and an aliquot taken for protein estimation was done by Bicinchoninic (BCA) method

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(ThermoFisher Pierce). The uptake of 2-deoxyglucose was then quantified by liquid scintillation counter and expressed per mg of protein.

Results

Table 1: Sucralose glucose uptake

Group Sucralose	Subgroup	N	Mean	SD	p value	AOV2. Group
Untreated	**Basal**	6	13.83	1.47	0.029*	
Untreated	**Stimuated**	6	18.50	4.23		
1nM	**Basal**	6	13.33	1.63		
1nM	**Stimuated**	6	15.17	1.17		
1□M	**Basal**	6	11.83	1.72		
1□M	**Stimuated**	6	13.33	0.82		
1mM	**Basal**	6	11.17	1.17		
1mM	**Stimuated**	6	13.50	0.84		
Group Sacchari n	**Subgroup**	**N**	**Mean**	**SD**	**p value**	**AOV2. Group**
Untreated	**Basal**	6	13.83	1.47	0.029*	
Untreated	**Stimuated**	6	18.50	4.23		
1nM	**Basal**	6	13.67	1.21	0.67	0.019*
1nM	**Stimuated**	6	14.00	1.41		
1□M	**Basal**	6	11.5	1.05	0.032*	
1□M	**Stimuated**	6	14.33	2.58		
1mM	**Basal**	6	12.83	1.94	0.00*	
1mM	**Stimuated**	6	17.5	1.05		

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Table 2: Saccharin glucose uptake

Table 3: Cyclamate glucose uptake

Group Cyclamate	Subgroup	N	Mean	SD	p value	AOV2. Group
Untreated	**Basal**	6	13.83	1.47	0.029*	
Untreated	**Stimuated**	6	18.50	4.23		
1nM	**Basal**	6	10.5	1.76	0.044*	
1nM	**Stimuated**	6	14.33	3.67		
1□M	**Basal**	6	11.0	2.0	0.002*	0.033*
1□M	**Stimuated**	6	15.83	1.94		
1mM	**Basal**	6	12.5	1.76	0.001*	
1mM	**Stimuated**	6	17.33	2.07		

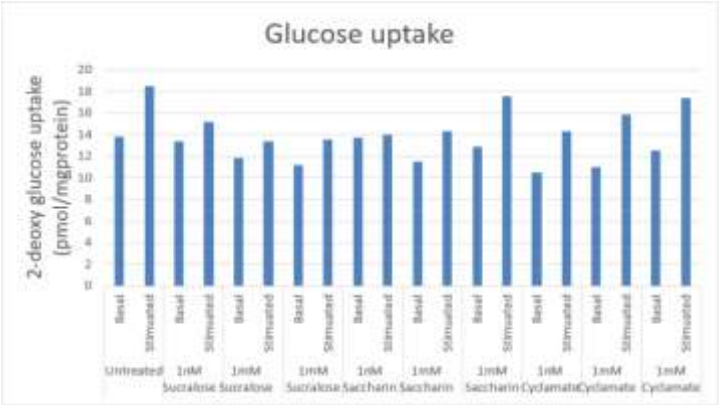

Figure 1: Glucose uptake with varying AS concentration

2-way Anova was done. Almost all of them showed significant increase in uptake in stimulated condition compared to basal. Cyclamate and saccharin showed an increase in uptake with increase in concentration. But sucralose shows a significant decrease in uptake with increase in concentration.

Discussion

The sweet-taste receptor is a transmembrane protein present in the cell membrane that is coupled to a G-protein (second messenger) system. Binding of a sweet substrate to the receptor causes a conformation change in the receptor protein that affects its association with the G-protein. The G-protein associated with the sweet-taste receptor is □-gustducin, which like most G-proteins comprises □, β and γ subunits, and is on the cytoplasmic side of the cell membrane. Binding of a sweet compound to the receptor causes dissociation of □-gustducin from the receptor, which triggers intracellular events such as the opening of ion channels or the generation of other bio- chemical signals. For sweetness perception, two G-protein-coupled transmembrane receptor proteins, T1R2 and T1R3, dimerise to form the sweet-taste receptor. Stimulation of the T1R2 + T1R3 taste receptor activates peripheral gustatory nerves

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and, in turn, brain gustatory pathways[9].

Mace *et al.*[10], Li *et al.*[11] and Margolskee *et al.*[12] have studied effect of various AS on glucose uptake in intestinal cells and shown that the glucose uptake is increased. Pepino *et al.* in a review has mentioned that sucralose increases plasma glucose concentration after sucralose ingestion[13]. In our study, compared to other AS, sucralose decreased glucose uptake by 3T3 cells in a dose dependent manner. However Becky *et al.*[14] have shown that sucralose increased glucose uptake by 3T3 cells.

We had shown a that sucralose decreased glucose uptake in L6 myotubes[15].

The other 2 AS studied- Saccharin and cyclamate increased glucose uptake by 3T3 cells. This is in accordance with the findings of Becky *et al.*[14].

Becky *et al.* have shown that these effects are seen even when knockout of T1R2 and T1R3 was done indicating that AS might act through hitherto unknown receptors. More studies are required to show these effects before we can conclude whether AS are detrimental to health. Studies are also required to identify the receptors through which these AS act.

Conclusion

AS are frequently consumed by people who have insulin resistance due to diabetes or due to obesity and metabolic syndrome. All AS do not have similar effect on glucose uptake by cells. Sucralose decreases the glucose uptake by cells and may not be the AS of choice in people who already have decreased glucose uptake. Whereas saccharin and cyclamate increase glucose uptake and could be beneficial in individuals with insulin resistance. AS are not metabolically inert, and it will be necessary to choose the right AS based on the metabolic status of the individual.

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