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Regular Low-calorie Sweetener Consumption is Associated with Increased Secretion of Glucose-dependent Insulinotropic Polypeptide

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Abstract

Low-calorie sweeteners (LCS) are widely used for weight control despite limited evidence of their effectiveness and studies linking LCS consumption with incident obesity. We tested the hypothesis that regular LCS consumption is associated with higher post-prandial glucose-dependent insulinotropic polypeptide (GIP) secretion, which has been linked to obesity. We used data from Baltimore Longitudinal Study of Aging participants who had completed a diet diary, had at least one visit with an oral glucose tolerance test (OGTT), and had no diabetes. Of 232 participants, 166 contributed one, 39 contributed two, and 27 contributed three visits; and 96 (41%) reported using LCS. Plasma OGTT samples were analyzed for glucose, insulin and GIP. Fasting glucose, insulin or GIP were no different between LCS users and non-users. The association of LCS use with 2-hr OGTT responses after adjustment for covariates were non-significant for glucose ($P=0.98$) and insulin ($P=0.18$), but significant for greater increase in GIP in users ($P=0.037$). Regular consumption of LCS is associated with greater increases in GIP secretion following food intake, which may potentially lead to weight gain through lipogenic properties of GIP.

INTRODUCTION

The worldwide prevalence of obesity has increased dramatically over the past several decades.¹ For over 40 years, use of low-calorie sweetener (LCS) as a dietary strategy to reduce carbohydrate and total energy intake for weight management has been recommended based on the understanding that they have negligible calories.^{2,3} Accordingly, LCS

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consumption has increased dramatically over the same period.³ Despite increased use of LCS, the prevalence of obesity has continued to rise.

Several longitudinal studies have found LCS consumption associated with higher incident and prevalent obesity.^{4,5} Randomized controlled trials of LCS for weight loss have not found compelling or consistent evidence for their effectiveness as weight-loss aides.² Several factors may explain the unexpected findings of these trials, namely the lack of control of other compensatory health behaviors that may offset the transient reduction of caloric intake as well as direct effect of LCS on metabolic dysregulation. Plausible mechanisms explaining the link between LCS consumption and metabolic dysregulation have begun to emerge. A recent study found that LCS consumption induced dysbiosis and altered the composition and function of intestinal microbiota to those associated with weight gain and glucose intolerance.⁶ One possible pathway linking dysbiosis and metabolic dysregulation could be the interactions between intestinal microbiota and the enteroendocrine system.

Whether the dysbiosis induced by LCS affects enteroendocrine physiology has not been systematically investigated. Most human studies have been single exposure experiments in which LCS is given once in the form of diet soda which has not been found to induce any increase in glucose-dependent insulintropic polypeptide (GIP) or glucagon-like peptide-1 (GLP-1).⁷ If the enteroendocrine system is indeed part of the causal pathway involving LCS use and dysbiosis, the potential effects of LCS on the enteroendocrine system would only emerge after regular exposure. In this study, we focus on the effects of LCS intake on GIP because it promotes triglyceride storage in adipocytes, enhances lipoprotein lipase activity in human adipocytes, and promotes preadipocytes differentiation.⁸ Circulating GIP levels are also significantly higher in obese individuals.⁹

We hypothesize that regular LCS consumption leads to higher post-prandial GIP secretion. To test this hypothesis, we used stored plasma samples from the Baltimore Longitudinal Study of Aging (BLSA) to compare plasma GIP levels in LCS users versus non-users during 75g oral glucose tolerance testing (OGTT).

RESEARCH DESIGN AND METHODS

Study population

The BLSA is an ongoing observational continuous-enrollment cohort study of normative aging in community-dwelling volunteers aged 20 years and older, conducted at and sponsored by the US National Institute on Aging (NIA) since 1958. BLSA participants are healthy at study entry, followed for life, and undergo medical, physiological, and psychological examinations at age-specific intervals. The BLSA protocol has been approved by the Intramural Research Program of NIA and the Institutional Review Board of the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. All participants provided written informed consent at each visit.

Assessment of LCS Use

In the BLSA, between 1984 and 2008, dietary intake was assessed using a 7-day dietary record.⁵ Participants were designated as LCS users if they reported consuming any food or

drink containing LCS (aspartame, saccharin, acesulfame potassium, or sucralose) at the visit at which the OGTT was performed or the most recent visit preceding the OGTT if no concurrent dietary record was available. We selected this operationalization based on our previous reported data that demonstrated most (79%) BLSA participants with multiple diet diaries do not transition between user and non-user.⁵

75g Oral Glucose Tolerance Test

After a 10-hr overnight fast, participants consumed a 75-g glucose solution and plasma samples were collected just before glucose ingestion and then every 20 minutes for 120 minutes. A participant was diagnosed as having diabetes if any of the following criteria were met: (1) history of diabetes; (2) taking hypoglycemic medications; or (3) fasting plasma glucose (FPG) ≥ 126 mg/dL and/or 2-hr OGTT glucose (2hrG) ≥ 200 mg/dL. Participants with no diabetes were selected for this study because GIP secretion during OGTT is significantly higher in individuals with diabetes.¹⁰ OGTT samples collected after April 2001 contained dipeptidyl peptidase-4 (DPP4) inhibitors which enabled reliable measurement of GIP. Sample collection methods and assays used are provided in the Supplemental Methods Section.

The present study included all BLSA participants who had at least one visit with OGTT after April 2001 and at least one visit at or no more than five years before the visit with OGTT in which they completed a diet diary. Of 232 participants who met these criteria, 166 contributed one visit, 39 contributed two visits, and 27 contributed three visits, with a total of 325 person-visits to this analysis.

Covariates

Covariates included age, sex, race and lifestyle factors including physical activity (sedentary, low, moderate or high intensities based on reported frequency and duration of vigorous and moderate physical activity) and smoking status (current smoker versus former and never smoker). Body mass index (kg/m^2) was determined from measured weight and height. These covariates were measured at the visit concurrent with the dietary assessment used in the study. In analysis, we also accounted for the year of recruitment, the year of study visit, and the number of years from dietary assessment to OGTT assessment.

Statistical analysis

Covariates were compared between LCS users and non-users with the chi-square or Fisher's exact test (categorical covariates) and the t-test or Wilcoxon rank-sum test (continuous covariates). We also compared mean values of fasting glucose, fasting insulin, and fasting GIP using t-tests when assessing the first visit with OGTT and clustered t-tests when assessing all person-visits.

Linear mixed effects models estimated the association of LCS use with 2-hour glucose, insulin, and GIP responses in 325 person-visits among 232 participants. Random intercepts and slopes accounted for within-participant correlation and within-visit correlation nested within participants between measures at 0, 20, 40, 60, 80, 100, and 120 minutes. Separate

models were fitted for glucose, insulin, and GIP adjusted for covariates. P-value < 0.05 was considered statistically significant for all analyses.

RESULTS

Participant characteristics

Of 232 participants, 96 (41%) reported using LCS. Table 1 shows that LCS users were more likely Caucasian ($P=0.04$). No other differences were observed with respect to smoking, physical activity, age, BMI, year of recruitment, or time between diet diary assessment and first OGTT. No differences were observed for fasting glucose, insulin or GIP using either the first OGTT visit or considering all person visits (135 person-visits among LCS users, 190 person-visits among non-users) (supplemental Table 1).

Association of LCS consumption with 2-hour glucose, insulin, and GIP responses

Figure 1 illustrates the association of LCS use with 2-hr OGTT glucose, insulin, and GIP responses after adjustment for covariates. Glucose peaked at 40 minutes within both groups then declined and was virtually identical over the two-hour assessment ($P=0.98$). In contrast, insulin peaked at 60 minutes then plateaued and began declining at 80 minutes among LCS users and peaked at 40 minutes among non-users and began declining at 80 minutes; however, the two-hour change in OGTT did not significantly differ between the groups ($P=0.18$). Lastly, GIP increased at 20 minutes and stayed essentially constant over the remainder of the two hours in both groups; however, the LCS users had a greater increase in GIP than non-users ($P=0.037$).

DISCUSSION

Using data on 325 visits from 232 non-diabetic men and women, we found regular LCS consumption associated with a greater increase in glucose-induced GIP secretion over two hours. GIP promotes triglyceride storage in adipocytes, enhances lipoprotein lipase activity in human adipocytes, and promotes preadipocytes differentiation.⁸ GIP levels are also significantly higher in obese individuals.⁹ Therefore, modulation of the enteroendocrine system by LCS consumption which leads to greater post-prandial GIP secretion, may be one mechanism linking LCS use to the development of obesity.

To our knowledge, this is the first investigation where higher post-prandial GIP response is associated with regular LCS consumption in humans. Direct links between LCS use and enteroendocrine hormones in humans has not been systematically investigated. Enteroendocrine K and K/L cells secrete GIP upon activation of specific transporters and receptors located on intestinal lumen by carbohydrates, fat, protein and cannabinoid receptor agonists.^{9,11} In rodents, LCS induces GIP secretion by activating sweet taste receptors on K cells.¹² Therefore, LCS, even though non-caloric, may induce GIP secretion from K and K/L cells, but the response may depend on cumulative or regular exposure to LCS, as a single dose in the form of diet soda did not produce increased secretion of GIP in humans.⁷

Another possible mechanism linking LCS consumption and metabolic dysregulation may be through effects of LCS on gut microbiota. Suez et al. reported that LCS consumption in

mice and humans altered the composition and function of intestinal microbiota to those associated with glucose intolerance and obesity; however, incretins were not measured.⁶ Emerging evidence links gut microbiota with enteroendocrine function.¹³ In mice, 11-week of LCS consumption resulted in altered gut microbiota with over-representation of glycan degradation pathways leading to increased formation of short-chain fatty acids (SCFAs).⁶ Petersen et al. showed that SCFAs, through increasing expression of transcription factor neuronal differentiation 1 (*Neurod1*), increase the number of L cells in mouse and human intestinal epithelium (*in vitro*) resulting in increased GLP-1 secretion.¹⁴ The number of K cells was also increased in the human intestinal epithelium although the increase, with N = 4, did not reach significance.¹⁴ The same group also showed that blocking the NOTCH signaling pathway with dibenzazepine, a γ -secretase inhibitor, led to elevated expression of Neurod1 and subsequent increase in L and K cell numbers as well as increased GLP-1 and GIP secretion.¹⁵

Our study has multiple strengths including a well-characterized cohort with detailed dietary records collected over multiple visits and carefully characterized metabolic profile. Despite these strengths, some limitations must be acknowledged. First, BLSA is an observation study, therefore we cannot rule out the possibility of unmeasured confounders. However, we mitigated this possibility by including many relevant covariates. Second, classification of LCS users and non-users was made using dietary data collected up to 5 years before OGTT samples were obtained. However, previously reported data showed that 79% of BLSA participants with multiple diet diaries did not transition between use and non-use.⁵

Our findings where association of regular LCS consumption with greater increases in nutrient-stimulated GIP secretion corroborate and weave together findings from *in vitro* and animal studies presented above.^{6,14,15} One plausible pathway in which LCS consumption may lead to weight gain and obesity is as follows: (1) LCS alters the composition of gut microbiota resulting in increased SCFAs formation; (2) greater SCFAs in the intestine lead to increased *Neurod1* expression which ultimately leads to greater K and K/L cell number, and increased GIP secretion; (3) Increased GIP secretion promotes more triglyceride storage in adipocytes, enhances lipoprotein lipase activity in human adipocytes, promotes preadipocytes differentiation, and ultimately weight gain and obesity. This hypothesis needs to be tested in intervention studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The association of regular LCS use (red = user; blue = non-user) with 2-hr OGTT plasma glucose (A), insulin (B), and GIP (C) after adjustment for covariates (age, sex, BMI, race, physical activity, smoking status, year of recruitment, the year of study visit, and the number of years from dietary assessment to OGTT assessment).

Table 1

Characteristics of the LCS users versus non-users

Characteristic	LCS user (N=96)	LCS non-user (N=136)	P value
Female Sex	72 (75.0)	86 (63.2)	0.064
Age, years	65.6 ± 12.1	63.4 ± 14.4	0.25
BMI, kg/m ²	26.2 ± 4.1	25.9 ± 4.5	0.58
Smoking			0.43
Never	53 (55.2)	68 (50.0)	
Former	40 (41.7)	66 (48.5)	
Current	3 (3.1)	2 (1.5)	
Physical Activity			0.39
Sedentary	3 (3.1)	9 (6.6)	
Low Intensity	39 (40.6)	49 (36.0)	
Moderate Intensity	26 (27.1)	46 (33.8)	
High Intensity	28 (29.2)	32 (23.5)	
Race			0.040
Caucasian	64 (66.7)	68 (50.0)	
African-American	18 (18.8)	41 (30.2)	
Other	14 (14.6)	27 (19.8)	
Year of Recruitment	1994 (1987, 1999)	1997 (1991, 1999)	0.17
Years from diet assessment to first OGTT	0 (0, 0) (range: 0 to 4.5)	0 (0, 0) (range: 0 to 4.9)	0.07
First OGTT assessment concurrent with diet assessment	79 (82.3)	103 (75.7)	0.26

Data presented as N (%), means ± SD or median (IQR)