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Nutrient-conditioned intake stimulation does not require a distinctive flavor cue in rats

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Abstract

The postnatal actions of nutrients in rodents can stimulate intake and condition flavor preferences through an appetition process. Appetition is revealed in rodents by their increased intake of and preference for a flavored solution paired with intragastric (IG) nutrient infusions. Here we determined if IG 16% maltodextrin (MD) infusions can stimulate intake and preference in the absence of a distinctive flavor cue. Rats implanted with IG catheters were given chow and water 2 h/day followed, 2 h later, by 20-h oral access to water paired with IG MD infusions. Other rats were given bitter sucrose octaacetate solution (SOA) paired with IG MD infusions 20 h/day. Over 8 test days, the SOA rats increased their total 20-h fluid intake (oral + IG) from 26 to 119 g/20 h and Water rats increased their intake from 31 to 96 g/20 h. When infused IG with water instead of MD in a 4-day extinction test, the SOA and Water groups reduced their fluid intakes to 45–48 g/20 h. When oral fluids were again paired with IG MD infusions, the SOA and Water groups increased their intakes to 115 and 109 g/20 h, respectively. In two-bottle tests, the SOA rats drank more SOA paired with IG MD than water paired with IG water. Water rats given the choice of a water bottle paired with IG MD and water bottle paired with IG water did not consistently prefer the H₂O/ID MD bottle. Instead they displayed side or sipper tube preferences although neither cue was consistently paired with IG MD during one-bottle training.

Keywords

Maltodextrin; Appetition; Preference; Intragastric conditioning; Sucrose octaacetate; Extinction

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Author contributions

Both authors participated in planning, supervising local research assistants and interpreting the results. A.S. analyzed the data and drafted the manuscript. Both authors revised the manuscript and approved the final version.

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1. Introduction

Rodents rapidly associate the flavor of a new food (or fluid) with the food's (or fluid's) postoral positive nutritional consequences. This has been demonstrated in the laboratory by training animals to consume a flavored nonnutritive solution (the CS+, e.g., grape-saccharin) that is paired with intragastric (IG) nutrient infusions (e.g., 16% glucose) and a different flavored solution (CS-, e.g., cherry-saccharin) with IG water infusions. In many cases, the animals drink more of the CS+/nutrient source than of the CS-/IG water source during training and strongly prefer the CS+ to CS- in a two-bottle test with or without concurrent IG nutrient infusions. The process responsible for the increase in CS+ intake and preference has been referred to as appetite to distinguish it from the satiation process that reduces food consumption (Sclafani, 2013). The appetite process is influenced by several factors including flavor quality (e.g., sweet flavors are more effective than non-sweet flavors) (Ackroff & Sclafani, 1994; Sclafani & Glendinning, 2003), nutrient type (e.g., glucose is more effective than fructose) (Sclafani *et al.*, 1999; Zukerman *et al.*, 2013a; Ackroff & Sclafani, 1994; Sclafani & Glendinning, 2003) and concentration (e.g., 16% glucose is more effective than 2% glucose) (Zukerman *et al.*, 2013b), and deprivation state (e.g., hungry vs. sated) (Yiin *et al.*, 2005). Postoral appetite can occur in the absence of a specific flavor cue, as in the case of mice licking an empty sipper tube in order to obtain IG nutrient infusions (e.g., glucose, soybean oil) (Ferreira *et al.*, 2012; Sclafani & Ackroff, 2016). Postoral appetite can even occur in the absence of a nutrient, as in the case of mice increasing their intake of a flavored saccharin solution paired with IG infusion of a nonmetabolizable glucose analog (Zukerman *et al.*, 2013a).

The present study investigated the importance of flavor cues to the appetite process in rats. In particular, we determined if rats would increase their intake of unflavored water paired with IG maltodextrin infusions. This was of interest because an early study reported that rats did not readily drink plain water paired with IG infusions of a high-fat or high-carbohydrate liquid diet even though it was their only source of nutrition (Warwick & Weingarten, 1995). However, the rats readily consumed a palatable saccharin solution paired with the IG diet infusions. Because rats are very familiar with the taste of water (Zocchi *et al.*, 2017), they may be resistant to acquiring an association between water taste and postoral nutrients via a latent inhibition process (García-Burgos *et al.*, 2013). Consistent with this view, Ramirez (1996) reported that giving rats two days access to a saccharin solution paired with IG water infusions prevented them from subsequently increasing their saccharin intake when the solution was paired with IG maltodextrin infusions. We previously observed that food-restricted rats substantially increased their intake of relatively unpalatable solutions (sour cherry or grape Kool-Aid, sour citric acid, bitter sucrose octaacetate (SOA)) paired with IG carbohydrate infusions during 20 h/day sessions (Pérez *et al.*, 1998; Myers & Sclafani, 2003; Yiin *et al.*, 2005). These solutions are less preferred than plain water to naïve rats but provide distinctive flavor cues that rats come to prefer to water after pairing with IG nutrient infusions. In the present experiment we determined (a) if food-restricted rats would increase their intake of plain water when it was paired with IG carbohydrate infusions 20 h/day. A control group was trained with an SOA solution paired with IG carbohydrate infusions as in prior studies (Myers & Sclafani, 2003; Pérez *et al.*, 1998; Yiin *et al.*, 2005). Given that water

taste was very familiar to the rats, we predicted it would support less nutrient stimulation of intake than the novel, but unpalatable SOA taste. We also determined if the water-trained rats (b) differed from SOA-trained rats in extinction of nutrient-conditioned stimulation of intake; (c) preferred drinking water from a bottle paired with IG maltodextrin over water from a bottle paired with IG water in a choice test, and (d) licked a dry sipper tube paired with IG carbohydrate infusions.

2. Method

2.1 Subjects

Adult female rats (n=20, Sprague Dawley strain, Charles River) weighing 295–345 g were used as in our prior IG conditioning studies using SOA solutions (Pérez *et al.*, 1998; Myers & Sclafani, 2003; Yiin *et al.*, 2005). The rats were individually housed in standard wire-mesh cages in a test room maintained at 21°C under a 12:12 h light:dark cycle (lights on at 0800 hours). Experimental protocols were approved by the Institutional Animal Care and Use Committee at Brooklyn College and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2 Surgery

The rats were anesthetized with a mixture of ketamine HCl (63 mg/kg) and xylazine (9.4 kg/kg), and implanted with a stainless-steel gastric cannula used to attach the infusion catheters as described previously (Elizalde & Sclafani, 1990). Briefly, the cannula was inserted into the fundus of the stomach and secured with a purse-string suture, polypropylene mesh and dental cement. The shaft of the cannula was passed through a small incision in the abdominal wall and skin. When not in use, the cannula was kept closed with a stainless steel screw.

2.3 Apparatus

Intragastric infusions were accomplished using an “electronic esophagus” apparatus described in detail elsewhere (Elizalde & Sclafani, 1990). In brief, the rats were housed in standard stainless steel cages with powdered chow available from a food cup accessible through a hole in the back wall of the cage. Drinking fluids were available from one or two stainless steel sipper tubes (Ancare, Bellmore, NY) located through two holes (19 mm) at the front of the cage. Spill cups were located outside the cage below the sipper tubes. A slot in the cage floor permitted two catheters attached to the rat’s gastric cannula to be connected to a dual-channel infusion swivel located below the cage; the catheters were protected by a flexible stainless steel spring. Tygon tubing connected the swivel to two peristaltic infusion pumps. The pumps were operated automatically by lickometer circuits and a microcomputer whenever the rat lick the sipper tubes. The flow rate of the pumps was 1.3 ml/min; they were operated for 3 s for each 20 licks at a spout, which resulted in ~1 ml of fluid infused IG for each 1 ml consumed orally. For some rats the lick criterion was increased or decreased so as to match the oral and IG intakes. The number of licks emitted during 3 sec bins were recorded by the microcomputer and stored for subsequent drinking pattern analysis.

2.4 Solutions

The drinking fluids were deionized water and a 0.03% sucrose octaacetate (SOA; ICN Pharmaceuticals, Cleveland, OH, USA) solution prepared with deionized water. The IG infusions were deionized water or 16% maltodextrin (MD, Maltrin QD 580, Grain Processing, Muscatine, IA) solution prepared with deionized water.

2.5 Procedure

Ten days after surgery, the rats were transferred to the test cages and given ad libitum access to powdered chow (Laboratory Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) and deionized water (hereafter, water). Five days later two infusion catheters were attached to the gastric cannula and for 3 days the rats were infused with water IG as they drank water from a sipper tube. They were next adapted for 3 days to a 2-2-20 h feeding schedule in which chow and water were available for 2 h (10–12 noon), followed by 2 h without food and water, and then 20-h access to water paired with IG water infusions (food + water Baseline). During the subsequent 3-day period food, but not water, was available during the 2-h feeding period while water (paired with IG water) remained available during the daily 20-h periods (food-only Baseline). Table 1 summarizes the test procedures.

One-Bottle Test 1.—Following the food-only baseline period, the rats were divided into two groups (n=10 each), equated for body weight, 2-h food intake and 20-h water intake. The SOA group was given daily 20-h access to the SOA solution paired with IG infusions of 16% MD for 8 test days and the Water group was given water paired with IG MD infusions during the 20-h period. On test days 1–4, water was not available during the daily 2-h feeding period while on test days 5–8 and for the remainder of the experiment water (not paired with IG infusions) was available during the daily 2-h feeding period. This sequence was used because in a prior study some rats drank very little SOA during initial test days when water was available during the 2-h feeding period (Pérez *et al.*, 1998). The left (L) and right (R) positions of the SOA and Water bottles varied over one-bottle test days according to a quasi LRRL sequence (see Fig. 1). Throughout baseline periods, one-bottle test and initial two-bottle test phases each rat had the same sipper tube placed on the left side ('left' tube) and a different sipper tube ('right' tube) on the right side of the cage such that both tubes were associated with IG MD infusions. This was done because prior findings indicate that in the absence of a unique flavor cue, rats could learn to associate a specific sipper tube with the IG nutrient infusion (Elizalde & Sclafani, 1990).

One-Bottle Extinction Test.—During this 4-day phase the SOA and Water rats had their 20-h intakes of SOA and water, respectively, paired with IG water infusions. We previously reported that rats rapidly reduced their intake of an SOA or Kool-Aid solution when infused with water rather than carbohydrate and this test determined if rats trained with Water + IG MD showed an attenuated or potentiated extinction response (Drucker *et al.*, 1994; Pérez *et al.*, 1998).

One-Bottle Test 2.—The two groups had their 20-h intakes of SOA and Water again paired with IG MD infusions during this 13-day period.

Two-Bottle Tests.—During *Test 1*, the SOA group was given 20-h two-bottle access to SOA paired with IG MD infusions and water paired with IG water infusions for 4 days. The left-right positions of the two bottles alternated daily. The Water group was given 20-h access to water paired with IG MD infusions (H₂O/IG MD bottle) and another water bottle paired with IG water infusions (H₂O/IG H₂O) bottle); the two bottles are referred to as H₂O-1 and H₂O-2, respectively. The left-right positions of the bottles (H₂O-1 or SOA) paired with MD infusions and those paired with water alternated daily. During this test, the ‘left’ and ‘right’ sipper tubes were always on the left and right sides, respectively, as during training. In *Test 2*, the two groups were again given 20-h access to their respective test fluids presented on alternate sides for 4 days. However, the so-called ‘left’ and ‘right’ sipper tubes also alternated over days such that the ‘left’ sipper tube was always paired with IG MD infusions, and the ‘right’ sipper tube was always paired with IG water infusions. The reason for this procedure change is explained in the results.

Limited One-Bottle Test.—Following the second two-bottle test, the SOA and Water groups were given one-bottle, 20-h access to SOA and water, respectively, with paired IG MD infusions for two days. This was followed by a 4-day test in which the rats were given access to only 20 ml of their respective oral fluids (SOA or H₂O) during the 20-h period. Oral intakes were paired with IG MD infusions. After drinking the 20 ml of fluid, if the rats licked the empty sipper tube they would continue to receive IG MD infusions. This test determined if the rats had acquired an operant licking response that allowed them to obtain nutrient infusions in the absence of oral fluid intake. The left-right position of the SOA and Water bottles alternated daily while the position of the ‘left’ and ‘right’ sipper tubes were fixed on the left and right sides, respectively, as during one-bottle training.

2.6 Data Analysis

One-bottle and two-bottle fluid intakes were calculated as oral, IG infused or total (oral + IG) values and analyzed using analysis of variance procedures with individual differences evaluated using simple main effects tests and Newman-Keuls tests according to Winer (1962). Two-bottle data were also expressed as percent preference (e.g., SOA intake/total intake $\times$ 100) and analyzed with t-tests. Drinking patterns (bout size, bout number) were analyzed with a bout being defined as a period of drinking containing at least 10 licks and interlick intervals no longer than 5 min (Yiin *et al.*, 2005).

3. Results

One-Bottle Tests.

Figure 1 presents the total 20-h fluid intakes (oral + IG) of the SOA and Water groups during baseline periods, One Bottle Test 1, Extinction Test, and Test 2. During the food and water baseline and food-only baseline the 20-h total (oral + IG) water intakes were 23.7 and 33.0 g/20-h, respectively. Compared to the food-only baselines, the MD infusions increased the total 20-h total fluid intakes similarly in the two groups in the first 4 one-bottle test days, when water was not available during the 2-h feeding period (Days main effect, $F(4,72) = 86.4$, $P < 0.001$). Intakes exceeded ($P < 0.01$, Newman-Keuls tests) baseline intakes on test days 2 to 4. The groups continued to increase their 20-h fluid intakes during one-bottle test

days 5–8 when water was available during the daily feeding periods (Days main effect, $F(3,54) = 14.9$, $P < 0.001$). Total 20-h fluid intakes tended to be higher in the SOA group than the Water group on days 5–8 (98.9 vs. 86.8 g/20 h) but this difference was not significant. The SOA and Water groups consumed similar amounts of chow during the 2-h feeding periods (29.4 vs. 29.7 kcal/2-h) and they were similar in their total energy intakes (chow + MD) during Test 1 (52.1 vs. 55.5 kcal/22 h). The 20-h oral intakes and bout patterns were compared for the last two food + water baseline days (days 2–3) and one-bottle Test 1 days 7 and 8. As indicated in Table 2, The Water and SOA groups showed very similar increases in 20-h oral fluid intakes (Days main effect, $F(1,18) = 113.5$, $P < 0.001$), mean bout numbers ($F(1,18) = 68.3$, $P < 0.001$), and mean bout sizes ($F(1,18) = 44.3$, $P < 0.001$).

During the Extinction Test, when 20-h fluid intakes were paired with IG water infusions, total fluid intakes rapidly declined in both SOA and Water groups, relative to the last two reinforced tests, from 92.9 to 46.4 g/20 h (Days main effect, $F(4,72) = 47.9$, $P < 0.001$); the two groups did not differ on this measure. Total 20-h intakes on extinction days 2 to 4, but not day 1, were lower than the reinforced test intakes ($P < 0.01$, Newman-Keuls tests).

In One-Bottle Test 2, when oral intakes were again paired with IG MD infusions, the SOA and Water groups rapidly increased their 20-h total fluid intakes (Days main effect, $F(12,216) = 17.3$, $P < 0.001$). Overall, 20-h total fluid intakes were higher for the SOA than Water rats (107.8 vs. 93.7 g/20 h) but this difference was not significant. Analysis of energy intakes during the 13-day Test 2 period indicated that the 2-h chow intakes were higher and 20-h MD intakes were lower for the Water rats than SOA rats, but these differences were not significant (chow: 37.7 vs. 30.7 kcal/2 h; MD: 30.7 vs. 35.9 kcal/20 h). Total 22-h calorie intakes (chow + MD) were very similar for the Water and SOA groups (68.4 vs. 69.4 kcal/22 h). In the last two days of Test 2, the Water rats consumed slightly but not significantly more water during the 2-h feeding periods than did the SOA rats (11.8 vs. 9.9 g/2 h).

Two-Bottle Tests.

Figure 2 presents the results of the 20-h, two-bottle preference tests. A between-group ANOVA of Test 1 data revealed that the Water and SOA groups did not differ in their overall fluid intakes or intakes over days, but did differ in their intakes of specific fluids (Group x Fluid interaction, $F(1,18) = 73.8$, $P < 0.001$). That is, the Water group overall consumed similar amounts of the H2O-1/MD and H2O-2/H2O fluids, while the SOA group consumed much more SOA/MD than H2O/H2O. There was also a Group by Fluid by Days interaction ($F(3,54) = 4.2$, $P < 0.01$). To clarify these differences, separate within-group ANOVAs were conducted. The SOA rats consumed significantly more SOA/IG MD than H2O/IG H2O in Test 1 (Fluid main effect, $F(1,9) = 118.3$, $P < 0.001$) and their intakes did not vary over days; mean percent preference for SOA/MD was 84%. In contrast, the Water rats did not consistently drink more H2O-1/IG MD than H2O-2/IG H2O in Test 1. Instead, they drank more from the left-side bottle than the right-side bottle (Fluid x Day interaction, $F(3,27) = 11.2$, $P < 0.001$). As a consequence, their mean percent preference for H2O-1/IG MD relative to H2O-2/IG H2O was 52%, while their mean preference for the left-side over right side bottle was 74%. The intakes from the H2O-1/IG MD and H2O-2/IG H2O bottles were

similarly high when they were on the left (81.6 vs. 75.5 g/20 h) and low when they were on the right side (34.7 vs. 28.0 g/20 h).

Because sipper tube positions were fixed in Two-Bottle Test 1, it was not clear if the Water animals were displaying a preference for the left side or for the left sipper tube. Prior studies indicate that animals can learn both side and sipper tube preferences in the absence of unique flavor cues depending upon training conditions (Elizalde & Sclafani, 1990; de Araujo *et al.*, 2008). To resolve this question, in Two-Bottle Test 2 the 'left' sipper tube was always paired with the H2O-1/IG MD (and SOA/IG MD) bottle and the 'right' sipper tube was always paired with the H2O-1/IG H2O (and H2O/IG H2O) bottle. The left-right position of the two bottles alternated daily as in Test 1. A between-group ANOVA of Test 2 revealed that overall both groups consumed more of the fluid paired with MD infusions than of the fluid paired with water infusions (Fluid main effect, $F(1,18) = 196.0$, $P < 0.001$). However this difference was significantly greater for the SOA group (126.2 vs. 7.7 g/day) than for the Water group (69.7 vs. 33.1, g/day; Group x Fluid interaction, $F(1,18) = 54.7$, $P < 0.001$). Consequently, the preference of the SOA rats for their SOA/IG MD source was substantially greater than the preference of the Water rats for their H2O-1/IG MD source (94% vs. 66%, $t(18) = 6.2$, $P < 0.001$).

Further analysis revealed that half of the Water rats ($n=5$) preferred the H2O-1/IG MD source in Test 2 while the other rats ($n=5$) did not (74% vs. 58%, $t(8) = 3.7$, $P < 0.01$) (see Fig. 3). Instead, the second subgroup continued to prefer the left-side to right-side bottle by 81%. In Test 1, both subgroups preferred the left-side bottle (and left sipper tube) rather than the H2O-1/IG MD to the H2O-2/H2O bottle, but the side/tube preference tended to be stronger in the second subgroup than the first subgroup (82% vs. 67%).

Limited One-Bottle Test.

This test determined if the rats would continue to lick the empty sipper tube in order to obtain IG MD infusions after they consumed all of the orally available fluid (SOA or water). Fig. 4 shows the IG MD self-infused intakes during the unlimited oral access baseline (2-day mean) and during the 4-day test when oral intakes were limited to 20 g/day. In the baseline period the SOA rats self-infused more MD than did the Water group (61.8 vs. 46.3 g/20 h, $P < 0.05$) but during the limited access test the two groups self-infused equivalent amounts (4-day means, 18.7 vs. 19.9 g/20 h; Group x Test interaction, $F(1,18) = 6.7$, $P < 0.05$). During the 4-day test, the overall MD self-infusions of the Water and SOA groups closely matched their limited oral fluid intakes (19.0 vs. 18.5 g/20 h). This demonstrates that once the rats consumed all of the orally available fluid they did not continue to lick the empty tube to self-infuse more MD.

4. Discussion

The present experiment revealed three main findings: First, in the one-bottle tests the IG maltodextrin infusions increased the 20-h total fluid intakes similarly in the Water and SOA groups; both groups also showed similar reductions in 20-h intakes in the extinction test. Second, in two-bottle tests SOA rats displayed strong preferences for SOA solution paired with IG maltodextrin infusions over water paired with IG water infusions, whereas the Water

rats did not consistently prefer the water bottle paired with the IG maltodextrin infusions. Third, in the limited access tests, the Water and SOA rats stopped licking the sipper tube for IG maltodextrin infusions after consuming all of the available oral fluid. These results are discussed in detail below.

Consistent with prior experiments, rats given daily 20-h access to a bitter SOA solution paired with IG carbohydrate (MD or glucose) rapidly increased their solution intakes and thereby their carbohydrate infusions (Pérez *et al.*, 1998; Myers & Sclafani, 2003; Yiin *et al.*, 2005). Similar results have been obtained with rats given 20-h access to sour citric acid or sour fruit-flavored (Kool-Aid) solutions (Pérez *et al.*, 1998; Ackroff *et al.*, 2001; Sclafani, 2004; Yiin *et al.*, 2005). Contrary to our prediction, rats given daily 20-h access to water paired with IG maltodextrin infusion rapidly increased their 20-h fluid intake to a degree similar to that of the SOA rats. Furthermore, like SOA rats, the Water rats increased both their drinking bout size and number. Both the SOA and Water groups then showed identical reductions in 20-h oral intakes during the 4-day extinction test when infused IG with water. These findings demonstrate that a novel flavor cue is not required for the postoral stimulation of intake by maltodextrin nor is it required for the decline in oral intakes produced by IG water infusions during extinction testing. Note that is possible that the SOA and Water groups may have displayed differential intakes if the extinction test was prolonged.

The similar increases in total fluid intakes, bout sizes and numbers displayed by the Water and SOA rats during one-bottle tests suggest that the Water group had acquired a conditioned increased attraction to the taste of water (but see below). Alternatively, the IG MD infusions may have produced a nonspecific stimulation of fluid intake perhaps, in part, to facilitate the digestion and metabolism of the infused carbohydrate. Prior findings argue against this interpretation. As previously noted, experimental rats given daily access to a saccharin solution paired with concurrent IG MD infusions substantially increased their saccharin intake compared to control rats given saccharin paired with IG water infusions (Ramirez, 1996). Rats in a yoked control group were also given access to saccharin and IG MD infusions, but their infusions were not paired with their saccharin drinking bouts but rather were yoked to those of the experimental rats. The yoked control rats did not increase their saccharin intake even though they received ID MD infusions identical to the experimental rats. These data suggest that IG carbohydrate infusions do not have a nonspecific stimulatory effect on oral fluid intake. As noted below, the extinction results also support this interpretation.

Our finding that IG MD infusions substantially increased 20-h fluid intake in the Water group would appear to conflict with the prior report that rats given two days experience with saccharin + IG H₂O subsequently failed to show elevated intakes when the saccharin was paired with IG MD infusions (Ramirez, 1996). In a recent study, however, mice trained to drink a flavored saccharin solution paired with IG water infusions for three daily 1-h sessions significantly increased their intake of the same flavored saccharin solution when it was paired with IG glucose infusions for five 1-h daily sessions (Ackroff & Sclafani, 2015). Furthermore the mice tested with the same flavor increased their intake by a similar amount as did mice tested with a new flavor paired with IG glucose infusions. These results are

consistent with the present data indicating that a novel flavor is not required to observe postoral stimulation of intake by MD or glucose infusions.

In marked contrast to the one-bottle findings, the SOA and Water groups dramatically differed in their two-bottle preference responses. The SOA rats displayed strong preferences for SOA over water in Two-Bottle Tests 1 and 2, which confirms prior experiments (Pérez *et al.*, 1998; Yiin *et al.*, 2005). In contrast, in Test 1 the Water rats displayed a preference for the left-side bottle/sipper tube rather than for the bottle paired with IG MD infusions. Five of the Water rats continued to prefer the left-side bottle in Test 2, while the other five rats now preferred the 'left' sipper tube which was attached to the H2O-1/IG MD bottle. This left-side or 'left'-tube preference was unexpected because during one-bottle reinforced testing, the IG MD infusions were paired with the left side/'left' tube on 10 days and the right side/'right' sipper tube on 11 days. Conceivably, the left side/'left' sipper tube preference occurred because on the last two reinforced days preceding the two-bottle test the H2O-1/IG MD bottle was placed on the left side. The reason why some rats in Test 2 preferred to drink from the left side and other from the 'left' sipper tube is not certain. It may be that the rats preferring the 'left' sipper tube happened to have left and right sipper tubes that were more distinguishable by texture cues than the other rats; note that the sipper tubes were all from the same source.

We previously reported that rats trained with one flavor (CS+, e.g., cherry) and sipper paired with IG MD infusions and a second flavor (CS-, e.g., grape) and sipper tube paired with IG water infusions subsequently strongly preferred the CS+ flavor in two-bottle reinforced and extinction tests (Elizalde & Sclafani, 1990). In tests with both bottles containing the same CS+ flavor, the rats preferred to drink from the bottle with the original 'CS+' sipper tube rather than the bottle associated with IG MD infusions. Because the Water rats in the present experiment did not have a unique flavor cue, sipper tube, or bottle position paired with the IG MD infusions during 21 one-bottle training days, we thought that they would attend to postoral nutrient cues and prefer to drink from the water bottle paired with the IG MD infusions. Their failure to do so indicates that postoral appetite cues generated by the MD infusions, while very effective in conditioning strong flavor or sipper tube preferences, are not sufficiently salient to directly control preference behavior.

Overall, in Two-Bottle Test 1 the Water rats consumed as much from the H2O-1/IG MD bottle as from the H2O-2/IG H2O bottle. This is consistent with the finding that the Water rats, as well as the SOA rats consumed similar amounts on the first day of extinction as during the preceding reinforced test days, which confirms prior results obtained with SOA and citric acid solutions (Pérez *et al.*, 1998). The fact that IG water did not reduce total fluid intakes in the first 20-h extinction session indicates that the elevated intakes were the result of a conditioned drinking response ("conditioned acceptance") and that the IG MD infusions do not directly drive oral intakes (Sclafani, 2001). Furthermore, the behavior of the Water rats in the two-bottle tests suggests that the conditioned acceptance response could be maintained over several test day by partial reinforcement, i.e., by alternating IG infusions of water and maltodextrin. This would be analogous to the maintenance of a nutrient-conditioned satiety response observed in rats given alternating sham vs. real feeding sessions (Davis & Smith, 2009).

Although IG MD infusions substantially increased 20-h water and SOA intakes in One-Bottle Test 1, intakes were not stimulated on the first test day, which is consistent with prior findings (Pérez *et al.*, 1998; Yiin *et al.*, 2005). Together, these results suggest that the IG MD infusions did not have an immediate stimulating effect on oral intake but rather required 20 h to be effective. Yet, other findings indicate that IG glucose infusions can have rapid stimulatory actions in rats and mice (Ackroff & Sclafani, 2014; Myers *et al.*, 2013; Zukerman *et al.*, 2013a; Zukerman *et al.*, 2013b). In one study, rats that were given access to a CS+ flavored solution paired with IG 16% glucose showed stimulation of intake during the very first 20 h session (Ackroff *et al.*, 2001). Furthermore, the IG glucose infusions stimulated peak 20-h total (oral + IG) intakes that exceeded those in the present experiment (190 g vs. 130 g/20 h). While glucose rather than maltodextrin infusions were used in these studies, published data indicate that intestinally infused maltodextrin is rapidly digested to glucose and is as rapidly absorbed as infused glucose (Daum *et al.*, 1978). Furthermore, IG glucose and maltodextrin infusions condition comparable flavor preferences in rats (Ackroff *et al.*, 2012; Sclafani *et al.*, 1993; Sclafani & Nissenbaum, 1988). Rather than the form of glucose infused, differences in the flavor of the oral stimulus appear to account for different intake stimulatory effects observed in the present and prior studies. In particular, greater and more rapid stimulation of oral intakes by IG carbohydrate infusions has been obtained with saccharin-sweetened than with unsweetened solutions such as SOA, citric acid and Kool-Aid flavors (Ramirez, 1994; Ramirez, 1996; Ackroff & Sclafani, 1994; Ackroff & Sclafani, 2004; Sclafani & Glendinning, 2003; Sclafani & Glendinning, 2005). Other palatable flavors (e.g., maltodextrin, fat) can also potentiate the appetite-stimulating actions of infused nutrients (Ramirez, 1996; Sclafani *et al.*, 2010).

Throughout one-bottle testing, the Water rats did not consume much more water during the daily 2-h feeding period than did the SOA rats. This suggests that the Water rats' high 20-h water intakes did not represent an increase in their attraction to the orosensory properties of water, per se. Instead, their 20-h oral intakes may have represented an operant licking response to obtain IG maltodextrin infusions. The limited access one-bottle test investigated this possibility by determining if the Water rats would continue to lick the sipper tube to trigger IG infusions after they had consumed the daily 20 ml oral water ration. This did not occur. Instead, the Water rats, like the SOA rats, stopped licking once they consumed the 20-ml fluid ration. Clearly, the availability of water (or SOA) in the sipper tube was essential to maintain the rats' licking response. Consistent with this finding, in a recent experiment thirsty rats trained 20 min/day to lick water from a sipper tube that was paired with IG glucose infusions extinguished their licking response over sessions when oral water was no longer available (Patrono *et al.*, 2017). It may be that the presence of water in the drinking tube represents a contextual cue that becomes associated with IG nutrient reinforcement (González *et al.*, 2012). In addition, the presence of chow in the cage may become a contextual cue (or occasion setter), that signifies that water consumption is not associated with IG MD infusions during the 2-h feeding periods (Puente *et al.*, 1988). Additional research is needed to explore these possibilities.

While the present and prior (Patrono *et al.*, 2017) findings do not provide evidence that rats will lick an empty (or dry) sipper tube for IG nutrient infusions, other studies indicate that mice readily do so. In particular, hungry mice vigorously licked an empty sipper tube during

1 h/day sessions to obtain IG infusions of sugar (sucrose, glucose) or fat (soybean oil) (Ferreira *et al.*, 2012; Tellez *et al.*, 2013; Sclafani *et al.*, 2015; Sclafani & Ackroff, 2016). These mice, however, were never initially trained to lick fluid from the sipper tube, but rather were induced to lick a dry sipper tube that contained food pellets which could be smelled and tasted but not consumed. During initial 1 h/day training sessions with the food-baited sipper tube, licking was reinforced with IG infusions of concentrated nutrient (20–30% Intralipid, 16% sucrose). In subsequent sessions with a dry sipper tube without food, the mice continued to lick for the IG nutrient infusions. Their licking response extinguished, however, when licks were paired with IG water or no infusions. Presumably this mouse training procedure could be used to train rats to lick a dry sipper tube for IG nutrient infusions, but this remains to be demonstrated. The present findings also suggest that, contrary to an early report (Warwick & Weingarten, 1995), rats could be trained to lick plain water for IG diet infusions as their sole source of nutrition if, during initial training days, they were given limited access to chow without water 2 h/day to induce them to drink water paired with IG diet infusions in subsequent hours.

The increased water intake induced by IG MD infusions would appear to be analogous to the conditioned increased intake of oral maltodextrin or glucose solutions observed in taste ageusic knockout (KO) mice. For example, KO mice missing subunits of the sweet receptor (T1R2, T1R3) are indifferent to sucrose in brief lick tests but show intake stimulation when offered concentrated (10–32%) sucrose solutions in 24-h intake tests (Zhao *et al.*, 2003; Zukerman *et al.*, 2009a; Zukerman *et al.*, 2009b; Brasser *et al.*, 2010). The elevated sucrose intakes are attributed to postoral appetite because T1R3 KO mice learn to prefer a flavored solution paired with IG sucrose infusions (Sclafani *et al.*, 2010). KO mice missing critical downstream taste cell ion channels (TRPM5, CALHM1) or postsynaptic receptors (P2X2/P2X3) are ageusic to sugars and maltodextrins but show stimulation of intake when offered concentrated sugar solutions in long-term tests (Sclafani *et al.*, 2007; Zukerman *et al.*, 2013c; Sclafani & Ackroff, 2014; Sclafani *et al.*, 2014). However, unlike the Water rats in the present experiment, ageusic KO mice acquire strong preferences for sugar and/or maltodextrin solutions in long-term two-bottle tests based on a learned attraction to the residual flavor (taste, odor, texture cues) provided by the carbohydrates (Zukerman *et al.*, 2009b; Zukerman *et al.*, 2013c; Sclafani & Ackroff, 2014; Sclafani *et al.*, 2014). Presumably, taste KO and WT mice would show similar behavioral responses as the Water rats in the present experiment if tested with oral water paired with IG maltodextrin or glucose infusions.

In summary, distinctive flavor cues are not essential for the stimulation of oral intake by IG nutrient infusions. IG maltodextrin infusions can stimulate the intake of plain water, and other findings show that IG fat and sugar infusions can stimulate the licking response of mice to dry sipper tubes (Ferreira *et al.*, 2012; Tellez *et al.*, 2013; Sclafani *et al.*, 2015; Sclafani & Ackroff, 2016). Postoral conditioned two-choice preferences, however, are maximized by distinctive flavor cues, although subtle sipper tube textural (Elizalde & Sclafani, 1990), visual (Beeler *et al.*, 2012), or side cues (de Araujo *et al.*, 2008) can support preference conditioning. A limitation of this study is that only female rats were tested. IG nutrient conditioning is readily produced in male rats but some sex differences have been observed (Ackroff & Sclafani, 2004).

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Ethics Statement:

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Reference List

- Ackroff K, Drucker DB & Sclafani A (2012). The CS-US delay gradient in flavor preference conditioning with intragastric carbohydrate infusions. *Physiology and Behavior*, 105, 168–174. [PubMed: 21840327]
- Ackroff K & Sclafani A (1994). Flavor preferences conditioned by intragastric infusions of dilute Polycose solutions. *Physiology and Behavior*, 55, 957–962. [PubMed: 8022918]
- Ackroff K & Sclafani A (2004). Fructose-conditioned flavor preferences in male and female rats: effects of sweet taste and sugar concentration. *Appetite*, 42, 287–297. [PubMed: 15183920]
- Ackroff K & Sclafani A (2014). Rapid post-oral stimulation of intake and flavor conditioning in rats by glucose but not a non-metabolizable glucose analog. *Physiology and Behavior*, 133, 92–98. [PubMed: 24811140]
- Ackroff K & Sclafani A (2015). Flavor change and food deprivation are not critical for post-oral glucose appetite in mice. *Physiology and Behavior*, 140, 23–31. [PubMed: 25484359]
- Ackroff K, Touzani K, Peets TK & Sclafani A (2001). Flavor preferences conditioned by intragastric fructose and glucose: differences in reinforcement potency. *Physiology and Behavior*, 72, 691–703. [PubMed: 11337001]
- Beeler JA, McCutcheon JE, Cao ZF, Murakami M, Alexander E, Roitman MF & Zhuang X (2012). Taste uncoupled from nutrition fails to sustain the reinforcing properties of food. *European Journal of Neuroscience*, 36, 2533–2546. [PubMed: 22712685]
- Brasser SM, Norman MB & Lemon CH (2010). T1r3 taste receptor involvement in gustatory neural responses to ethanol and oral ethanol preference. *Physiological Genomics*, 41, 232–243. [PubMed: 20145204]
- Daum F, Cohen MI, McNamara H & Finberg L (1978). Intestinal osmolality and carbohydrate absorption in rats treated with polymerized glucose. *Pediatric Research*, 12, 24–26. [PubMed: 643370]
- Davis JD & Smith GP (2009). The conditioned satiating effect of orosensory stimuli. *Physiology and Behavior*, 97, 293–303. [PubMed: 19358860]
- de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MAL & Simon SA (2008). Food reward in the absence of taste receptor signaling. *Neuron*, 57, 930–941. [PubMed: 18367093]
- Drucker DB, Ackroff K & Sclafani A (1994). Nutrient-conditioned flavor preference and acceptance in rats: Effects of deprivation state and nonreinforcement. *Physiology and Behavior*, 56, 701–707. [PubMed: 7800736]
- Elizalde G & Sclafani A (1990). Flavor preferences conditioned by intragastric Polycose infusions: A detailed analysis using an electronic esophagus preparation. *Physiology and Behavior*, 47, 63–77. [PubMed: 2109327]
- Ferreira JG, Tellez LA, Ren X, Yeckel CW & de Araujo IE (2012). Regulation of fat intake in the absence of flavor signaling. *Journal of Physiology*, 590, 953–972. [PubMed: 22219333]
- García-Burgos D, Gonzalez F & Hall G (2013). Motivational control of latent inhibition in flavor preference conditioning. *Behav. Processes*, 98, 9–17. [PubMed: 23624026]

- González F, García-Burgos D & Hall G (2012). Associative and occasion-setting properties of contextual cues in flavor-nutrient learning in rats. *Appetite*, 59, 898–904. [PubMed: 22967816]
- Myers KP & Sclafani A (2003). Conditioned acceptance and preference but not altered taste reactivity responses to bitter and sour flavors paired with intragastric glucose infusion. *Physiology and Behavior*, 78, 173–183. [PubMed: 12576114]
- Myers KP, Taddeo MS & Richards EK (2013). Sensory-specific appetite: Postingestive detection of glucose rapidly promotes continued consumption of a recently encountered flavor. *Physiology and Behavior*, 121, 125–133. [PubMed: 23562868]
- Patrono E, Matsumoto J, Nishimaru H, Takamura Y, Chinzorig IC, Ono T & Nishijo H (2017). Rewarding effects of operant dry-licking behavior on neuronal firing in the nucleus accumbens core. *Frontiers in Pharmacology*, 8, 536. [PubMed: 28860992]
- Pérez C, Lucas F & Sclafani A (1998). Increased flavor preference and acceptance conditioned by the postingestive actions of glucose. *Physiology and Behavior*, 64, 483–492. [PubMed: 9761222]
- Puente Gr. P., Cannon DS, Best MR & Carrell LE (1988). Occasion setting of fluid ingestion by contextual cues. *Learning and Motivation*, 19, 239–253.
- Ramirez I (1994). Stimulation of fluid intake by carbohydrates: interaction between taste and calories. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 266, R682–R687.
- Ramirez I (1996). Stimulus specificity in flavor acceptance learning. *Physiology and Behavior*, 60, 595–610. [PubMed: 8840924]
- Sclafani A (2001). Postingestive positive controls of ingestive behavior. *Appetite*, 36, 79–83. [PubMed: 11161347]
- Sclafani A (2004). Oral and postoral determinants of food reward. *Physiology and Behavior*, 81, 773–779. [PubMed: 15234183]
- Sclafani A (2013). Gut-brain nutrient signaling: appetite vs. satiation. *Appetite*, 71, 454–458. [PubMed: 22664300]
- Sclafani A & Ackroff K (2014). Maltodextrin and fat preference deficits in “taste-blind” P2X2/P2X3 knockout mice. *Chemical Senses*, 39, 507–514. [PubMed: 24833134]
- Sclafani A & Ackroff K (2016). Operant licking for intragastric sugar infusions: Differential reinforcing actions of glucose, sucrose and fructose in mice. *Physiology and Behavior*, 153, 115–124. [PubMed: 26485294]
- Sclafani A, Cardieri C, Tucker K, Blusk D & Ackroff K (1993). Intragastric glucose but not fructose conditions robust flavor preferences in rats. *American Journal of Physiology -Regulatory, Integrative and Comparative Physiology*, 265, R320–R325.
- Sclafani A, Fanizza LJ & Azzara AV (1999). Conditioned flavor avoidance, preference and indifference produced by intragastric infusions of galactose, glucose and fructose in rats. *Physiology and Behavior*, 67, 227–234. [PubMed: 10477054]
- Sclafani A, Glass DS, Margolskee RF & Glendinning JI (2010). Gut T1R3 sweet taste receptors do not mediate sucrose-conditioned flavor preferences in mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 299, R1643–R1650.
- Sclafani A & Glendinning JI (2003). Flavor preferences conditioned in C57BL/6 mice by intragastric carbohydrate self-infusion. *Physiology and Behavior*, 79, 783–788. [PubMed: 12954423]
- Sclafani A & Glendinning JI (2005). Sugar and fat conditioned flavor preferences in C57BL/6J and 129 mice: Oral and postoral interactions. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 289, R712–R720.
- Sclafani A, Marambaud P & Ackroff K (2014). Sucrose-conditioned flavor preferences in sweet ageusia T1r3 and Calhm1 knockout mice. *Physiology and Behavior*, 126, 25–29. [PubMed: 24384370]
- Sclafani A & Nissenbaum JW (1988). Robust conditioned flavor preference produced by intragastric starch infusions in rats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 255, R672–R675.
- Sclafani A, Touzani K & Ackroff K (2015). Intragastric fat self-administration is impaired in GPR40/120 double knockout mice. *Physiology and Behavior*, 147, 141–148. [PubMed: 25911263]

- Sclafani A, Zukerman S, Glendinning JI & Margolskee RF (2007). Fat and carbohydrate preferences in mice: The contribution of α -gustducin and Trpm5 taste signaling proteins. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 293, R1504–R1513.
- Tellez LA, Ferreira JG, Medina S, Land BB, DiLeone RJ & de Araujo IE (2013). Flavor-independent maintenance, extinction, and reinstatement of fat self-administration in mice. *Biological Psychiatry*, 73, 851–859. [PubMed: 23587200]
- Warwick ZS & Weingarten HP (1995). Determinants of high-fat diet hyperphagia: Experimental dissection of orosensory and postingestive effects. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 269, R30–R37.
- Winer BJ (1962). *Statistical Principles in Experimental Design*. New York: McGraw Hill.
- Yiin Y-M, Ackroff K & Sclafani A (2005). Food deprivation enhances the expression but not acquisition of flavor acceptance conditioning in rats. *Appetite*, 45, 152–160. [PubMed: 15946767]
- Zhao GQ, Zhang Y, Hoon MA, Chandrasekar J, Erlenbach I, Ryba NJP & Zuker CS (2003). The receptors for mammalian sweet and umami taste. *Cell*, 115, 255–266. [PubMed: 14636554]
- Zocchi D, Wennemuth G & Oka Y (2017). The cellular mechanism for water detection in the mammalian taste system. *Nature Neuroscience*, 20, 927–933. [PubMed: 28553944]
- Zukerman S, Ackroff K & Sclafani A (2013a). Post-oral appetite stimulation by sugars and nonmetabolizable sugar analogs. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 305, R840–R853.
- Zukerman S, Ackroff K & Sclafani A (2013b). Post-oral glucose stimulation of intake and conditioned flavor preference in C57BL/6J mice: A concentration-response study. *Physiology and Behavior*, 109, 33–41. [PubMed: 23200639]
- Zukerman S, Glendinning JI, Margolskee RF & Sclafani A (2009a). T1R3 taste receptor is critical for sucrose but not Polycose taste. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 296, R866–R876.
- Zukerman S, Glendinning JI, Margolskee RF & Sclafani A (2013c). Impact of T1r3 and Trpm5 on carbohydrate preference and acceptance in C57BL/6 mice. *Chemical Senses*, 38, 421–437. [PubMed: 23547138]
- Zukerman S, Touzani K, Margolskee RF & Sclafani A (2009b). Role of olfaction in the conditioned sucrose preference of sweet-ageusic T1R3 knockout mice. *Chemical Senses*, 34, 685–694. [PubMed: 19736224]

Figure 1.

Mean ($\pm$ SEM) total (oral + IG) 20-h/day fluid intakes of SOA and Water groups during one-bottle baseline (BL), Test 1, Extinction, and Test 2 phases. During the 2 days prior to and first 4 days of Test 1, water was not available during the daily 2-h feeding periods. The horizontal bar in the BL period represents the mean 20-h total fluid intake during the last 2 days of the food + water baseline period. During the baseline and Extinction tests, 20-h oral intakes were paired with concurrent IG infusions of water. During Tests 1 and 2 the 20-h oral intakes were paired with concurrent intakes of 16% maltodextrin (MD). The left and right positions of the drinking bottle each day are indicated by the L and R symbols along the X axis.

Figure 2.

Mean ($\pm$ SEM) total (oral + IG) 20-h/day fluid intakes of the Water (top graph) and SOA group (bottom graph) during Two-Bottle Tests 1 and 2. In Test 1, the 'left' and 'right' sipper tubes were presented on the left and right sides of the cages, respectively. In Test 2, the 'left' sipper tube was attached to the bottles (H2O-1/IG MD, SOA/IG MD) paired with IG maltodextrin infusions, and the 'right' sipper tube was attached to the bottle (H2O-2/IG H2O, H2O/IG H2O) paired with IG water infusions. The left and right positions of the bottle paired with IG MD infusions each day are indicated by the L and R symbols along the X axis.

Figure 3.

Mean ($\pm$ SEM) total (oral + IG) 20-h/day fluid intakes of the 'Side Preferrers' (top graph) and 'Sipper Preferrers' (bottom graph) Water subgroups ($n=5$ each) during Two-Bottle Tests 1 and 2. In Test 1, the 'left' and 'right' sipper tubes were presented on the left and right sides of the cages, respectively. In Test 2, the 'left' sipper tube was attached to the H2O-1/IG MD bottle paired with IG maltodextrin infusions, and the 'right' sipper tube was attached to the H2O-2/IG H2O bottle paired with IG water infusions. The left and right positions of the bottle paired with IG MD infusions each day are indicated by the L and R symbols along the X axis.

Figure 4.

Mean ($\pm$ SEM) daily 20 h/day IG maltodextrin (MD) intakes of SOA and Water groups prior to and during one-bottle Limited Intake Test. Day 0 represents the two-day mean IG intake when rats had unlimited oral access to SOA or water during the 20 h/day sessions. Days 1 to 4 represents the daily IG MD infusion intakes when the rats were limited to 20 ml of SOA or water but IG infusions were unlimited during the 20-h drinking periods.

Table 1.

Experimental procedures

Days	2-h period	20-h period
<i>Baseline</i>		
1 – 3	Food + Water	H ₂ O → IG H ₂ O
4 – 6	Food only	H ₂ O → IG H ₂ O
<i>One-Bottle Test 1</i>		
1 – 4	Food only	H ₂ O or SOA → IG MD
5 – 8	Food + Water	H ₂ O or SOA → IG MD
<i>Extinction Test</i>		
1 – 4	Food + Water	H ₂ O or SOA → IG H ₂ O
<i>One-Bottle Test 2</i>		
1 – 13	Food + Water	H ₂ O or SOA → IG MD
<i>Two-Bottle Test 1</i>		
1 – 4	Food + Water	Water group: H ₂ O-1 → IG MD, H ₂ O-2 → IG H ₂ O
'Left' sipper/left side; 'Right' sipper/right side		SOA group: SOA → IG MD, H ₂ O → IG H ₂ O
<i>Two-Bottle Test 2</i>		
1 – 4	Food + Water	Water group: H ₂ O-1 → IG MD, H ₂ O-2 → IG H ₂ O
'Left' sipper/IG MD, 'Right' sipper/IG H ₂ O		SOA group: SOA → IG MD, H ₂ O → IG H ₂ O
<i>Limited One-Bottle Test</i>		
1 – 2	Food + Water	H ₂ O or SOA → IG Water, unlimited oral intake
1 – 4	Food + Water	H ₂ O or SOA → IG Water, 20 g limited oral intake

Notes: The rats had no food or fluid during the 2 h between the 2- and 20-h periods. IG = intragastric, SOA = 0.03% sucrose octaacetate solution, MD = 16% maltodextrin solution.

Table 2.

Mean ($\pm$ SEM) 20-h oral intakes, mean bout numbers, and mean bout sizes of Water and SOA groups during Baseline days 2–3 and Test days 5–8 when food and water were available during the 2-h daily feeding periods.

Groups	Oral Intake (g)		Bout Number		Bout Size (licks)	
	Baseline (2–3)	Test (5–8)	Baseline (2–3)	Test (5–8)	Baseline (2–3)	Test (5–8)
Water	12.7 $\pm$ 1.7	43.6 $\pm$ 5.4 *	15.0 $\pm$ 2.0	26.0 $\pm$ 1.7 *	211.6 $\pm$ 31.0	440.7 $\pm$ 40.4 *
SOA	12.9 $\pm$ 1.2	48.5 $\pm$ 3.3 *	14.4 $\pm$ 0.9	26.7 $\pm$ 1.4 *	211.9 $\pm$ 17.7	554.1 $\pm$ 72.9 *

* indicates significant difference between Test and Baseline periods.