Early milk feeding influences taste acceptance and liking during infancy[1–5]

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ABSTRACT

Background: We identified a model system that exploits the inherent taste variation in early feedings to investigate food preference development.

Objective: The objective was to determine whether exposure to differing concentrations of taste compounds in milk and formulas modifies acceptance of exemplars of the 5 basic taste qualities in a familiar food matrix. Specifically, we examined the effects of consuming hydrolyzed casein formulas (HCFs), which have pronounced bitter, sour, and savory tastes compared with breast milk (BM) and bovine milk–based formulas (MFs), in which these taste qualities are weaker.

Design: Subgroups of BM-, MF- and HCF-fed infants, some of whom were fed table foods, were studied on 6 occasions to measure acceptance of sweet, salty, bitter, savory, sour, and plain cereals.

Results: In infants not yet eating table foods, the HCF group ate significantly more savory-, bitter-, and sour-tasting and plain cereals than did the BM or MF groups. HCF infants displayed fewer facial expressions of distaste while eating the bitter and savory cereals, and they and BM infants were more likely to smile while they were eating the savory cereal. In formula-fed infants eating table foods, preferences for the basic tastes reflected the types of foods they were being fed. In general, those infants who ate more food displayed fewer faces of distaste.

Conclusions: The type of formula fed to infants has an effect on their response to taste compounds in cereal before solid food introduction. This model system of research investigation sheds light on sources of individual differences in taste and perhaps cultural food preferences. *Am J Clin Nutr* 2009;90(suppl):780S–8S.

INTRODUCTION

Many cultures place great importance on establishing patterns of food habits early in life (1, 2). The types of foods and flavors mothers eat during pregnancy and lactation and then feed to their children during the transition from an all-milk diet to one containing foods of the table are part of the traditions of many cultures (2). Experimental research conducted during the past few decades has revealed that first experiences with food flavors occur long before the first "taste" of solid foods because flavor volatiles and taste compounds from the maternal diet are transmitted to and flavor amniotic fluid and human milk (3). Thus, culturally determined flavor preferences, one of the most enduring characteristics of an ethnic group (1), can be understood in the context of early flavor exposure and thus may provide the foundation for cultural differences in cuisine.

Psychophysical studies of human milk revealed that breastfed infants consume a milk that has a predominant taste quality of sweetness, contains volatile food odors, and varies from mother to mother (4–6). In marked contrast, formula-fed infants are usually exposed to constant flavors, because most mothers who use formulas often feed their infants a single type of formula (7, 8). The flavors of the various types and brands of formulas differ from each other substantially, however, and these flavors too are detected by infants (9–11). Formula flavors, which are due to composition and processing, can range from low levels of sweet and sour tastes in milk-based formulas to savory, sour, and bitter tastes and unpleasant odor volatiles (to adults and older children) in protein hydrolysate formulas (11, 12). The strong flavor of hydrolysate formulas is due, in part, to their high amino acid content (13, 14). These formulas contain protein nutrients in a "predigested" form as amino acids and small peptides (12, 15, 16), and like milk-based formulas, are fortified with certain amino acids to provide a balanced amino acid profile that complies with the standards for nutrient content of infant formulas (16).

From the perspective of taste, infants who consume different types of milk during early life are exposed to pronounced differences in levels and patterns of taste experience, because each contains variable amounts of compounds that have specific taste qualities. Perhaps the most striking from a sensory perspective is the difference in the taste-active amino acid, glutamate, which occurs naturally in many foods, such as meats, cheeses, broths, and tomatoes (17), and imparts a savory taste (umami). Glutamate is the most abundant free amino acid in human milk; it is

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40-fold higher in milk relative to plasma (18) and accounts for >50% of the total free amino acid content (19). Although glutamate concentrations in human milk (20, 21) are several times greater than those in cow milk (21) and bovine milk–based formulas, concentrations in hydrolysate formulas are >300 times greater (12, 22, 23).

The pronounced differences in nutrient content and flavor profiles of early milk feedings provide an ideal model system to test the hypothesis that infants exposed to higher levels of certain taste compounds exhibit elevated taste preferences for them (24–26). After the transition to milk feedings at birth, the next largest change in the human diet occurs at weaning, when an infant moves from a mainly milk-based diet to a mixed diet containing the foods of the table. This dietary change is accompanied by major changes in the content and context of flavor experiences, leading to the hypothesis that major changes in preferences would coincide with this major shift in feeding regimen. For example, elevations in preferences for aqueous salt solutions coincide with weaning and the initiation of a mixed, table food diet (LJ Stein, BJ Cowart, and GK Beauchamp, unpublished observations, 2009). Consequently, the present study assessed infants' preferences for the basic tastes before and after the introduction of a mixed diet.

The present study was designed to compare responses to basic taste compounds in a familiar infant food in 3 groups of infants: those fed breast milk (BM), those fed bovine milk–based formulas (MF), and those fed hydrolyzed casein formula (HCF). We tested the hypothesis that these differences in early milk consumption result in different responses to the basic tastes as a consequence of differential taste familiarity. Furthermore, to test the hypothesis that consuming a diet containing weaning foods modifies infant taste preferences, we also recruited subgroups of infants who were still consuming human BM or formulas and who were either not yet being fed table foods (NT) or were being fed table foods (T).

SUBJECTS AND METHODS

Subjects

Mothers whose infants were between the ages of 4 and 9 mo were recruited from advertisements in local newspapers and from Women, Infant and Children Programs in Philadelphia between 2004 and 2007. All infants were born full term, were healthy at the time of testing as reported by their mothers, and had been eating baby cereal from a spoon for ≥2 weeks (*see* reference 27 for methods). From a pool of 97 mother-infant dyads (44% black, 30% white, and 26% mixed race/other), groups were formed on the basis of the milk-consuming history of the infants [group fed BM, group fed MF, and group fed HCF] and whether or not children were fed table foods (NT and T; *see* **Table 1**). Thirteen additional infants were tested but were excluded from these analyses because they ate the maximum amount of food on the majority of test days (n = 6), were sick (n = 3), or their mothers withdrew from the study (n = 4). Although we recruited an excess of breastfed infants, we were unable to get a sufficient number who were eating both cereal and table foods for statistical analyses. All procedures were approved by the Office of Regulatory Affairs at the University of Pennsylvania, and written informed consent was obtained from each mother.

Food stimuli

The type (rice, oatmeal, or barley) and brand (Gerber, Fremont, MI; Beechnut, Canajoharie, NY; or Earth's Best, Boulder, CO) of cereal used during testing were those the infants were being fed at home and thus were familiar with. The cereals were prepared by mixing 40 g of cereal with 160 mL of distilled water (plain cereal) or a similar volume of the following solutions: 0.56 mol D-lactose/L (sweet cereal), 0.1 mol sodium chloride/L (salty cereal), 0.24 mol urea/L (bitter cereal), 0.006 mol citric acid/L (sour cereal), and 0.02 mol monosodium glutamate/L (savory cereal). The concentrations were selected on the basis of previous studies of infant reactivity to the basic tastes [eg, urea (28), salt (29), and glutamate (29)]. We aimed for a weak intensity of flavors to best represent the flavor of human milk (4).

Before infant testing, a trained sensory panel of 9 adults (7 women and 2 men), who were between the ages of 25 and 31 y [mean (±SEM) age: 28.8 (0.7) y], evaluated the 6 cereal mixtures using the general Labeled Magnitude Scale (30), which is a psychophysical tool that allows subjects to rate perceived intensity along a vertical axis labeled according to the following adjectives: no sensation = 0, barely detectable = 1, weak = 6, moderate = 16, strong = 33, very strong = 50, and strongest imaginable = 95. The adjectives are spaced semilogarithmically, on the basis of experimentally determined intervals, to yield data that parallel magnitude estimation (30, 31). Panelists were offered each cereal individually and asked to rate its sweetness, saltiness, bitterness, sourness, savoriness, and overall intensity and rinse their mouths ≥3 times between each tasting. After completing the ratings, they were again offered each cereal sample and asked to indicate whether the predominant taste quality was sweet, salty, bitter, sour, or savory.

Procedures

The methodologies used were developed and validated at the Monell Chemical Senses Center and designed to control for a number of factors to allow for the evaluation of infants' hedonic responses independently of the caregiver and experimenter (27). To this end, we first accustomed infants to various aspects of the study procedures before testing. Mothers were sent bibs, spoons, and masks to use while feeding their infants at home for the 3 d before the first testing session. They were asked to refrain from introducing additional foods or beverages to their infants before and during the experimental period. To encourage compliance, mothers kept a daily record of what they fed their infants.

Testing occurred at approximately the same time of day and 30 to 60 min before the infants' next scheduled feeding so that intake and facial reactivity were not affected by hunger or satiation but rather reflected hedonic responses to the food. During the test sessions, the mothers wore a mask and refrained from talking to eliminate any influence of their facial or verbal responses on the infants' behaviors (32, 33). Testing occurred in a well-ventilated closed room specifically designed for sensory testing and under naturalistic conditions in which infants determined the pacing and duration of the feeding. The experimenter was out of view of the mother-infant dyad, and infants were videotaped as their mothers fed them at their customary pace. Analyses of the videotapes revealed that the infants were not distracted by their mothers wearing a mask because they had become familiar with

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the mask before the actual testing. The feeding ended when a child rejected the cereal ≥3 consecutive times or ate the maximum amount (≈200 g). All food that spilled onto the tray or bib was placed in the bowl before weighing. The amount of food consumed was measured by weighing each bowl immediately before and after each feeding on a Mettler balance (Mettler Instrument Corp, Hightstown, NJ) accurate to 1.0 g. Immediately after each session, mothers rated their infants' enjoyment of the food on a 9-point scale (9 = extreme liking).

All but 2 mothers completed a 95-item questionnaire that measured their perceptions of their infants' temperament (34). All mothers were queried about which specially prepared infant foods and table foods their infants had been exposed to as well as the frequency of consumption of these foods. On the first day of testing, each infant was measured and weighed; z scores for weight, length, and weight-for-length were then calculated using the World Health Organization Anthro software (version 2.02; Geneva, Switzerland) (35).

Videotape analyses

In addition to calculating intake, videotapes were analyzed to evaluate the length of the feeding and the types of facial expressions made during feeding, the latter of which were measures of hedonic responses or liking (5, 6, 36). Each videotape was subjected to frame-by-frame analysis using an IBM-based event recorder program, The Observer (Noldus Inc, Wageningen, Netherlands). A trained rater, who was certified in Ekman and Friesen's Facial Action Coding System (37) and who was unaware of the infants' group designation and experimental treatment, scored the first 2 min of each test session. Not all feeding sessions were scored for length of feeding for 2 infants and for facial reactivity for 10 infants because of technical difficulties or because the baby cried throughout that particular testing session. The scoring focused on facial expressions of liking (eg, smiling) and distaste, the latter of which included brow movements (eg, brow lowering and inner brow raises), nose wrinkling, upper lip raising, squinting, lip tightening, chin raising, and gaping (6, 36, 38). Because of marked individual differences in the display of the faces, statistical analyses focused on the total number of facial expressions of distaste made per spoonful offered as well as the incidence of specific facial responses (eg, smiles, squints, and gapes) during feeding. More detailed analysis of the facial reactivity data will be reported elsewhere.

Data analyses

For each infant, we calculated the total intake (g), length (min), and rate (g/min) of each feeding, number of facial expressions of distaste made per spoonful offered, and mother's perception of the infant's enjoyment of the cereals during each test session. The first set of analyses focused on differences between the groups of NT infants. To this end, separate analyses of variance (ANOVAs) were conducted for each measurement with milk- and formula-fed groups (BM, MF, and HCF) as the between-subjects factor. Planned comparisons were conducted using Bonferroni-corrected *t* tests to evaluate whether the HCF group differed from the MF or BM groups. Statistical tests also focused on relative responses that were compared within the groups. We ranked each of the 6 cereals on the basis of how much each subject ate (1 = greatest consumption) to eliminate absolute differences that may be due to other factors. From these data, we compared the percentage of infants within each group who ranked each of the cereals as first to third most preferred on the basis of intake using a chi-square analyses. A composite ranking score was computed on the basis of the relative intake of sour, savory, and bitter cereals, which was then compared between groups using a one-factor ANOVA. In addition, we computed the percentage of children who gaped, squinted, or smiled during feeding, and separate chi-square analyses were conducted to calculate if the likelihood of expressing these behaviors differed between groups. Correlational analyses were conducted to determine whether the types of facial responses made during the initial minutes of the feeding were related to how much the infants ate of the different tasting cereals.

The second set of analyses focused on whether acceptance of the basic tastes interacted with the type of milk experienced when infants were eating table food. Because we were unable to get a sufficient number of breastfed infants who were still eating cereal once they started experiencing table foods, this approach evaluated responses in formula-fed infants only. Separate ANOVAs were conducted with the MF and HCF groups and the NT and T groups as the between-subjects factors and age as the covariate (*see* Subject Characteristics). Significant interactions were probed by simple main effects analyses.

The third set of analyses was conducted by using data obtained from the T infants to determine whether the types of foods experienced by infants correlated with their acceptance of the different tasting cereals. Separate ANOVAs were conducted whether or not the infant had been exposed to a particular table food as the between-subjects factor and age as the covariate. All summary statistics are expressed as mean ± SEM.

RESULTS

Subject characteristics

HCF infants were fed a milk- or soy-based formula during their first months (1.2 ± 0.2 mo) of life and then, usually according to their pediatrician's recommendation, switched to hydrolysate formulas (eg, Nutramigen; Mead Johnson, Evansville, IN, or Alimentum; Ross Products Company, Abbot Park, IL). The vast majority (91.7%) of the HCF infants began being fed hydrolysates during the first 3 mo of life. The BM infants had little or no experience with formulas and neither they nor the MF infants had ever tasted hydrolysate formulas. Breastfed infants were perceived by their mothers as being more rhythmic [ie, more regular in bodily functioning; $F_{(2,83)} = 4.55$, $P < 0.02$], adaptable [$F_{(2,83)} = 7.68$, $P < 0.001$], and approachable [$F_{(2,83)} = 7.99$, $P < 0.001$] and less distracted [$F_{(2,83)} = 10.62$, $P < 0.001$] than those infants fed formula (data not shown). As shown in Table 1, in the NT infant group, BM infants were older than MF infants and were introduced to cereal later than were HCF infants. In the formula-fed infant groups, T infants were older and weight-for-age *z* scores were larger than for NT infants. Because of these differences, all subsequent analyses covaried age.

The majority of infants were fed cereals prepared with ingredients other than water. In line with recommendations from baby food manufacturers, many lactating mothers prepared their

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TABLE 1
Subject characteristics[1]

	NT			T		*P* value	
	BM (n = 37)	MF (n = 16)	HCF (n = 13)	MF (n = 12)	HCF (n = 11)	One-factor analyses for NT groups[2]	Two-factor analyses for MF and HCF groups[3]
Infant characteristics							
Age (mo)	5.9 ± 0.1[4]	5.2 ± 0.1[5]	5.5 ± 0.3	7.2 ± 0.3	7.1 ± 0.3	0.01	0.001[6]
Girls (%)	54.1	50	30.8	41.7	45.5	NS	NS
Weight-for-age z scores	0.5 ± 0.2	−0.04 ± 0.2	0.3 ± 0.3	0.5 ± 0.3	0.8 ± 0.3	NS	0.04[6]
Age at cereal introduction (mo)	4.6 ± 0.1	3.4 ± 0.2[5]	3.5 ± 0.4[5]	2.7 ± 0.4	4.0 ± 0.4	0.001	NS
Cereal preparation methods[7]							
Added BM (%)	75.7	0[5]	0[5]	0	0	0.001	NA
Added formula (%)	8.1	93.8[5]	100[5]	100	81.8	0.001	NS
Added sugar/syrup/honey/fruit (%)	35.1	56.3	30.8	75.0	90.9	NS	0.01[6]

[1] BM, breast milk group; MF, milk-based formula group; HCF, hydrolyzed casein formula group; NT, no table food group; T, table food group; NA, not applicable.
[2] *P* values obtained after either Pearson chi-square or one-factor ANOVA with milk experience group (BM, MF, or HCF) as the between-subjects factor for NT infants. If significant, post hoc tests were conducted to calculate differences between groups.
[3] *P* values obtained after 2-factor ANOVA with milk experience group (MF or HF) and table food group (NT or TF) as the between-subjects factors for formula-fed infants.
[4] Mean ± SEM (all such values).
[5] Significantly different from BM-NT, $P < 0.05$ (Fisher's least significant difference test with Bonferroni correction).
[6] Significant main effect of T group.
[7] Columns do not total 100% because some mothers may add more than one ingredient.

infants' cereal with BM, and nonlactating mothers prepared the cereals with the type of formula their infants were fed. Sugars, syrups, and fruit juices were added to the cereal of the vast majority of T infants and to the cereal of approximately one-third of the NT infants. Preliminary analyses were conducted to determine whether experience with these sweet tastes in cereals affected the NT infants' cereal acceptance. These analyses indicated that infants consumed similar amounts of plain or sweetened cereals during testing, regardless of how their cereal was prepared at home (all P values > 0.22). Once table foods were introduced to the infants' diet, there were large individual differences in the types of foods experienced (eg, meats, dairy products, pizza, macaroni, cheeses, puddings, desserts, vegetables, and fruit).

Sensory panel evaluation of cereals

As shown in **Figure 1**, cereals to which lactose, salt, urea, citric acid, and glutamate had been added tasted more sweet [$F_{(5,40)} = 17.93$, $P < 0.001$], salty [$F_{(5,40)} = 19.86$, $P < 0.001$], bitter [$F_{(5,40)} = 6.93$, $P < 0.001$], sour [$F_{(5,40)} = 8.49$, $P < 0.001$], and savory [$F_{(5,40)} = 5.46$, $P = 0.002$], respectively. The taste qualities of all but the plain cereals were correctly identified by the majority of the panelists. Approximately half (44%) of the panelists incorrectly identified the plain cereal, the majority of whom thought it tasted bitter, sour, or savory. The intensity ratings of the characteristic taste qualities [mean (±SEM): 6.7 (1.2)] and the overall intensity [mean (±SEM): 1.6 (0.3)] were in the moderate or weak range, respectively.

Taste acceptance in infants with no experience with table foods

As shown in **Table 2**, the HCF-NT group ate significantly more of the savory-flavored cereal than did both the BM-NT and MF-NT groups and ate all but the plain cereal at a faster pace than the BM-NT group. The HCF-NT infants also ate more of the sour- and bitter-flavored cereals and the cereal to which distilled water had been added. Separate repeated-measures ANOVAs on HCF-NT group data revealed that they ate significantly more savory cereal than the bitter and sour cereals [$F_{(5,45)} = 2.65$, $P < 0.04$]. Mothers were aware of their infants' acceptance because it was indicated by their significantly higher scores of liking for the savory and plain cereals in the HCF-NT group.

In addition to eating more, HCF-NT infants squinted less while they were fed the bitter [0.4 ± 0.1 compared with 0.9 ± 0.1/spoonful; $F_{(2,57)} = 3.48$, $P < 0.04$] and savory [0.4 ± 0.1 compared with 0.7 ± 0.1/spoonful; $F_{(2,60)} = 4.08$, $P < 0.03$] cereals and tended to make fewer facial expressions of distaste overall while they were fed the bitter- and savory-flavored cereals when compared with the BM-NT infants. Although 38% of the BM-NT infants and 25% of the MF-NT infants gaped while eating the bitter-flavored cereal, none of the HCF-NT infants made this facial expression of distaste [chi-square$_{(2)} = 6.74$, $P < 0.04$]. Although there were no differences in the intake of the different cereals between the BM-NT and MF-NT infants, the BM-NT and HCF-NT infants were more likely to smile while eating the savory cereal than were the MF-NT infants [chi-square$_{(2)} = 6.39$, $P < 0.05$]. In the BM-NT infants, the more savory cereal eaten, the fewer faces of distaste made while they were fed [$r_{(33)} = -0.36$, $P < 0.04$]. Significantly more of the HCF-NT infants (85%) ranked, on the basis of intake, the savory-flavored cereal as first to third most preferred when compared with the BM-NT infants [49%; chi-square$_{(1)} = 4.43$, $P < 0.04$] or the MF-NT infants [44%; chi-square$_{(1)} = 5.08$, $P < 0.03$; **Figure 2**]. Analyses of the total ranking score on the basis of the intake of the savory, sour, and bitter cereals revealed that these cereals were preferred more by the HCF-NT infants (9.7 ± 0.5) than by both the MF-NT (11.8 ± 0.6) and BM-NT (12.0 ± 0.2) infants [$F_{(2,62)} = 6.29$, $P = 0.003$]. The MF-NT and BM-NT infants also preferred the sweet cereal but the salty and sour

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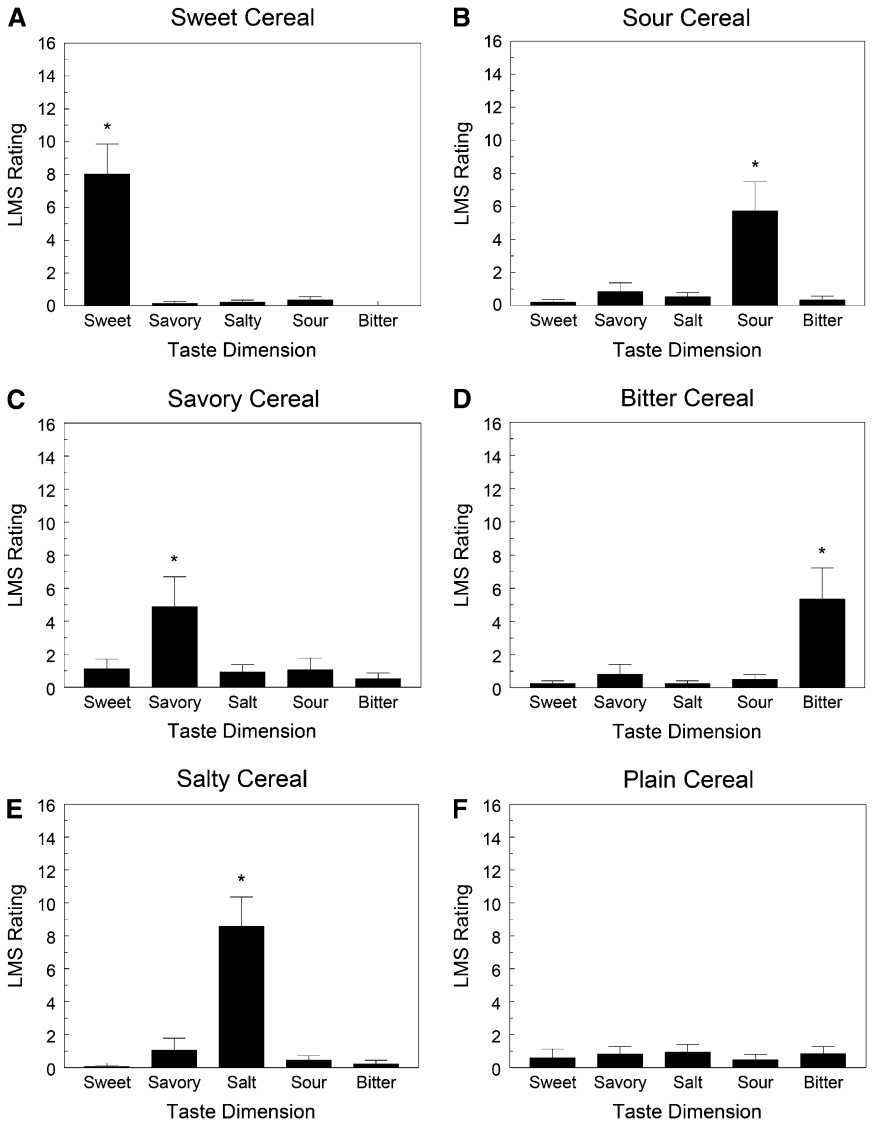

FIGURE 1. Mean (±SEM) sensory evaluation of the stimuli used during infant testing. A trained panel (n = 9) rated the sweetness, savoriness, saltiness, sourness, and bitterness of the cereals to which lactose (A), citric acid (B), glutamate (C), urea (D), sodium chloride (E), or distilled water (F) had been added by using the general Labeled Magnitude Scale (LMS), a psychophysical tool that allows subjects to rate the perceived intensity of sensations that are arranged semilogarithmically and range from "no sensation" to "strongest imaginable." The data yielded general LMS ratings in the weak range. For reference, values of 0 = no sensation, 1 = barely detectable, 6 = weak, and 16 = moderate. *$P < 0.05$ when compared with other taste qualities by using a one-factor analysis with Fisher's least significant difference test.

cereals were also highly ranked for the MF-NT infants and the savory for the BM-NT infants.

Taste acceptance comparisons between formula-fed infants with and without table food experience

As a group, formula-fed T infants ate significantly less of the cereals [52.4 ± 9.3 g compared with 81.1 ± 8.5 g; $F_{(1,42)} = 5.22$, $P = 0.027$) and spent a shorter amount of time feeding [8.2 ± 1.1 min compared with 12.3 ± 1.0 min; $F_{(1,40)} = 8.04$, $P = 0.007$) when compared with formula-fed NT infants. As shown in Table 2, the greater consumption (and in some cases faster rate of eating) in HCF-NT infants relative to MF-NT infants was not apparent in those eating table foods [HCF-T compared with MF-T; $F_{(1,20)} = 0.02$, $P = 0.89$]. As a group, formula-fed T infants were perceived by their mothers as enjoying the cereals, in particular the savory and plain, less than those who were not eating table foods. However, HCF-T infants were perceived as liking the savory cereal more than were the MF-T infants (*see* Table 2).

There was a great deal of variability in the types of table foods proffered, and experiences with some of these table foods were related to the liking for particular tastes in cereal during testing. For example, those infants who were eating cheese at home ate more of the salty cereals (79.6 ± 24.9, n = 9) than did those infants who were not eating cheese at home [42.1 ± 12.7, n = 14; $F_{(1,20)} = 4.43$, $P = 0.048$]. Eating bitter green vegetables, such as broccoli, was associated with greater acceptance of the bitter-flavored cereal [94.0 ± 19.9, n = 5 compared with 31.0 ± 10.5, n = 18; $F_{(1,20)} = 7.56$, $P = 0.01$] and eating pasta and other foods that contained cheese or tomatoes, which are high in glutamate, was associated with greater acceptance of the savory cereal [62.1 ± 14.6, n = 7 compared with 33.6 ± 9.6, n = 16; $F_{(1,20)} = 6.19$, $P = 0.02$].

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TABLE 2
Acceptance of the basic tastes in a cereal matrix on the basis of the type of milk or formula that infants are currently consuming and whether they are eating table foods[1]

	NT			T		*P* value	
	BM (n = 37)	MF (n = 16)	HCF (n = 13)	MF (n = 12)	HCF (n = 11)	One-factor analyses for NT groups[2]	Two-factor analyses for MF and HCF groups[3]
Lactose							
Intake (g)	71.7 ± 7.9[4]	89.5 ± 18.5	95.8 ± 14.4	73.8 ± 15.4	78.9 ± 19.7	NS	NS
Length of feeding (min)	13.2 ± 1.1	12.9 ± 1.9	12.6 ± 1.5	12.0 ± 2.2	11.8 ± 1.5	NS	NS
Rate of intake (g/min)	5.8 ± 0.6	6.4 ± 0.8	8.3 ± 1.4[5]	6.4 ± 0.9	6.6 ± 1.4	0.03	NS
Distaste expressions (no./spoonful offered)	2.2 ± 0.3	2.0 ± 0.3	2.2 ± 0.5	2.1 ± 0.3	1.6 ± 0.2	NS	NS
Mothers' rating of liking[6]	6.4 ± 0.3	7.3 ± 0.5	6.6 ± 0.7	6.4 ± 0.6	7.1 ± 0.8	NS	NS
Glutamate							
Intake (g)	49.6 ± 6.9	63.7 ± 14.2	114.4 ± 15.6[5,7]	59.0 ± 17.6	41.0 ± 9.9[8]	0.0004	0.05[9] 0.04[10]
Length of feeding (min)	9.6 ± 0.9	11.4 ± 1.8	16.6 ± 1.8[5,11]	9.0 ± 1.9	7.0 ± 1.1	0.006	0.05[9] 0.06[10]
Rate of intake (g/min)	5.1 ± 0.5	5.4 ± 0.7	7.1 ± 1.1[12]	6.0 ± 1.0	6.1 ± 1.0	0.05	NS
Distaste expressions (no./spoonful offered)	2.4 ± 0.2	1.8 ± 0.3	1.6 ± 0.2	2.0 ± 0.3	2.1 ± 0.3	NS	0.06[9]
Mothers' rating of liking[5]	6.1 ± 0.4	5.6 ± 0.7	7.6 ± 0.4[5,7]	4.8 ± 0.8	5.4 ± 0.6	0.05	0.06[13]
Salt							
Intake (g)	67.6 ± 9.8	80.2 ± 16.0	76.4 ± 17.1	65.4 ± 17.4	64.0 ± 15.6	NS	NS
Length of feeding (min)	12.5 ± 1.2	14.0 ± 2.1	10.9 ± 1.9	10.4 ± 2.3	9.7 ± 1.3	NS	NS
Rate of intake (g/min)	5.2 ± 0.6	5.4 ± 0.7	8.8 ± 2.3[12]	6.0 ± 0.7	7.4 ± 1.8	0.02	NS
Distaste expressions (no./spoonful offered)	2.2 ± 0.2	1.8 ± 0.4	1.9 ± 0.4	2.1 ± 0.3	1.9 ± 0.3	NS	NS
Mothers' rating of liking[6]	6.4 ± 0.4	6.7 ± 0.6	6.1 ± 0.6	5.6 ± 0.7	7.4 ± 0.5	NS	NS
Citric acid							
Intake (g)	45.5 ± 7.2	56.8 ± 8.6	89.0 ± 12.6[5]	69.5 ± 18.0	48.8 ± 9.8[8]	0.003	0.05[10]
Length of feeding (min)	9.1 ± 0.8	10.7 ± 1.4	12.5 ± 1.4	9.1 ± 2.0	8.3 ± 1.1	NS	NS
Rate of intake (g/min)	4.7 ± 0.5	5.3 ± 0.6	7.3 ± 1.1[5]	7.2 ± 0.7[7]	5.6 ± 0.8	0.01	0.05[10]
Distaste expressions (no./spoonful offered)	2.4 ± 0.4	2.0 ± 0.3	2.0 ± 0.5	2.0 ± 0.3	1.9 ± 0.2	NS	NS
Mothers' rating of liking[6]	5.1 ± 0.4	5.8 ± 0.6	6.0 ± 0.6	6.2 ± 0.7	5.3 ± 0.8	NS	NS
Urea							
Intake (g)	36.7 ± 6.2	44.0 ± 9.0	78.6 ± 14.6[5,11]	54.0 ± 17.6	47.5 ± 16.6	0.02	NS
Length of feeding (min)	8.3 ± 0.9	8.7 ± 1.4	11.5 ± 1.7	9.9 ± 2.4	9.6 ± 1.6	NS	NS
Rate of intake (g/min)	4.2 ± 0.4	5.1 ± 0.7	6.8 ± 0.8[5]	5.0 ± 0.7	5.0 ± 1.1	0.009	NS
Distaste expressions (no./spoonful offered)	3.2 ± 0.4	2.5 ± 0.4	2.0 ± 0.5	2.7 ± 0.3	2.1 ± 0.4	NS	NS
Mothers' rating of liking[6]	4.4 ± 0.3	5.7 ± 0.5	6.4 ± 0.8[5]	4.6 ± 0.9	5.0 ± 0.7	NS	NS
Plain							
Intake (g)	46.6 ± 7.0	57.0 ± 11.8	87.7 ± 14.1[5]	47.6 ± 11.4	50.5 ± 16.0	0.02	NS
Length of feeding (min)	9.1 ± 0.8	10.4 ± 1.6	12.9 ± 1.1[5]	9.9 ± 2.5	9.0 ± 1.7	NS	NS
Rate of intake (g/min)	4.8 ± 0.5	5.2 ± 0.5	6.8 ± 0.9	5.2 ± 0.5	5.4 ± 0.9	NS	NS
Distaste expressions (no./spoonful offered)	2.4 ± 0.3	2.1 ± 0.3	2.1 ± 0.4	1.8 ± 0.3	1.6 ± 0.3	NS	NS
Mothers' rating of liking[6]	5.1 ± 0.4	6.2 ± 0.4	7.8 ± 0.3[5,7]	5.3 ± 0.7	5.0 ± 0.8	0.002	0.02[9]

[1] BM, breast milk group; MF, milk-based formula group; HCF, hydrolyzed casein formula group; NT, no table food group; T, table food group.
[2] *P* values obtained after one-factor ANOVA with milk/formula groups (BF, MF, or HCF) as the between-subjects factor for NT infants. If significant ($P < 0.05$), planned comparisons between HCF-NT and MF-NT and between HCF-NT and BM-NT were performed by using Bonferroni-corrected *t* tests.
[3] *P* values obtained after 2-factor ANOVA with milk/formula groups (MF or HCF) and table food groups (TF or NT) as the between-subjects factors for formula-fed infants only. If significant ($P < 0.05$), post hoc tests were conducted to calculate differences between groups.
[4] Mean ± SEM (all such values).
[5] Significantly different from BM-NT, $P < 0.05$.
[6] Values ranged from 1 to 9 (1 = did not like, 9 = liked very much).
[7] Significantly different from MF-NT, $P < 0.05$.
[8] Significantly different from HCF-NT, $P < 0.05$.
[9] Main effect of table food groups.
[10] Interaction effect of table food groups × milk/formula groups.
[11] Indicates a trend when compared with MF-NT ($P < 0.06$).
[12] Indicates a trend when compared with BM-NT ($P < 0.06$).
[13] Main effect of milk/formula groups.

Facial reactivity and intake

There were significant inverse relations between how much infants ate and the number of expressions of distaste displayed during the initial minutes of feeding with each of the cereals [sweet cereal: $r_{(84)} = -0.28$, $P = 0.009$; salty cereal: $r_{(79)} = -0.28$, $P = 0.015$; savory cereal: $r_{(84)} = -0.30$, $P = 0.006$; sour

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cereal: $r_{(83)} = -0.22$, $P = 0.047$; bitter cereal: $r_{(82)} = -0.26$, $P = 0.019$; and plain cereal: $r_{(84)} = -0.39$, $P = 0.001$]. The more a child ate, the fewer facial expressions of distaste the child displayed. A repeated-measures ANOVA was conducted by using intake data of the 6 cereals for all formula-fed infants with taste as the within-subjects factor and subgroup as the grouping factor. Taste was an important determinant of the facial reactivity response made during feeding [$F_{(5,350)} = 4.33$, $P < 0.0001$]. In general, babies made more faces of distaste while being fed the bitter cereal when compared with being fed the other cereals (all P values < 0.02).

DISCUSSION

The NT infants exhibited preferences for the taste qualities experienced in their formulas as we hypothesized. Infants fed HCF ate more of the cereals that tasted savory, sour, and bitter and at a faster rate than did MF and BM infants. That they liked the savory and bitter tastes more is suggested by the display of fewer facial expressions of distaste during feeding. In the HCF-NT infants, the savory cereal was ranked, on the basis of intake, as one of the more preferred tastes. They also ate more of the plain cereal than did the BM-NT infants, which may be a reflection of a greater appetite or liking for the weak bitter taste of distilled water (39). Because the majority of infants, regardless of milk-consumption history, had experience eating cereal prepared with ingredients other than water (*see* reference 40), it seems unlikely that the HCF-NT infants ate more of the plain cereal because it was novel to them.

Compared with milk-based formulas, and presumably BM (4), hydrolysate formulas have more pronounced savory, bitter, and sour tastes and stronger odors (11). Thus, infants who regularly are fed formulas that contain casein hydrolysate have more experiences with these taste and flavor qualities. The current results show that they also display their preferences for these flavors in a food matrix (eg, cereal), which is salient to them. However, in infants who have been introduced to the wide range of tastes and flavors in solid foods, their preferences for the basic tastes reflect the types of foods they are being fed. Those who were eating foods that tasted savory, salty, or bitter ate more of cereals containing those respective tastes.

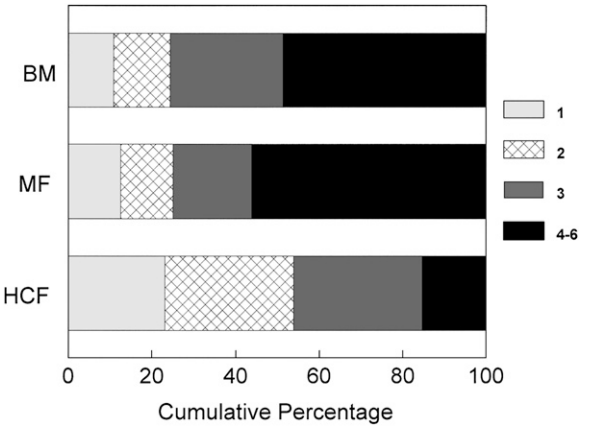

FIGURE 2. The cumulative percentage of infants who were not yet eating table foods (NT) but were consuming either breast milk (BM, top panel; $n = 37$), milk-based formula (MF, middle panel; $n = 16$), or hydrolyzed casein formula (HCF, bottom panel; $n = 13$) whose intake of the savory cereal was ranked first (most preferred), second, third, or higher. A higher percentage of the HCF-NT infants (85%) ranked, on the basis of intake, the savory-flavored cereal as first to third most preferred than did the BM-NT infants (49%; chi-square$_{(1)} = 4.43$, $P < 0.04$) or MF-NT infants (44%; chi-square$_{(1)} = 5.08$, $P < 0.03$).

The relation between feeding history and cereal intake during the taste preference tests was not evident in formula-fed infants who were tested after they had been fed table foods for several weeks to months. Once table foods were introduced, the acceptance of the different flavored cereals was similar in the MF and HCF groups. One explanation for the absence of a relation is that enhanced preference induced by early experience during formula feedings (due to flavor differences between MFs and HCFs) disappears after the introduction to table foods. However, this explanation is unlikely to be valid on the basis of our previously published observations. Children, ages 4 to 5 y, who were fed hydrolysate formulas during their infancy exhibited more positive responses to foods and beverages containing the sensory attributes associated with them (eg, sour taste, hydrolysate aroma, broccoli) several years after their last exposure to the formula (41, 42). Thus, an alternative and likely explanation for the absence of the relation is that that the taste variation in the cereals was too small to be attended to and to drive differential consumption for infants used to eating highly flavored table foods. As shown in Figure 1, the flavor intensity levels, as judged by adult observers, were weak for all added tastes and thus were likely to be considerably less intense than those flavor intensity levels with which infants exposed to table foods were familiar.

Because human milk contains more free glutamate than do milk-based formulas, we also hypothesized that BM infants would show elevated umami preferences. Although the BM infants did not consume more of the savory cereal, they exhibited more positive (eg, smiling) facial responses to that taste than did MF infants, an observation consistent with previous research (43). One explanation for this dissociation is that consummatory and hedonic responses are controlled by separate neural structures in the brain (*see* reference 44 for review). Another explanation is that these measures differ in their sensitivity and, consequently, measures of consumption were not sensitive enough to detect the breastfed infants' preference for the savory cereal.

It is important to acknowledge that although glutamate is the most abundant free amino acid in human milk (21, 45), there is a great deal of individual variation, and concentrations in milk (20, 21, 45) are just at or slightly below glutamate detection thresholds (46). However, nucleotides, which are present in higher amounts in human than in bovine milk (47), may act synergistically with glutamate to enhance its savory flavor. What causes variation in glutamate concentrations in human milk is unknown, although there is some evidence, albeit weak, that the variation is not due to the glutamate content of the maternal diet (48).

In the foregoing discussion, we assumed that differences in responses to tastes are caused by infants' differential feeding histories. But because infants in the present study were not randomly assigned to different feeding regimens, this conclusion can only be tentative. In one area, comparison of breastfed with formula-fed infants, we know that there may be many other differences. That we could not identify sufficient numbers of breastfed infants who were eating both table foods and infant cereal highlights the fact that these infants differ in more ways than the type of milk they consume. Not only are breastfed infants

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perceived differently by their mothers (49), but they are typically introduced to solid food at a later age (50, 51) than those who are fed formula. Mothers who breastfeed also exert less control during feeding than mothers who formula feed (52). Grouping breastfed infants on the basis of flavor experiences early in life also presents difficulties in interpretations, because there is a great degree of individual variation in the taste quality (eg, sweetness) of the breast milk (4) as well as flavor experiences due to the transmission of volatiles from a mother's diet to milk (3, 5, 6) and amniotic fluid (53). Consequently, even during very early development, there is a great deal of individual variation in the variety of taste and flavor compounds sampled by breastfed infants before table foods are introduced to their diets.

Whether the glutamate content in human milk serves to enhance the flavors of the transmitted volatiles from a mother's diet as it does for other foods (54–56) is an important area of future research. Also unknown is how early taste experiences during milk feedings modify acceptance of table foods. For the volatile flavor component of foods, we have shown that preferences for flavors will be displayed in other food matrixes, especially when the flavor resembles that experienced in mothers' milk (3). For infants fed hydrolysates, we would expect that the effect of their early taste experiences with formula will be magnified when they are offered table foods that taste savory, sour, or bitter and reflect the flavor profile of their cultures' cuisine. Examples of such foods include vegetable soup with rice flour, meat, and Parmesan cheese (high in umami flavor), which is fed to infants in parts of Italy (57), and miso soup in Japan.

The sensory world of infants is ever changing and dynamic and most evident as they begin the transition from an all-milk diet to one containing solid foods. Although much of the taste research on human infants has been devoted to the study of sweet and salty perception, less attention has been paid to the other basic tastes of sour, bitter, and umami. The present observations add to this body of research and reveal that early experiences in milk and solid foods affect infants' taste acceptance patterns and that infants communicate their acceptance by both intake and facial displays (5, 6, 58). An appreciation of the role of early experiences and complexity of early feeding and a greater understanding of the different ways infants communicate their liking of tastes and flavors will aid in the development of evidence-based strategies to facilitate healthy eating by children. (Other articles in this supplement to the Journal include references 59–87.)

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