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Vitamin-supplemented chewing gum can increase salivary and plasma levels of a panel of vitamins in healthy human participants

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ABSTRACT

A number of commercially-available chewing gums contain health-related ingredients including vitamins. The ability of chewing gum to deliver these ingredients to the plasma has not been well-studied. We examined the release and plasma levels of a panel of vitamins from two supplemented gums in 15 healthy human participants. We examined the release of vitamins from the gums into the saliva using a single-blind randomized design, and then determined the acute impact of chewing vitamin-supplemented gums on plasma vitamin concentrations in a single-blind, placebo-controlled, crossover study. Retinol, thiamine, riboflavin, niacinamide, pyridoxine, folic acid, cyanocobalamin, ascorbic acid, and α-tocopherol were released into the saliva by chewing. Plasma vitamin concentrations were increased for retinol (75–96%), pyridoxine (906–1077%), ascorbic acid (64–141%) and α-tocopherol (502–418%) after chewing the supplemented gums, compared to baseline. To our knowledge, this is the first study examining the delivery of vitamins using chewing gum in humans.

1. Introduction

Despite the growing availability of vitamin-fortified foods as well as single and multivitamin dietary supplements (Andrews et al., 2017), chronic or transient vitamin deficiencies still exist in the United States (US). An analysis of the National Health and Nutrition Examination Survey (NHANES, 1999–2006) found that approximately 10% and 6% of persons (> 1 year old) in the US were deficient in vitamin B6 and vitamin C, respectively (U.S. Centers for Disease Control and Prevention, 2012). In developing countries, vitamin deficiency among children has been recognized as an epidemic (Ahmed, Hossain, & Sanin, 2012; Angurana & Mahajan, 2016; Caulfield, Richard, & Black, 2004; Dror & Allen, 2011). It is well-established that severe vitamin deficiencies cause a range of problems including developmental abnormalities, growth deficiencies, visual impairment, and compromised immune and cognitive functions (Kohlmeier, 2003). Vitamin supplements represent a potential means to ensure that consumers do not develop deficiencies of these key micronutrients when dietary vitamin content is insufficient (Deruelle & Baron, 2008).

Chewing gum is a popular confectionary product with approximately 50% of the US population currently using chewing gum (Anonymous, 2018; Mintel Group Ltd, 2015). In 2015, chewing gum sales in the US exceeded USD 4.8 billion (Mintel Group Ltd, 2015). Functional chewing gums containing ingredients such as botanical extracts and vitamins are commercially available and represent approximately 14% of the market (Nieburg, 2014). There are several potential advantages to using chewing gums as delivery vehicles for vitamins compared to bolus formulations (i.e. pills, capsules, and gummies). First, because chewing gums are used habitually, these products can be supplemented with much lower levels of vitamins than bolus dosage forms, potentially reducing the risk of overdose and facilitating better titration of doses (Aslani & Rostami, 2015). Second, the popularity of chewing gum could increase compliance compared to other supplement forms (Imfeld, 1999).

Although vitamin-containing chewing gums are commercially available, the extent to which these products release their component vitamins, and their efficacy in influencing blood levels of vitamins has not been examined. We have previously observed that chewing gum is an effective vehicle to deliver phytochemicals from green tea to the oral cavity (Blair, 2010). While it is possible that vitamins in chewing gums

Abbreviations: $AUC_{0\rightarrow 10h}$, area under curve; BHT, butylated hydroxytoluene; ECD, electrochemical detection; HPLC, high-performance liquid chromatography; L_{max}, maximum level; ROUT, robust regression and outlier removal; TCA, trichloroacetic acid

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have similar bioavailability to vitamins delivered by foods, these fortified products are physically and chemically different from the source foods. These differences may affect vitamin release and bioavailability *in vivo*. It is, therefore, necessary to establish the release efficiency and bioavailability of novel vitamin supplement formulations.

The objectives of this study were to determine the extent to which a subset of water-soluble and fat-soluble vitamins are released from the gum matrix into the saliva during a typical chew, and to evaluate the impact of chewing these supplemented gums on acute plasma levels of the constituent vitamins in a population of healthy human participants. This study will provide data that can be used in the design of future human intervention studies to assess the efficacy of these products for the mitigation or correction of vitamin deficiencies, and to guide the development of novel formulations of vitamin-supplemented chewing gums with improved performance characteristics.

2. Participants and methods

2.1. Reagents

Thiamine, riboflavin, nicotinamide, pyridoxine, folic acid, cyanocobalamin, ascorbic acid, DL- α-tocopherol, and cholecalciferol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Retinol was purchased from Alfa Aesar (Ward Hill, MA, USA). DL-α-tocopherol acetate and retinyl palmitate were purchased from TCI, Co. (Portland, OR, USA) and MP Biomedicals, LLC (Solon, OH, USA), respectively. Stock solutions of thiamine, riboflavin, nicotinamide, pyridoxine, cyanocobalamin, and ascorbic acid were prepared in 2.4% aqueous acetic acid. A stock solution of folic acid was prepared in 5% aqueous sodium bicarbonate. Stock solutions of all fat-soluble vitamins except retinyl palmitate were made with ethanol containing 100 µg/L butylated hydroxytoluene [BHT]. Retinyl palmitate standards were prepared in dimethyl sulfoxide. All other solvents and reagents were of the highest grade commercially-available.

2.2. Test materials

Vitamin-supplemented chewing gums (Sport and Immunity) and a placebo chewing gum were provided by the study sponsor (Vitaball, Inc., Fort Thomas, KY, USA). The Sport gum was grape-flavored and packed in opaque plastic tubes. The Immunity and placebo chewing gums were peppermint-flavored and presented in foil-backed blister packs. The vitamin content and nutrient information for each gum is shown in Table 1.

2.3. Study protocol

2.3.1. Subject recruitment and experimental design

Our study was approved by the Institutional Review Board of the Pennsylvania State University (University Park, PA, protocol no. STUDY00001013). The study has been registered with Clincaltrials.gov (NCT03230369). Participants were excluded from the study if they had pre-existing oral diseases or iron deficiency, were pregnant or lactating, suffered from phenylketonuria, or had medical, social, or economic circumstances which are likely to prevent adherence to the protocol. Informed consent was collected prior to enrollment in the study. All female participants of reproductive age were given a urine pregnancy test prior to enrollment in the study and prior to each phase of the study. Healthy participants (age: 22–52 y, 6 men and 9 women) were recruited. Signed informed consent was obtained from all participants. The mean body mass index (± SD) of the participants was 24.0 ± 4.1 kg/m^2 which is within the healthy range. Our overall study design included two experimental phases. In Phase I, a randomized study design was employed to examine the release of constituent vitamins from the gum matrix into saliva during a typical chew (Fig. 1). Phase II employed a randomized, single-blind, placebo-controlled, three-period crossover design to examine the acute impact on plasma levels of vitamins of chewing vitamin-supplemented gums over the course of the day (Fig. 1).

Table 1
Vitamin content (per serving) in the experimental gums.[a]

Vitamin	Placebo	Sport (% RDA)	Immunity (% RDA)	Upper Limit[b]
Ascorbic acid (mg)	0	15 (25)	62.5 (40)	2000
Biotin (µg)	0	11.25 (38)	75 (250)	N/E
Calcium d-panothenate (mg)	0	2.5 (50)	10 (200)	N/E
Cholecalciferol (IU)	0	100 (25)	200 (50)	N/E
Cyanocobalamin (µg)	0	1.5 (63)	5 (208)	N/E
dl-tocopherol acetate (IU)	0	7.5 (25)	20 (67)	1100
Folic acid (µg)	0	100 (25)	200 (50)	1000
Niacinamide (mg)	0	5 (31)	10 (62)	35
Pyridoxine HCl (µg)	0	500 (25)	1 (50)	100
Retinyl palmitate (IU)	0	1250 (25)	2000 (40)	10,000
Riboflavin (µg)	0	425 (33)	0	N/E
Thiamine mononitrate (µg)	0	375 (31)	0	N/E

[a] Analysis provided by Vitaball, Inc. based on lot-specific quality assurance analysis. Recommended daily allowance (RDA) for each vitamin is shown in parenthesis. N/E = not established.
[b] Upper limit of intake values are for adults (aged 19 years +) and are from the Dietary Reference Intakes (https://www.nal.usda.gov/fnic/vitamins-and-minerals).

2.3.2. Salivary release profile of vitamin-supplemented gums

Participants were provided with a new toothbrush and toothpaste (Colgate Total, Colgate-Palmolive Company, New York, USA) by the investigators and brushed their teeth prior to the start of the experimental session. They were then instructed to drink 250 mL of bottled water (Deer Park Water, Arbutus, MD, USA). An unstimulated, baseline saliva sample was collected immediately prior to chewing the experimental gum. Participants were assigned to chew either 2 pieces of Sport gum (n = 8) or 2 pieces of Immunity gum (n = 7) using a random number generator (odds to Sport, evens to Immunity). Participants were randomized after enrollment by WYK. Participants chewed their assigned gums for 30 min and expectorated all saliva for the following time periods 0–2, 2–5, 5–15, and 15–30 min into separate centrifuge tubes (Fig. 1). The chewing duration of 30 min was chosen to simulate typical gum chewing behavior (Barabolak, Hoerman, Kroll, & Record, 1991; Hearty, Lau, & Roberts, 2014). It has also been shown that salivary flow rates during a 30 min gum chewing session peak within first 2 min of chewing (Polland, Higgins, & Orchardson, 2003). This observation coupled with the expectation that water-soluble vitamins will be released rapidly into the saliva prompted us to collect more time points within the first 15 min of chewing. Saliva samples were then aliquoted into 1.5 mL centrifuge tubes. Spent gum samples were also collected. Saliva and gum samples were stored at −80 °C for until further analysis.

2.3.3. Impact of vitamin-supplemented gums on plasma levels of vitamins

To determine the impact of chewing vitamin-supplemented gums on plasma levels of vitamins, a participant-blinded, placebo-controlled, three-period crossover design was employed in Phase II of this study (Fig. 1). The protocol involved three visits during which participants chewed Sport, Immunity, or placebo gum (one type of gum per visit). There was a one-week washout period between each study visit. Participants were instructed to stop using vitamin supplements one week prior to the start of the study and to abstain from using such products for the duration of the study. Participants were assigned to the initial treatment arm (n = 5 per arm) using a random number generator (1–5 to placebo, 6–10 to Sport, 11–15 to Immunity). Assignment to treatment in the second period of the study was accomplished in a similar