Kaisu Keskitalo

A Matter of Taste

Genetic and Environmental Influences on Responses to Sweetness

Publications of the National Public Health Institute A 5/2008

Department of Molecular Medicine
National Public Health Institute, Helsinki, Finland
and
Department of Food Technology
Faculty of Agriculture and Forestry
University of Helsinki, Finland

Helsinki, Finland 2008

Kaisu Keskitalo

A MATTER OF TASTE

GENETIC AND ENVIRONMENTAL INFLUENCES ON RESPONSES TO SWEETNESS

ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Agriculture and Forestry, University of Helsinki, for public examination in Walter Hall, Viikki, on March 7th, 2008, at 12 noon.

Department of Molecular Medicine
National Public Health Institute, Helsinki, Finland
and
Department of Food Technology
Faculty of Agriculture and Forestry
University of Helsinki, Finland

Helsinki 2008

Publications of the National Public Health Institute
KTL A5 / 2008

Julkaisija-Utgivare-Publisher

Kansanterveyslaitos (KTL)
Mannerheimintie 166
00300 Helsinki
Puh. vaihde (09) 474 41, telefax (09) 4744 8408

Folkhälsoinstitutet
Mannerheimvägen 166
00300 Helsingfors
Tel. växel (09) 474 41, telefax (09) 4744 8408

National Public Health Institute
Mannerheimintie 166
00300 Helsinki, Finland
Telephone +358 9 474 41, telefax +358 9 4744 8408

ISBN 978-951-740-781-6
ISSN 0359-3584
ISBN 978-951-740-782-3 (pdf)
ISSN 1458-6290 (pdf)

Kannen kuva - cover graphic: Jussi Vuokko

Yliopistopaino
Helsinki 2008

Supervised by

Professor Hely Tuorila
University of Helsinki
Helsinki, Finland

and

Docent Markus Perola
National Public Health Institute
Helsinki, Finland

Reviewed by

Dr. Dennis Drayna
National Institutes of Health
Rockville, MD, USA

and

Professor Mika Kähönen
University of Tampere
Tampere, Finland

Opponent

Dr. Danielle R. Reed
Monell Chemical Senses Center
Philadelphia, PA, USA

”Tell me what you eat, and I will tell you what you are”

Anthelme Brillant-Savarin, The Physiology of Taste, 1825.

Kaisu Keskitalo, A matter of taste: genetic and environmental influences on responses to sweetness
Publications of the National Public Health Institute, A5/2008, 80 Pages
ISBN 978-951-740-781-6; 978-951-740-782-3 (pdf-version)
ISSN 0359-3584; 1458-6290 (pdf-version)
http://www.ktl.fi/portal/4043

ABSTRACT

Diet is a major factor in the maintenance of health and the onset of many diseases of public health importance. Choice of food composing the diet is known to be largely influenced by sensory preferences. However, in many cases, it is unclear whether these preferences and dietary behaviors are innate or acquired.

The aim of this work was to determine the extent to which individual differences in dietary responses, especially in liking for sweet taste, are influenced by genetic factors. Several traits measuring the responses to sweetness and eating behavior were examined in four populations: in British (TwinsUK) and Finnish (FinnTwin12 and FinnTwin16) twin cohorts and in a Finnish migraine family study. All subjects were adults and they participated in chemosensory measurements and filled in food behavior questionnaires. Further, it was evaluated, whether the correlations among variables are mediated by genetic or environmental factors and where in the genome the genes influencing heritable traits are located.

A study of young adult Finnish twins (FinnTwin16, n=4388) revealed that around 40% of food use is attributable to genetic factors and that the common childhood environment does not affect food use even briefly after moving out from the parental home. Both the family study (n=146) and the twin studies (British twins, n=663) showed that around half of the variation in the liking for sweetness is inherited. The same result was obtained by both the chemosensory measurements (heritability 41-49%) and questionnaire variables (heritability 31-54%). By contrast, the intensity perception of sweetness or the responses to saltiness were uninfluenced by genetic factors. Further, a locus influencing the use-frequency of sweet foods was identified on chromosome 16p. A closer examination of the relationships among the variables based on 663 British twins revealed that several genetic and environmental correlations exist among the different measures of liking for sweetness. However, these correlations were not very strong (range 0.06-0.55), implying that the instruments used measure slightly different aspects of the phenomenon. In addition, the assessment of the associations among responses to fatty foods, dieting behaviors, and body mass index in twin populations (TwinsUK n=1027 and FinnTwin12 n=299) showed that dieting behaviors (cognitive restraint, uncontrolled eating, and emotional eating) mediate the relationship between obesity and diet.

In conclusion, this work contributed to the understanding of the factors underlying human eating behavior. Genetic effects were shown to underlie the variation of many dietary traits, such as liking for sweet taste, use of sweet foods, and dieting behaviors. However, responses to salty taste were revealed to be mainly determined by environmental factors and thus should more easily be modifiable by dietary education, exposure, and learning than sweet taste preferences. Although additional studies are needed to characterize the genetic element located on chromosome 16 that influences the use-frequency of sweet foods, these results underline the importance of inherited factors on human eating behavior.

Keywords: sweet taste, eating behavior, genetics, twins, family study, heritability

Kaisu Keskitalo, Makuasioista ei voi kiistellä: geneettisten ja ympäristötekijöiden vaikutus makean maun kokemiseen
Kansanterveyslaitoksen julkaisuja, A5/2008, 80 sivua
ISBN 978-951-740-781-6; 978-951-740-782-3 (pdf-versio)
ISSN 0359-3584; 1458-6290 (pdf-versio)
http://www.ktl.fi/portal/4043

TIIVISTELMÄ

Ruokavalio on tärkeä terveyteen ja monien kansanterveyden kannalta tärkeiden sairauksien puhkeamiseen vaikuttava tekijä. Ruokavalinnat määräytyvät pitkälti makumieltymysten perusteella. Kuitenkin monissa tapauksissa on epäselvää, ovatko makumieltymykset ja syömiskäyttäytymismallit synnynnäisiä vai opittuja.

Tämän tutkimuksen tavoitteena oli selvittää, kuinka suurelta osin makean mieltymykset ja muut ruokaorientaatiota kuvaavat tekijät periytyvät. Useita makean maun kokemista ja ruokakäyttäytymistä mittaavia testejä käytettiin neljässä tutkimuksessa: suomalaisessa migreeniperhetutkimuksessa sekä brittiläisessä (TwinsUK) ja suomalaisissa (FinnTwin12 ja FinnTwin16) kaksostutkimuksissa. Tutkimusten koehenkilöt olivat aikuisia ja he sekä osallistuivat aistitutkimuksiin että täyttivät syömiskäyttäytymiskyselyitä. Jatkoanalyyseissa tutkittiin, johtuivatko mittareiden väliset korrelaatiot geneettisistä vai ympäristötekijöistä sekä pyrittiin paikantamaan periytyviin ominaisuuksiin vaikuttavia tekijöitä genomissa.

Suomalaisia nuoria aikuisia (FinnTwin16, n=4388) käsitellyt tutkimus osoitti, että n. 40 % eroista ruokien käytössä selittyi perinnöllisillä tekijöillä ja että lapsuuden perheympäristö ei vaikuta ruokien käyttöön tilanteessa, jossa suurin osa koehenkilöistä oli vastikään muuttanut pois vanhempiensa luota. Sekä perhetutkimus (n=146) että kaksostutkimukset (brittikaksoset, n=663) osoittivat, että noin puolet eroista makean mieltymyksissä johtui geneettisistä tekijöistä. Sama tulos saatiin sekä aistitestauksilla että kyselylomakkeilla. Sen sijaan suolaisen maun aistimukset ja mieltymykset eivät olleet periytyviä. Lisäksi saatiin selville, että kromosomissa 16 sijaitsee geneettinen tekijä, joka vaikuttaa makeiden ruokien käytön useuteen. Muuttujien yhteyksien tarkempi tarkastelu 663 brittikaksosen aineistossa osoitti, että samat geneettiset ja ympäristötekijät vaikuttavat eri makean mittareilla saatuihin tuloksiin. Nämä korrelaatiot eivät olleet kovin suuria (vaihteluväli 0.06-0.55), joten eri makean mieltymysten testit mittaavat ilmiötä hieman eri näkökulmasta. Kun edelleen tarkasteltiin rasvaisten ruokien arvioiden, ruokakäyttäytymisen ja painoindeksin välisiä yhteyksiä kaksospopulaatiossa (TwinsUK n=1027 ja FinnTwin12 n=299) havaittiin, että yhteys ruokavalion ja lihavuuden välillä kulkee ruokakäyttäytymismallien (syömisen rajoittaminen, kontrolloimaton syöminen, tunnesyöminen) kautta.

Yhteenvetona voidaan todeta, että työ lisäsi tietämystä ihmisten syömiskäyttäytymisen taustasta. Perimällä osoitettiin olevan suuri vaikutus moniin ravitsemuksellisiin tekijöihin, kuten makean mieltymyksin, makeiden ruokien käyttöön ja ruokakäyttäytymiseen. Toisaalta mieltymys suolaiseen makuun osoitettiin olevan pääosin ympäristön määräämää ja suolamieltymysten tulisikin siis olla muokattavissa ravitsemuskoulutuksen, altistuksen ja oppimisen avulla helpommin kuin makean mieltymysten. Kromosomissa 16 sijaitsevan geneettisen tekijän havaittiin vaikuttavan makeiden elintarvikkeiden käyttöön. Vaikka lisätutkimuksia tarvitaan, jotta vaikuttava geeni ja sen yhteydet muihin ominaisuuksiin saadaan selville, tutkimuksen tulokset viittaavat siihen, että periytyvillä tekijöillä on merkitystä ihmisten ruokakäyttäytymiseen.

Asiasanat: makeus, ruokakäyttäytyminen, genetiikka, kaksoset, perhetutkimus, perinnöllisyys

CONTENTS

ABBREVIATIONS

A	additive genetic effects
a^2	proportion of variance explained by additive genetic effects
BMI	body mass index
bp	base pair
C	common (shared) environmental effects
c^2	proportion of variance explained by common environmental influences
cM	centiMorgan
D	genetic dominance effects
d^2	proportion of variance explained by genetic dominance effects
DNA	deoxyribonucleic acid
DZ	dizygotic
e^2	proportion of variance explained by specific environmental influences
FFQ	food-frequency questionnaire
GWA	genome-wide association
GPCR	G-protein-coupled receptor
h^2	heritability (narrow sense)
HRR	haplotype relative risk
HTAS	Health and Taste Attitude Scales
JAR	just-about-right scale
LAM	labeled affective magnitude scale
LMS	labeled magnitude scale
LD	linkage disequilibrium
LOD	logarithm of odds
M	Morgan

MZ	monozygotic
NaCl	sodium chloride
PCR	polymerase chain reaction
PROP	6-*n*-propylthiouracil
PTC	phenylthiocarbamide
QTL	quantitative trait locus
r	Pearson correlation coefficient
r_{DZ}	dizygotic within-pair correlation
r_{MZ}	monozygotic within-pair correlation
RFLP	restriction fragment length polymorphism
SNP	single nucleotide polymorphism
TAS1R1	taste receptor family 1, member 1
TAS1R2	taste receptor family 1, member 2
TAS1R3	taste receptor family 1, member 3
TAS2R38	taste receptor family 2, member 38
TDT	transmission/disequilibrium test
TFEQ	Three-Factor Eating Questionnaire
Var_{MZ}	monozygotic within-pair variance of the mean difference
Var_{DZ}	dizygotic within-pair variance of the mean difference
Var(E)	environmental variance
Var(G)	genetic variance
Var(P)	phenotypic (total) variance

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals (I-IV):

I Keskitalo K, Silventoinen K, Tuorila H, Perola M, Pietiläinen KH, Rissanen A, Kaprio J. Genetic and environmental contributions to food use patterns of young adult twins. Physiology & Behavior 2008;93:235-42.

II Keskitalo K, Knaapila A, Kallela M, Palotie A, Wessman M, Sammalisto S, Peltonen L, Tuorila H, Perola M. Sweet taste preferences are partly genetically determined: identification of a trait locus on chromosome 16. American Journal of Clinical Nutrition 2007;86:55-63.

III Keskitalo K, Tuorila H, Spector TD, Cherkas LF, Knaapila A, Silventoinen K, Perola M. Same genetic components underlie different measures of sweet taste preference. American Journal of Clinical Nutrition 2007;86:1663-9.

IV Keskitalo K, Tuorila H, Spector TD, Cherkas LF, Knaapila A, Kaprio J, Silventoinen K, Perola M. Three-factor eating questionnaire, body mass index, and responses to sweet and salty fatty foods: a twin study of genetic and environmental associations. Submitted.

In addition, some unpublished material is included.

1 INTRODUCTION

Humans have an innate preference for sweet taste (Desor et al., 1973). Naturally sweet foods, such as fruits and berries, are good and safe sources of nutrients. Assumingly, this has resulted in an evolutionary development to prefer them (Rozin & Vollmecke, 1986), and these sweet foods still form an important part of a healthy diet. However, many sweet foods available today also have adverse health effects. Consumption of foods with refined sugar may lead to intake of extra calories and increased risk of dental caries. Thus, dietary guidelines generally discourage the consumption of added sugar (e.g. Dietary Guidelines for Americans, 2005; Finnish Nutrition Recommendations, 2005).

Though sweet taste is universally liked, great differences between individuals exist in the degree of liking for sweet foods (Pangborn, 1981). These individual differences have both environmental (e.g. experience, attitudes) and genetic (e.g. genetic liability to like sweet taste) determinants. The genetic predisposition to like sweetness at birth is modified by experience with sweet taste already during the first six months of life (Beauchamp & Moran, 1982). Taste preferences, in general, are a major determinant of food choices in modern societies (Drewnowski, 1997a). Food choice, in turn, has an effect on the development of many diseases of public health importance, such as obesity, diabetes, and cardiovascular diseases. In children, sweetness and familiarity are often the main determinants of food preferences (Benton, 2004). However, adults' food choices are also affected by attitudinal, cultural, situational, physiological, and economic factors.

Genetic studies on taste preferences have, until recently, mostly concentrated on bitter taste perception and on the effect of the bitter taste receptor gene TAS2R38 on various phenotypes. The genes encoding the two subunits of the human sweet taste receptor, first discovered in mice, are located on human chromosome 1p36. The dimer of the two subunits, taste receptor family 1, members 2 and 3 (TAS1R2 & TAS1R3) reacts with a range of different sweet tastants, from sugars to proteins. The effect of polymorphisms in the *Tas1r3* gene on the sweetness perception of mice has been investigated widely. In humans, no studies evaluating the effect of the variations in these genes on sweetness perception have been carried out.

Degree of liking for sweetness can be measured by several methods, divided into taste tests (chemosensory measurements) and questionnaire instruments. Chemosensory methods usually include rating of the intensity of and liking for sweet samples or ranking samples with varying sweetness levels. Aqueous solutions most often serve as the stimulus, but sweet foods can also be used. Questionnaire instruments measure the use-frequency of, liking for, or attitudes towards sweet foods. The intake of sugar, reflecting the amount of sweet foods consumed, can be obtained using food intake diaries or other dietary research methods. All of these

measures give slightly different estimates of sweet taste preference, but the outcomes are often correlated (Mattes & Mela, 1986).

The following literature review discusses the determinants and health consequences of a preference for sweetness. In addition, methods to measure hedonic responses to sweetness and to investigate quantitative genetic traits are described. The four original publications focus on the genetic background of sweet taste perception and the associations between dietary and chemosensory variables.

2 REVIEW OF THE LITERATURE

2.1 Mechanisms for perceiving sweet taste

2.1.1 Anatomy of tasting: taste buds and papillae

Human can perceive five tastes: sweet, sour, bitter, salty, and umami (or savory). Taste perception begins when a tastant encounters the taste receptor cells in the mouth. The taste receptor cells are located in taste buds (**Figure 1C**), each bud containing 50-150 taste receptors.

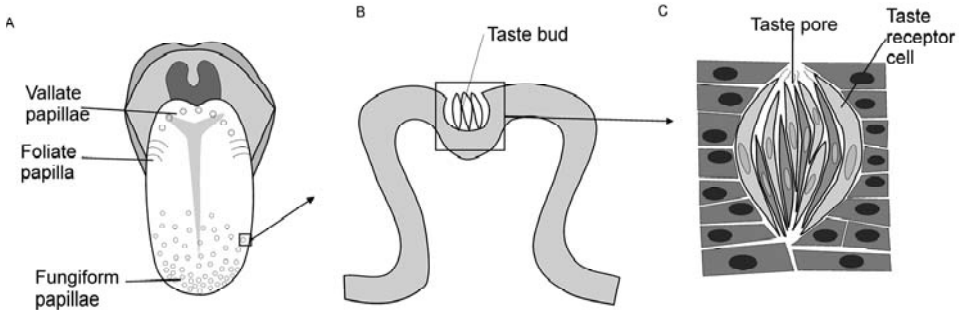


Figure 1. ***Location of papillae on the tongue (A), a fungiform papilla (B), and a taste bud (C). The tastant encounters the taste receptor cells through the taste pore (modified from Chandrashekar et al., 2006).***

Taste buds are located in the gustatory taste papillae of the tongue. There are three types of gustatory papillae: vallate, foliate, and fungiform papillae (Witt et al., 2003). The locations of different types of gustatory papillae on the tongue are presented in **Figure 1A**. Contrary to common belief, all areas of the tongue are responsive to all five tastes (e.g. Chandrashekar et al., 2006).

Vallate papillae, also known as circumvallate papillae, are located at the back of the tongue, forming a V-shaped line across the root of the tongue. They are round and 2-8 mm in diameter. The human tongue has, on average, nine vallate papillae (range 4-18), each containing over 200 taste buds. Foliate papillae lie along the sides of the tongue, next to the lower molar teeth, and consist of ridges and valleys. On average, they contain a total of more than 1300 taste buds per tongue. The foliate papillae are hard to see, as they flatten out when the tongue is extruded.

Fungiform papillae, by contrast, can be seen as pink elevations of ~0.5 mm in diameter at the front of the tongue. The distribution of fungiform papillae differs

markedly among individuals, as does the number of taste buds in them. Fungiform papillae density has been shown to be related to the perceived intensity (Miller & Reedy, 1990) and liking (Yeomans et al., 2007) of sweetness.

Taste buds can also be detected "extralingually" in the oral, pharyngeal, and laryngeal cavities. It is not clear whether these extralingual taste buds are sensitive to tastes. The taste buds of the epiglottis and/or uvula, for example, do not directly function in taste perception, but may be involved in the pharyngolaryngeal water response. In addition to gustatory papillae, two types of papillae, filiform and conical are nongustatory, meaning that they do not contain taste buds. These cone-shaped papillae are located on the dorsal surface of the tongue (Witt et al., 2003).

Differences exist between individuals in taste sensitivity. However, ageusia, a disorder of complete inability to taste, is very rare, presumably because taste information is carried by three different nerves. A few cases of aglycogeusia (inability to taste the sweetness of sucrose) have been reported. Two cases described by Henkin and Shallenberger (1970) both had congenital idiopathic hypoparathyroidism and low in calcium concentrations. The patients had normal detection and recognition thresholds for sour, bitter, salty, and sweet tastes, but neither of the patients could recognize sucrose solutions as sweet. From the sweet substances tasted, fructose and sucrose were perceived as sour, xylose as salty, and glucose, galactose, and cyclamate as bitter. Based on the follow-up of the patients, Henkin and Shallenberger suggested that the aglycogeusia was associated with hypoparathyroidism.

2.1.2 Sweet taste receptors and signal transduction

The five different taste modalities are recognized either by G-protein-coupled receptor (GPCR) dimers or through membrane channels. There are two taste receptor GPCR families. Family 1 consists of only three members, which mediate the perception of sweet and umami tastes. Family 2 is large, with around 30 different GPCRs, and the dimers of these make up the receptors for different bitter-tasting compounds. Salty and sour tastes have been suggested to be perceived by direct entry of Na+ and H+ through specialized membrane channels. However, the mechanisms of sour taste perception are not fully known (Chandrashekar et al., 2006).

Sweet taste is mediated by two class C GPCRs, members 2 and 3 of taste receptor family 1 (TAS1R2/TAS1R3). Members 1 and 3 together form the umami receptor, responding to amino acids and nucleotides (Chandrashekar et al., 2006). Human family 1 taste receptor genes 1, 2, and 3 are all located within a small region on chromosome 1p36 (Liao & Schultz, 2003).

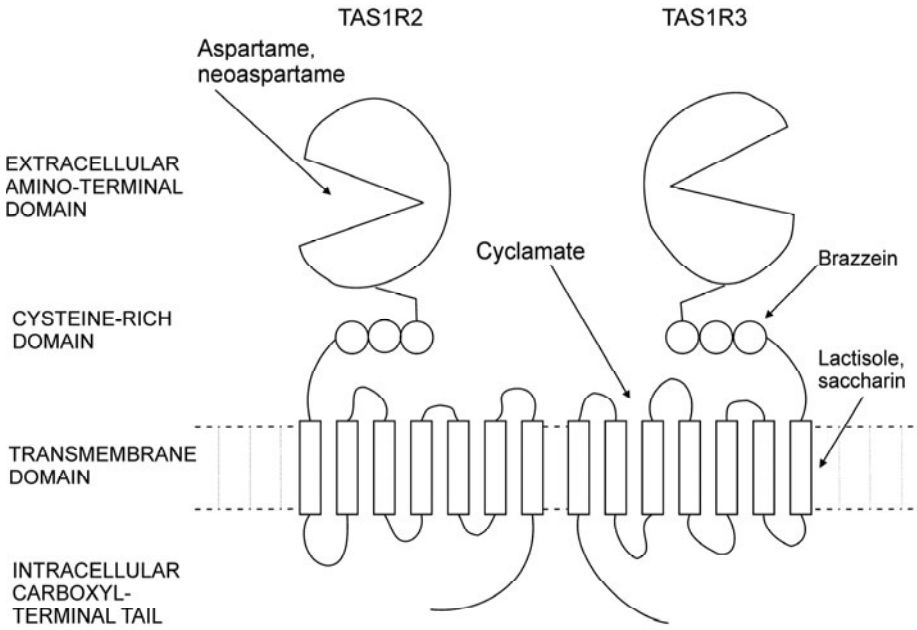


Figure 2. ***Schematic representation of a sweet taste receptor and the binding sites of different artificial sweet-tasting compounds (modified from Naim et al., 2006, and Roper, 2007).***

The heterodimeric combination of two proteins, TAS1R2 and TAS1R3, is needed to form a functional sweet taste receptor. This receptor complex responds to all kinds of sweet tastants, including sugars, artificial sweeteners (sucralose, saccharin), amino acids, alcohols (sorbitol, xylitol), peptides (aspartame), and proteins (brazzein, thaumatin), as well as to sweetness inhibitors (lactisole). The attempt to find common features among sweet-tasting compounds has produced the concept of "glycophore", which consists of an electronegative atom (A) that forms a hydrogen bond (H) with another electronegative atom (B). AH-B is found in all sweet compounds.

TAS1R2 and TAS1R3 have different functional roles in ligand recognition, and multiple ligand binding pockets are present on sweet taste receptors (Xu et al., 2004; Roper, 2007), as demonstrated in **Figure 2**. The large extracellular domain of the sweet taste receptor (TAS1R2/TAS1R3) has a so-called Venus flytrap motif. Binding sites for sugars have been found in both TAS1R2 and TAS1R3 extracellular amino-termini.

Signal transduction of sweet taste comprises interaction of the ligand (sweet tastant) with the GPCR and downstream intracellular second-messenger cascades. The taste

stimulation of GPRC initiates two cascades, a PLCβ2-signaling stream, in which the release of calcium opens a nonselective monovalent cation channel TRPM5, and a less understood stream in which cAMP (cyclic adenosine monophosphate) stimulates PKA (protein kinase A). After the signal transduction, the signal processing of the sweet taste includes secretion of the neurotransmitter ATP (Roper, 2007).

Two cranial nerves transmit the signals of the taste perception from the tongue to the brain. Chorda tympani, a branch of the facial nerve (cranial nerve VII), innervates the anterior two-thirds of the tongue, and the glossopharyngeal nerve (cranial nerve IX), the posterior one-third of the tongue. In addition, the vagus nerve (cranial nerve X) carries sensations from the back of the oral cavity. The taste information is first carried into the nucleus of the solitary tract in the brain stem. From there, the second-order taste neurons project it to the thalamic nucleus, and from the thalamic taste area (ventroposteromedial thalamic nucleus) to the primary taste cortex (frontal operculum/insula). This area also receives input from vision, olfaction, and touch (Rolls & Scott, 2003).

2.2 Determinants of liking for sweet taste

Sweet taste is generally liked by humans from birth (see Section 2.2.1). Naturally sweet foods, like fruits and berries, are good and safe sources of energy and nutrients (Rozin & Vollmecke, 1986). Thus, adaptive evolutionary development has preferred their consumption. Although sweet taste is generally enjoyed, differences exist among individuals in preference for and use of sweet foods (Pangborn, 1981; Conner et al., 1988). The major influences on sweet taste preferences and sweetness perception are presented in **Figure 3** and reviewed in the following sections. Despite interactions among the factors, each is discussed separately. Other factors, such as socioeconomic status or psychological factors (e.g. depression: Scinska et al., 2004) may also influence the degree of liking for sweetness, but no sound scientific evidence of the contribution of these factors is available.

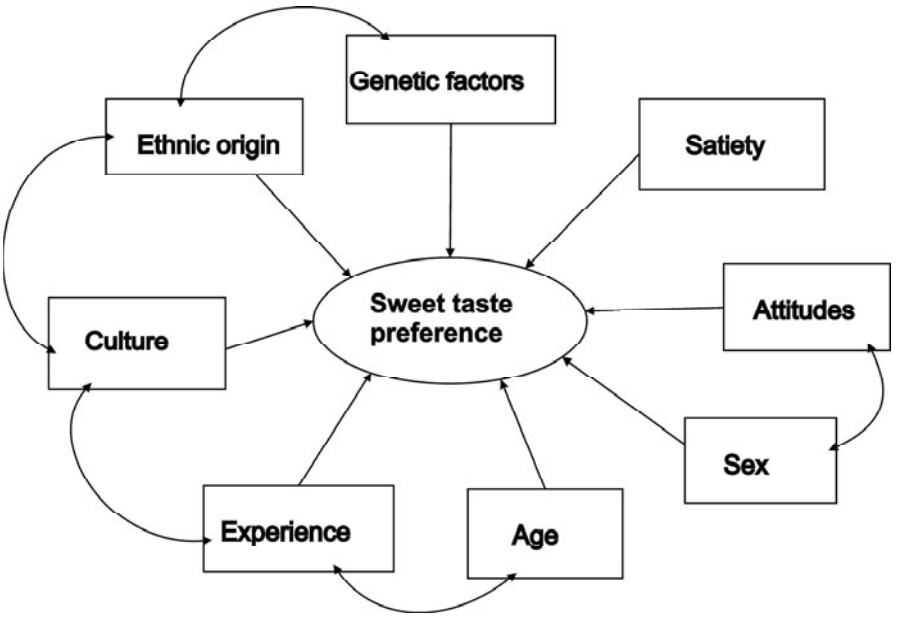


Figure 3. ***Major determinants of sweet taste preference.***

2.2.1 Age

Both ingestion and facial expression studies have shown that human newborns like sweet taste. The former method was used by Desor et al. (1973a), who offered 1- to 3-day-old infants (n=192) two bottles: a water bottle and a sugar solution bottle containing glucose, fructose, lactose, or sucrose. The infants ingested more sugar solutions than water, and the sweeter sugars, fructose and sucrose, were more highly preferred. These results were interpreted as evidence for a preference for sweet taste. The observation of facial expressions of newborn infants after inserting 0.2-0.5 ml of taste solution into the mouth confirms these findings (Steiner, 1977). Sweet stimuli are associated with lip licking, rhythmic sucking, and relaxed face/smiling, indicating pleasant perception (reviewed by Ganchrow & Mennella, 2003).

The strong preference for sweetness diminishes with age. Comparison of the taste preferences of 9- to 15-year-olds (n=618) with those of adults (n=140) showed that younger subjects preferred higher sweetness (sucrose and lactose) concentrations than adults (Desor et al., 1975). The trend of diminishing sweet taste preference with age was confirmed in a longitudinal study of 44 subjects whose sucrose preferences were measured twice: first at adolescence (11-15 years) and then in young adulthood (19-25 years). The concentration selected as most preferred decreased during the 9-

to 10-year period significantly, and no sex or race differences were observed (Desor & Beauchamp, 1987).

de Graaf & Zandstra (1999) compared the sweetness intensity and pleasantness ratings of a sucrose and orange lemonade sample among three age groups: children (9-10 years), adolescents (14-16 years), and adults (20-25 years). The optimal sucrose level and sensitivity for sweetness were shown to be lower in the older age groups, and the age effects were similar for the water and lemonade samples. In another study, Zandstra and de Graaf (1998) compared six age groups: children (6-12 years), adolescents (13-18 years), young adults (19-34 years), adults (35-49 years), older adults (50-65 years), and elderly (>65 years). The subjects rated the sweetness, sourness, and orange flavor intensity and the pleasantness of orange beverage samples with varying sucrose, citric acid, and orange flavor concentrations. In general, children and the elderly preferred higher sucrose concentrations than young adults. In addition, children and the elderly were less able to discriminate between sucrose concentrations than young adults (i.e. had a lower slope for psychophysical function). Although the results imply that the perception of sweetness is altered in the elderly, earlier studies have demonstrated that the ability to perceive sweetness does not decrease as much as the ability to perceive odors (Murphy, 1993) or bitter tastes (Gilmore & Murphy, 1989). In fact, Koskinen et al. (2003) found that elderly subjects (65-82 years) identified on average more sucrose solutions (ranging from 0.1% to 3.4%) correctly as sweet than young subjects (20-35 years), whereas for the other tastes tested (salty, sour, and bitter) the trend was the opposite.

2.2.2 Sex

Male and female infants do not differ in the degree of liking for sweet solutions (Desor et al., 1973; Beauchamp & Moran, 1984). However, in adults, males show a higher preference for sweetness than females. Conner and Booth (1988) measured the sweet taste preferences of 121 males and 223 females aged 6-65 years by determining their ideal concentration of sugar in a lime drink and by asking about their preferences for sweet versus nonsweet foods. Males expressed a liking for higher sugar content in the lime drink than females. In addition, males showed a greater tendency to add sugar to tea and coffee, interpreted by the authors as reflecting "less concern in males about excessive sugar consumption". The attitudinal differences, which will be discussed more detailed in the following section (Section 2.2.3), may thus underlie the sex differences.

Sex hormones may also influence sweet taste perception and the preferences or cravings for sweet foods. The recognition threshold of sucrose has been shown to be influenced by menstrual cycle: females have lowered thresholds (higher sensitivity) for sucrose before ovulation (Than et al., 1994). Bowen and Grunberg (1990) reported higher sweet food preferences and consumption during the premenstrual period. However, in their study, each of the 39 women participating was only tested

once during the menstrual cycle. Frye et al. (1994) examined male (n=12) and female (n=25) subjects who rated the pleasantness, sweetness, and fatness of 16 dairy products with varying fat and sucrose contents. The ratings were done over four consecutive weeks. Males, more than females, liked the sweetest samples. The preference ratings declined significantly over the four weeks of testing, but women who started the testing during premenstrual or menstrual weeks had increased preference ratings for the samples during this period. In addition, high degree of dietary restraint was associated with lower preference ratings for the less sweet samples in women.

2.2.3 Attitudes

In humans, attitudes towards food may also affect consumption. The relationship between attitudes towards sugar and the liking for sweet drinks was assessed by Tuorila-Ollikainen and Mahlamäki-Kultanen (1985) in a sample of 112 male and 112 female young adults. The participants rated the pleasantness of sweetness in drink samples with two sweetness levels and their sugar attitudes were measured by 12 statements. In general, females had more negative attitudes towards sugar, but reported a greater liking for sweet foods than males. The sugar attitudes were unrelated to pleasantness ratings of the higher sweetness level corresponding to the sweetness level generally found in commercial products. However, a positive correlation between the negative sugar attitudes and pleasantness ratings of the drink with a lower sucrose concentration was observed. The authors suggested that this was due to the "activation of an attitude"; the normal sweetness level was not a proper stimulus to activate cognitive processing, whereas an unusually low sweetness activated the attitudinal background.

Grogan et al. (1997) further examined the sex differences in the consumption of and attitudes towards sweet snacks in 65 women and 64 men aged 18-40 years by behavioral questionnaires. In line with the results of Tuorila-Ollikainen and Mahlamäki-Kultanen, females reported sweet snacks to be significantly less healthy, especially due to the believed weight gain, and more pleasant than did males. The males reported an intention to eat sweet snacks more often than females, but no sex differences in use-frequency of 16 sweet snacks were observed. Intended and actual use correlated significantly, although the intended frequency of consumption was significantly lower than the actual use-frequency. Both attitude dimensions, pleasantness and healthiness, correlated significantly with the intended use-frequency of sweet snacks.

In a study of 136 women, both liking and use of sweet snacks was related to attitudes towards the social and rewarding properties of sweetness (Lähteenmäki & Tuorila, 1994). However, the liking and use of soft drinks were also influenced by attitudes towards healthiness and restriction of eating. Based on the results of these

studies, it appears that the effect of attitudes and social pressure on sweetness preferences is complicated and clearly influenced by sex.

2.2.4 Experience

The innate preference for sweetness is modified already during the first months of life according to the infant's experiences with sweet foods. Beauchamp and Moran (1982) studied the infants' preferences for sucrose solutions and water after birth (n=199) and re-tested them at 6 months of age (n=140). The amount ingested was compared with infants' consumption of sweet foods and drinks obtained from 7-day diet records kept by their mothers. At 6 months of age, infants fed sugar-containing water during the 7-day diet history maintained the same level of preference for sucrose solution relative to water as they had at birth. By contrast, infants not fed sugar-containing water diminished the intake of sucrose compared with plain water during the 6 months.

Further, 63 of these children were tested again at 2 years of age (Beauchamp & Moran, 1984). Similar to previous results, children fed sugar-containing water during infancy ingested more sugar solutions than children never fed sugar-containing water. The number of months that sugar-containing water had been fed to an infant had no effect on the ingestion volume of the sugar solutions.

Experience and repeated exposure do have an effect on the sweetness preference, but this effect may be very food-specific. The 2-year-old children participating in the Beauchamp and Moran (1984) study were offered fruit-flavored drink (Kool-Aid). The ingestion volume of sweetened or unsweetened Kool-Aid was unrelated to sugar water feeding, but the effect of prior experience with Kool-Aid was significant; children who had never tried Kool-Aid consumed less of it, regardless of its sweetness level. Liem and Mennella (2002) also investigated the effects of food experiences on 4- to 7-year-old children's sweet taste preferences. The level of sweetness preferred in a juice was related to the sugar content of the child's favorite cereal and to the routine of adding sugar to the child's food, but not to the type of formula fed during infancy.

The effect of dietary experience on sweetness preferences of adults has not been studied extensively. Tuorila-Ollikainen and Mahlamäki-Kultanen (1985) examined the effect of experience on pleasantness ratings of soft drinks in young adults. Present and childhood use-frequency of and liking for soft drinks were related to pleasantness ratings of the sample soft drinks. However, the liking for or use-frequency of other sweet foods (candies, delicacies, pastry) did not correlate significantly with hedonic responses to the soft drink samples.

2.2.5 Culture and ethnicity

Differences in preference for sweetness among cultures and races/ethnic groups have been investigated by several studies. Some differences between races have emerged: African Americans have higher (Schiffman et al., 2000) and Pima Indians lower (Salbe et al., 2004) sweetness preference than European Americans. It is noteworthy that greater liking for sweetness does not inevitably lead to higher use of sucrose; in the study of Bertino and Chan (1986), Chinese subjects showed higher preferences for sweetness in water and in cookies than European Americans, but no differences in sugar intake were present. Often, racial differences are regarded as evidence of genetic effects on phenotype. However, the human genetic variations are structured by geographic origin rather than race (Jorde & Wooding, 2004). As racial groups often are not defined according to shared genetic variations (but by other, historical, criteria) and each individual's genome may have multiple historical sources, grouping by race frequently does not predict genetic similarities/differences between the groups (Foster & Sharp, 2004).

Differences in sweetness preferences between populations may also be due to environmental differences. These environmental factors include cultural differences, which, in turn, are related to experienced sweetness levels of food items. Food specificity of sweet taste preferences has been observed in many studies comparing two cultures. A comparison between Taiwanese and European American students revealed that while Taiwanese rated high sweetness in a water solution as more pleasant than Americans, the trend was the opposite with cookies (Bertino et al., 1983).

Studies of Australian and Japanese subjects highlight the importance of familiarity and experience with the product on the rating of its sensory properties, including pleasantness (Prescott, 1998); although no differences in hedonic responses to sucrose solutions or sweetness intensity ratings of foods were observed, the liking for sweetness in foods was highly product-specific. A similar observation was made by Holt et al. (2000) when they examined the difference between Australian and Malaysian students for sweetness preference in water, orange juice, custard, and biscuits, each with four different sucrose levels. Increasing sucrose levels provided different response patterns for each product. Differences between Australian and Malaysians were apparent, but very food-specific.

Differences between cultural groups may be also related to the lifestyle and urbanization. Studies on the effect of urbanization on sweet taste preferences have been conducted. Steiner et al. (1984) examined urban and rural adolescents among Bedouin citizens in Israel and Jamel et al. (1996) in children, adolescents, and young adults in Iraq. Both studies showed that individuals from the urban population have a greater preference for sweetness.

2.2.6 Satiety

The effect of satiety state on perception of and liking for sweetness is unclear. Zverev (2004) investigated the recognition thresholds of salty, bitter, and sweet tastes in 16 students after 14-16 h of fasting and 1 h after a meal. He found that the sensitivity for sucrose and salt was increased after fasting (mean 0.79% and 0.63%, respectively) compared with in the sated state (mean 1.36% and 1.12%, respectively). However, Pasquet et al. (2006) observed no such effect. They determined the recognition thresholds of sucrose, fructose, sodium chloride, quinine sulphate, PORP, and liquorice with 24 students. No significant difference in mean thresholds was noted between overnight fasting (for sucrose 1.42% and for NaCl 0.13%) and 1 h after a standard meal (for sucrose 1.37% and for NaCl 0.11%). The difference between the results may be due to differences in the length of fasting or in the method of recognition threshold determination. Zverev applied a two-alternative forced-choice method in which eight pairs of water and sucrose solution were presented in random order (concentration recognized twice correctly regarded as threshold), whereas Pasquet et al. used the staircase method in which the solutions were presented twice in an ascending order of concentration (threshold calculated as mean of the lowest recognized concentrations). In addition, the sample size in both studies was small.

The relationship between hedonic responses to sweetness and hunger was examined by Pangborn (1959). She conducted a large (n=11 456) consumer study on preferences for peaches with varying sweetness and acidity levels and compared these ratings with self-rated hunger. The phenomenon was further examined in a laboratory study of eight students in which preferences for apricot nectars varying in sweetness were determined at two satiety states; in the afternoon 1) after having a light lunch (not fasting) during the day and 2) after having only breakfast (fasting). In both studies, the hunger/fasting had only a minimal effect on preference ratings of the products.

The phenomenon was also investigated by Laeng et al. (1994). The 29 male and 28 female subjects were divided into sated and hungry groups based on whether they had had a meal within 2 h of the test. They were requested to rate the intensity and pleasantness of four lime drinks with varying sweetness. Contrary to the results of Pangborn, the hungry group gave higher pleasantness ratings to the drinks than the sated group. However, when the samples of males and females were separated, the effect remained significant only for females. The difference from the results of Pangborn may be due to rating of the degree of pleasantness of the samples instead of the preference between two samples. In addition, classification of subjects to hungry and sated groups was not done using similar criteria.

No studies on the effect of longer, controlled fasting on sweet taste preferences have been conducted. Biologically, however, it appears logical that in a state of hunger the hedonic response to foods with easily available energy, i.e. foods containing

sugars, would be elevated. However, a comparison of the effect of a food-based low-calorie diet and a supplement-based very-low-calorie diet on food cravings implied that the cravings diminish with caloric restriction (Martin et al., 2006). After 12 weeks of dieting, the craving for sweets had decreased in both groups, but the decrease in the very-low-calorie group was significantly greater than in the low-calorie group.

2.2.7 Genetic factors

The contribution of genetic effects to sweet taste preference measured by chemosensory testing had not been determined prior to our studies. However, phenotypes that reflect the degree of sweet taste preference have been used in some twin studies. de Castro (1998) reported a heritability of 42% for candy consumption and 48% for ice cream consumption using 7-day food intake diaries. Hur et al. (1998) found a heritability of 24% for intake of simple carbohydrates. Innate taste preferences are strong evidence of genetic liability for sweet taste preference. However, the genetic elements and the biology underlying the preference remain unclear. Studies on the genetic background of sweet taste perception and preference are reviewed herein.

2.2.7.1 Variations in sweet taste receptor genes

The effect of variations in sweet taste receptor genes *TAS1R2* and *TAS1R3* to sweetness perception in humans has not yet been studied. However, the first step towards estimating these variations has been taken; the occurrence of single nucleotide polymorphisms (SNPs) in human taste receptor family 1 genes was recently evaluated (Kim et al., 2006). Sequencing of the exons of the three genes of 88 individuals coming from eight different geographic locations (Cameroonians, Northern Europeans, Russians, Pakistanis, Hungarians, Native Americans, Chinese, and Japanese) revealed that the *TAS1R2* gene, encoding a protein to form the sweet taste receptor, has more polymorphic sites than the other two genes. The gene responsible for formation of both sweet and umami receptor dimers, *TAS1R3*, is the most conserved of the taste receptor family 1 genes. The sequence conservation may be due to the requirement of the protein product to be able to form a functional dimer with both *TAS1R1* and *TAS1R2*.

The effect of polymorphisms in sweet taste receptor gene *Tas1r3*, earlier known as a *Sac* (saccharin preference) locus (Bachmanov et al., 2001a), on the sweetness preferences of mice has been examined widely. Polymorphisms in the gene have been shown to be associated with saccharin preference in 30 mice strains (Reed et al., 2004). Several studies have compared mouse strain C57BL/6Byj (B6), referred to as “high-sweetener preferring” with the “low-sweetener-preferring” strain 129P3/J (129), which have different alleles of Tas1r3. B6 mice have lower detection thresholds and higher preferences for sugars, sweet-tasting amino acids, and some

noncaloric sweeteners (Bachmanov et al., 2001b) and greater neural response to sucrose (Inoue et al., 2001; McCaughey, 2007) than the 129 mice. However, the 129 mice are as much or even more motivated to obtain sucrose and more influenced by prior experience with sugar (Sclafani, 2006).

The effects of polymorphisms in *Tas1r2*, the more polymorphic gene in humans, on sweetness perception have not been examined extensively. The importance of the other subunit of the receptor is highlighted both genes being required for the sweetness perception (Zhao et al., 2003). *Tas1r3* knockout mice's sensitivity to sucrose is similar to that of wild-type mice, and only sensitivity to artificial sweeteners is affected by knockout of *Tas1r3* (Damak et al., 2003; Delay et al., 2006). In addition, the cat's insensitivity to sweetness has been shown to be due to pseudogenization (loss of expression) of *Tas1r2* (Li et al., 2005).

2.2.7.2 Variations in other loci

Genome-wide scans to locate genes combined with measurement of an individual's degree of sweet taste preference have not been performed before our study (II). The only related studies have concentrated on macronutrient intake, where intake of sucrose might serve as an indicator of sweet food consumption. Two studies thus far have performed genome-wide scans to identify quantitative trait loci (QTL) determining sucrose intake in humans. Collaku et al. (2004) found a promising linkage for sucrose intake on chromosome 1q43 (singlepoint p=0.002 and multipoint p=0.0018) in a population of 313 black subjects belonging to 126 families. This result was not obtained in the white population (n=514) of the same study. Another study to locate QTLs affecting sucrose intake was performed by Cai et al. (2004) in a population of 1431 subjects belonging to 42 Mexican American families. No significant linkage results were found for sucrose intake, the maximum multipoint LOD score being 1.60 at chromosome 2. In mice, QTLs influencing intake of energy from carbohydrates (as opposed to fat) have been identified on mouse chromosomes 17, 6, and X (Smith Richards et al., 2002).

More extensive research on taste genetics has concentrated on bitter tasting ability; several studies on the effect of the *TAS2R38* taste receptor gene on various variables have been conducted over the past few years. The receptor encoded by this gene recognizes bitter compounds with N-C=S group such as phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP). The studies evaluating the relationship between the tasting ability of mainly PROP and hedonic responses to sweet taste have yielded controversial results. In the studies of Drewnowski et al. (1997b; 1997c) and Ly and Drewnowski (2001), no relationship between PROP taster status and hedonic responses to sweetness was observed, but some studies have described an association between the two variables (Looy & Weingarten, 1992; Yeomans et al., 2007).

Identification of the three main SNPs of *TAS2R38* affecting PROP taster status (Kim et al., 2003), enabled the classification of subjects based on genotype. Mennella et

al. (2005) genotyped one of the variant SNPs, A49P (changing alanine to proline at position 49), in 143 children aged 5-10 years and their mothers (n=114) and determined their PROP sensitivity and sweet taste preferences. As expected, the genotype was a good predictor of PROP tasting ability. However, the genotype was related to sucrose preference only in children; children homozygous for the bitter-insensitive allele (AA) preferred significantly higher sucrose concentrations than the other children (with genotype AP or PP). Similarly, preferences for sweetness in cereals and beverages were affected by the genotype in children, but not in their mothers. In adults, the strongest determinant of sweet taste preferences in solutions and in foods was race (black/white).

2.3 Measuring responses to sweetness

Several methods are used to measure responses to sweet taste. When interested in the tasting ability for a certain compound, determination of subjects' detection or recognition threshold is most often used. However, in this section, the emphasis lies on methods measuring hedonic responses to suprathreshold sweetness concentrations and on behavioral responses to sweet foods.

The terms "liking" and "preference" are often used synonymously, although they have separate meanings. "Preference" is used when comparing products, and it does not always imply liking: when comparing two distasteful samples, a food may be preferred to another, although it is not liked. "Liking", by contrast, refers to hedonic response and is more difficult to measure than preference. Thus, if the preference of one food over other is measured, the results cannot be directly generalized to liking (Rozin, 1979).

2.3.1 Chemosensory tests

The preference for sweet taste is often measured by rating of the liking for a sweet water solution or a flavored drink. However, the strengths and weaknesses of such samples lie in their simplicity. While the experiences with certain foods or situations do not interfere with the ratings for an aqueous solution under laboratory conditions, the results are no generalizable to real eating or food choice situations. Thus, sometimes sweet foods are used as stimuli. Holt et al. (2000) performed a study in which 122 Australian Caucasian and Malaysian students were requested to rate the intensity of sweetness and the liking for the level of sweetness in a sucrose solution, orange juice, custard, and shortbread biscuit. The hedonic response to the sucrose solution was not a good predictor of liking for sweetness in the foods. As noted before, the preferred level of sweetness appears to be food-specific and related to experiences. In general, the predictive value of separate taste measurements in terms of nutrient intake may be limited (Mattes & Mela, 1986)

The methods to measure hedonic responses to sweetness in a sample vary according to the study objective. Rating of an overall liking for the sample or for the sweetness in the sample is common. Subjects are either asked to rate the liking using a scale or to state which of the samples is preferred. The latter, the paired preference test, is often used to determine the preferred sweetness level in a product and not so often when investigating differences between individuals in hedonic response to sweetness (Issanchou & Nicklaus, 2006).

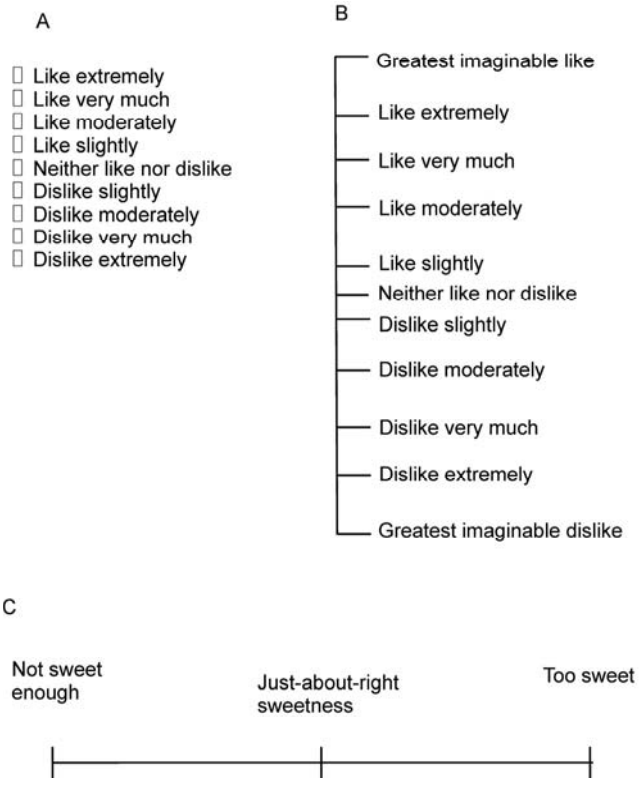


Figure 4. ***Scales used for evaluation of liking: A) 9-point hedonic scale, B) labeled affective magnitude scale, and C) just-about-right scale.***

The hedonic scales are used to assess the degree of liking for a sample and to idetify individual difference. However, the type of scale selected may affect the quality of the data. The traditional 9-point hedonic scale (Peryam & Pilgrim, 1957), presented in **Figure 4A,** is often applied, probably because it is easy for consumers to understand and use. Disadvantages of this scale are that it does not produce truly continuous data and that it may not separate the extreme ratings sufficiently well (ceiling effect). However, the largest problem is that the distances between the

categories are not equal which can be demonstrated by comparing the results with those obtained by magnitude estimation (Issanchou & Nicklaus, 2006). In magnitude estimation, an attribute (e.g. sweetness) of a sample is compared with that of a reference sample, which is given a value, e.g. 1. If the subject thinks that the sample is ten times sweeter than the references sample, he gives a value of 10. The results show that the distance between words at the ends of the 9-point hedonic scale is larger than the distance between words in the middle of the scale (Schutz & Cardello, 2000). Though providing continuous data with no ceiling or end-use-avoidance effects, magnitude estimation is rarely used, as it is difficult to use and thus requires training.

To combine the good properties of the 9-point hedonic scale and magnitude estimation, Schutz and Cardello (2001; Cardello & Schutz, 2004) developed a labeled affective magnitude (LAM) scale (**Figure 4B**). It corresponds to the labeled scale developed for rating of intensity (LMS, Green et al., 1993). The positions of the verbal labels were decided by magnitude estimation of the words describing liking. LAM is a semistructured scale with verbal labels making the rating easier than with magnitude estimation or unlabeled scales, while still producing continuous data.

Another method to study individual differences in sweet taste preferences is to ask the subjects to assess how far a sample is from ideal sweetness using just-about-right (JAR) response scales (**Figure 4C**). The JAR scales lead to lower optimal sweetness levels than the other methods (Issanchou & Nicklaus, 2006). A widely used method to evaluate the differences between individuals is to divide subjects into sweetness "likers" and "dislikers" by the shape of the relationship between concentration of a sweetener and liking (e.g. Yeomans et al., 2007), as described in **Figure 5**. Sweetness likers show an increasing liking for the sample with increasing concentration, whereas the sweetness dislikers show an increase in liking up to a certain concentration, with liking decreasing after this point.

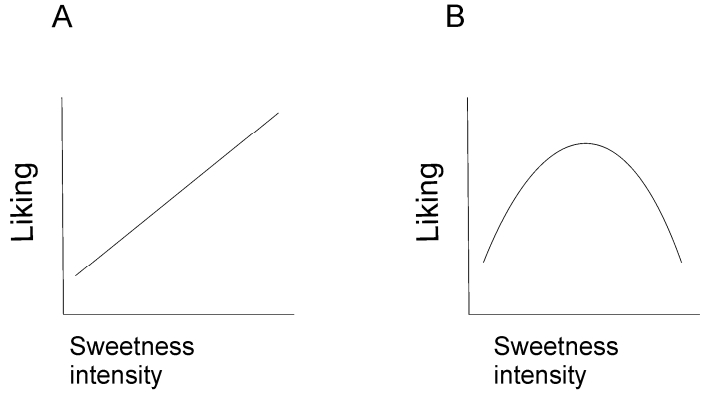


Figure 5. ***Relationship between sweetness intensity and liking for sweetness likers (A) and dislikers (B).***

2.3.2 Food behavior questionnaires

In addition to chemosensory testing, information about taste preferences and eating behavior can be obtained using postal or electronic questionnaires. To ascertain sweet taste preferences, questionnaires measuring the consumption of and/or liking for sweet food items are commonly used. For many foods, including sweet foods, the use-frequency and liking ratings of a food item are usually correlated (Lähteenmäki & Tuorila, 1994; Tuorila et al., 2008). However, for foods that are consumed very rarely or are highly preferred, the correlations between self-reported likings and use-frequencies are sometimes non-significant (Drewnowski & Hann, 1999). The ability of use-frequency and liking questionnaires to predict the actual intake of foods may be of limited value. In the study of Drewnowski et al. (1999), the liking ratings for sweet desserts were unrelated to intake of carbohydrates, fiber, or β-carotene, but the presumed main nutritional outcome of eating sweet foods, sucrose intake, was not measured. Sometimes the nonsweet counterparts of the food items are also included in the questionnaires (e.g. Conner & Booth, 1988). However, finding a sufficient number of pairs of sweet and nonsweet foods, with which most of the subjects are familiar, may be difficult.

Many questionnaires related to craving for sweet foods and attitudes towards sweet foods have also been developed. The "Craving for sweet foods" questionnaire is a subscale of the Health and Taste Attitude Scales (Roininen et al., 1999). It measures the tendency to crave sweet foods with 6 statements, each of which is evaluated using 7-point Likert scale (from strongly disagree to strongly agree). The ratings of the questionnaire have been shown to correlate with use-frequency and pleasantness of chocolate bars and pleasantness of soft drinks (Roininen et al., 2001). "Attitudes to Chocolate Questionnaire", developed by Benton et al. (1998), measures three dimensions of chocolate use 1) craving for chocolate and the tendency to seek comfort from chocolate under emotional stress ("craving"), 2) negative feelings associated with eating chocolate and dissatisfaction with weight and body image ("guilt"), and 3) the purpose of eating chocolate ("functional approach"). The craving factor was shown to be strongly associated with the number of chocolate bars consumed weekly.

A more general measure of attitudes towards sweetness was developed by Lähteenmäki and Tuorila (1994). Five dimensions of attitudes were composed: "social", "reward", "guilt", "health", and "restriction". The ratings on the social, health, and restriction scales were associated with sweetness ratings of strawberry-flavored yoghurts, highlighting the importance of social factors in food preferences.

2.4 Health effects of sweet taste preference

The sweet taste preference itself probably does not have any health effects, but as noted before, the liking for sweet foods is correlated with their use-frequency.

Dietary guidelines generally discourage the use of refined sugar (e.g. Dietary Guidelines for Americans, 2005; Finnish Nutrition Recommendations, 2005), and thus, many artificial sweeteners have been developed. The presumed consequences of consumption of sugar-containing foods are reviewed below.

2.4.1 Body weight

Consumption of sweet foods containing refined sugar may lead to extra caloric intake from carbohydrates. As an increase in body weight is regarded as a consequence of ingesting more energy than is expended, the consumption of sweet foods is often thought to underlie weight gain. However, the relationship between energy intake and body mass index is not so simple. Several groups have examined the effect of diet composition on increased body weight (reviewed by Hill & Prentice, 1995). Metabolic responsiveness is greater with carbohydrates than with fat; thus, if a meal containing carbohydrates and fats is ingested, the carbohydrates are oxidized first. The use of sugars therefore leads to a positive fat balance rather than to carbohydrates stored as fat (de novo lipogenesis), which is energetically a very expensive process.

Consequently, excess energy from fats results in higher fat storages, whereas excess energy from carbohydrates increases carbohydrate oxidation. De novo lipogenesis is expected to occur in humans only if the diet is very low in fat and rich in carbohydrates or in an exceptional state of carbohydrate intake exceeding oxidation capacity. As a result, it has been suggested that the carbohydrate-rich diet is not a major contributor to the development of obesity compared with a diet rich in fat (Hill & Prentice, 1995). However, it is noteworthy that foods that are both sweet and fatty are commonly preferred, and intakes of sugar and fat are generally associated. The excessive ingestion of these foods together with insufficient physical activity may lead to obesity which is linked to many diseases, such as type II diabetes and cardiovascular diseases.

Several studies have actually found a negative association between use of sugars and body weight. However, as Hill and Prentice note in their review, this correlation may be influenced by energy underreporting or a tendency to avoid consumption of sugars by overweight subjects. However, a review by Malik et al. (2006) concluded that intake of sugar-sweetened beverages is associated with weight gain in both children and adults. This is probably due to the body's incapability to estimate calories from fluids; if the same amount of energy is ingested from solid foods, the compensation for calories consumed would occur during subsequent meals, whereas fluids do not produce a similar feeling of satiety.

2.4.2 Dental caries

The relationship between the intake of sugars and the number of cariogenic microorganisms in the oral cavity is rather clear (Sheiham, 2001). Dental caries is one of the most common diseases and can seriously damage the teeth. In fact, Barkeling et al. (2001) used the counts of mutans streptococci and lactobacilli in saliva of obese and normal-weight women as an indirect measure of the intake of sweet foods. However, sugar consumption is not the only risk factor of dental caries. Meal frequency, education, motivation, socioeconomic group, ethnicity, oral hygiene, and use of fluoride and other preventive methods also play a role in the development of dental caries (Harel-Raviv et al., 1996), but were not considered by Barkeling et al.

2.4.3 Mood

Mood changes have been discussed both as a cause and a consequence of consuming sweet foods. However, the relationship between mood and sweet foods remains unclear. In the study by Reid and Hammersley (1995), no relationship between ingestion of a sucrose drink (with 40 g of cane sugar) and mood state measured using the Profile of Mood States form 30 and 60 min after intake was observed in a population of 31 males and 29 females. Yet, Kampov-Polevoy et al. (2006), who examined the associations between hedonic response to sweetness and the mood-altering effect of sweet foods, showed an association between liking for sweetness and expected mood improvements following the consumption of sweet foods. The participants (n=163) in their study rated the sweetness intensity of and liking for five sucrose solutions and filled in a questionnaire measuring two factors: 1) the mood altering effect of and 2) impaired control over eating sweet foods. Based on the liking ratings of the solutions, subjects were divided into sweetness-likers and -dislikers, the former rating higher on both factors. These studies were conducted by different methods and study designs, but their contradictory results warrant additional research on the relationship between mood and sweetness.

2.5 Genetic methods for investigating complex traits

2.5.1 Human genome

Genome refers to the total genetic information carried by an organism. The human genome consists of approximately 3 billion base pairs carried in 23 chromosomes in the nucleus of each cell. The exact number of genes in the human genome remains unknown, but the latest estimate is between 20 000 and 25 000 (International Human Genome Sequencing Consortium, 2004). Only 1.3% of the genome appears to code

for proteins, while the function of the rest of the DNA is less clear; a significant part of the noncoding sequences may have a functional role (Bejerano et al., 2004).

The human genome is diploid, meaning that it consists of 23 chromosome pairs, including 22 autosome pairs and the sex chromosome pair XY (males) or XX (females). Each chromosome has two arms: the shorter arm of the chromosome is denoted p (derived from "petit", small in French), and the longer q (next letter in alphabet after p). Further, each chromosomal arm can be divided into bands when stained, and these bands are numbered from the centromere, a region at which the chromosomal pairs appear to be attached to each other during cell division. Often the genetic location in the genome is labeled as the chromosome number, arm, and the band, e.g. 1p36. In addition to the chromosomes of the nucleus, humans carry DNA in the mitochondria. The mitochondrial DNA is circular and is always inherited maternally.

2.5.2 Inheritance and genetic variability

Genetic variability among individuals is based on human inheriting genetic material in the form of DNA from both parents. Humans inherit one of the homologs of each chromosome from the mother and one from the father. The diploid zygote, a cell from which all cells in a human are derived, is formed from two gametes: a mother's egg cell and a father's sperm cell. The gametes are haploid, i.e. that they contain only one copy of each chromosome: 22 autosomes and one sex chromosome. In egg cells, the sex chromosome is always X, but in sperm either X or Y.

The gametes are formed in meiosis, which is a special form of cell division. In mitosis, a normal form of the cell division, two daughter cells with identical, diploid genomes are produced. Whereas mitosis occurs in all tissues, meiosis occurs only in the primary spermatocytes of the testis and primary oocytes of the ovary. Meiosis produces haploid sperm and egg cells, and thus, only half of the genetic material of the diploid cell is passed on to daughter cells. The homologs of each chromosome are independently assorted to gametes, allowing 2^{23} (8.4×10^6) different combinations of parental chromosomes by one person. In addition, the genetic material of the chromatids is exchanged in a process called recombination, or crossing over (**Figure 6**). As a product, a recombinant chromatid, containing genetic material from both homologs of a chromosome is formed. Owing to both independent inheritance of homologous chromosomes and recombination, an individual is able to produce a virtually unlimited number of different gametes (Strachan & Read, 2004).

The probability of recombination of two loci is known as the recombination fraction, θ. Loci on different chromosomes are inherited independently of each other ($\theta=0.5$), but loci that are near each other on the same chromosome are co-inherited more often than in half of the meioses ($\theta < 0.5$) as the occurrence of crossover between

these loci during meiosis is less probable. Recombination fraction is used when calculating genetic distances between two loci. The unit of the genetic distance is Morgan (M). It is defined as the length of the DNA in which, on average, one crossover per meiosis is expected to occur. The recombination fraction is translated to Morgan units using a map function, such as the Haldane or Kosambi map function. The relationship of genetic distance to physical distance between two loci, measured in base pairs (bp), varies thorough the genome, as crossovers occur more often in some parts of the genome than in others. On average, a genetic distance of 1 centiMorgan (cM, i.e. 1% probability of recombination) corresponds to 1 million bp (Strachan & Read, 2004).

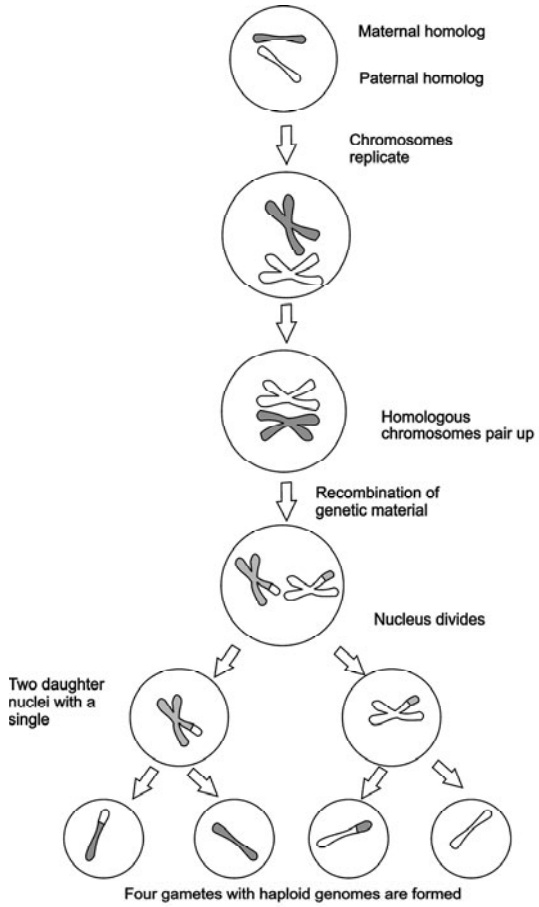


Figure 6. ***Meiosis with crossover (recombination) presented for one chromosome.***

2.5.3 Heritability analysis

Heritability is the proportion of variation of a trait attributable to genetic effects. Thus, the aim of the heritability analysis is to separate phenotypic variance into genetic and environmental components, expressed as an equation

$$Var(P) = Var(G) + Var(E)$$

in which Var(P) refers to phenotypic (total) variation, Var(G) to variation due to genetic factors, and Var(E) to variation due to environmental factors. According to the definition, heritability, h^2, can be expressed as

$$h^2 = Var(G)/Var(P)$$

Heritability refers to two different measures. Broad-sense heritability refers to the effect of all the genetic components, including epistasis, additive, and dominance genetic effects. Additive genetic variance consists of the sum of the allelic effects over all relevant loci, whereas dominance genetic variance includes interaction of alleles in the same locus. Epistasis, in turn, refers to interaction between genes. The other measure of heritability, narrow-sense heritability, includes only the additive genetic variance. Both of these measures are generally called heritability (Sham, 1998). The environmental and genetic factors may also interact, a phenomenon called gene-environment interaction (GxE). The gene-environment interaction means that individuals with different genotypes respond to the environment differently. Thus, decomposing the variation into genetic and environmental components may be an oversimplification.

Furthermore, in a family material with no family members reared apart, the heritability estimate represents all the variation that makes the family members more similar to each other, also called familial correlation. The familial correlation includes the effects of shared genes and common environment. In the case of food use, for example, part of the correlation among individuals belonging to the same family may be due to common eating habits and food culture, i.e. common environmental effects.

Heritability analysis is usually used as a starting point of genetic studies. Before laborious and expensive gene mapping experiments, it is worthwhile first to estimate to which extent the trait studied is inherited. Most behavioral traits do not have a direct measure, unlike measuring an individual's height or blood pressure, and thus several measures are generally used. These instruments may measure slightly different aspects of the phenomenon, and knowing which aspects are inherited is very useful.

2.5.4 Classical twin design

Twins provide a special study group for epidemiologic research of genetic and environmental factors. A twin pair shares their childhood environment even more closely than normal siblings because they developed in the same uterus and are of the same age. Genetic research of twins is based on differences between the two types of twins; the monozygotic (MZ, identical) twins are genetic clones of one another, sharing all segregating genes, while dizygotic (DZ, nonidentical, fraternal) twins share, on average, half of their segregating genes, as do siblings in general. DZ pairs can be either same- or opposite-sexed. The classical twin study compares the covariance of MZ twins with that of DZ twins. Basically, all factors making MZ pairs dissimilar are environmental, and thus if MZ twins resemble each other more than DZ twins, the trait is influenced by genetic effects.

Twin analysis assumes that the environment influences MZ and DZ twins similarly (i.e. equal environment assumption). The validity of this assumption in behavioral traits has been questioned; the bonding between MZ twins has been speculated to be more intense than between DZ twins. Tishler and Carey (2007) studied the validity of the assumption by determining whether the smoking prevalence was similar in MZ and DZ twins, considered as proof of equal environments. However, they observed that prevalence of smoking was generally higher in DZ than in MZ twins, and concluded that for subjective traits the environments of MZ and DZ twins may be unequal. Kaprio (2007) noted in his commentary to Tishler and Carey that in the re-analysis of one of the cohorts, taking into account intra-pair correlations, the difference between MZ and DZ twins was no longer significant. In addition, the results of Tishler and Carey were limited to one phenotype, and assessment of social environment should be included in all twin studies to explore the mechanisms of the zygosity difference.

In addition, epistatic effects, gene-environment interactions, and random mating with respect to the trait are assumed to be absent in the basic twin model. On the basis of these assumptions, the variance of a trait can be decomposed to genetic and environmental factors. In twin studies, the genetic factors can be divided into additive (A) and dominant (D) with the within-pair correlations for DZ twins being 0.5 and 0.25, respectively. As MZ twins share all their genes, their genetic correlation is 1. The environmental factors can be divided into common environmental effects (C), including all environmental effects making the twins in a pair more similar to each other (such as family environment and common friends), and specific environmental effects (E), including all effects making the twins in a pair more dissimilar to each other (including measurement error). Thus, the correlation of common environmental effects is 1 and of specific environmental effects 0 for both DZ and MZ twins (Neale & Cardon, 1992).

However, in a study including only twins reared together and not other family members, only three of these four variance components can be estimated. Thus, the

starting point for univariate modeling is either ACE- or ADE-model, as a model with a D component but without an A component is not biologically reasonable for traits influenced by many genes (Neale & Cardon, 1992). The choice between these is made according to the within-pair correlation patterns: if MZ correlations are twice the DZ correlations, the genetic effects are assumed to be additive, and if MZ correlations are more than half or less than half of the DZ correlations, the genetic effects are assumed to be dominant. If MZ and DZ correlations are similar, the resemblance of the twins is probably due to common environmental effects, and genetic effects do not play a role. Additionally, if the within-pair correlation of opposite-sexed DZ twins is much lower than that of same-sexed DZ twins, sex-specific genetic effects may underlie the trait.

Raw estimates of A, C, D, and E can be made from within-pair correlations (Posthuma et al., 2003):

Additive genetic influences, $a^2 = 2(r_{MZ} - r_{DZ})$

Dominant genetic influences, $d^2 = 2r_{MZ} + 4r_{DZ}$

Common environmental influences, $c^2 = 2r_{DZ} - r_{MZ}$

Specific environmental influences, $e^2 = 1 - r_{MZ}$

In the equations, r_{MZ} stands for MZ correlation and r_{DZ} for dizygotic correlation.

However, the estimation is not usually done by hand, but by computer program, most often Mx (Neale et al., 1999), which also allows inclusion of covariates.

2.5.5 Marker maps

Locating loci influencing a trait requires the genotyping of markers with a known location. Basically, any polymorphism in the genome with a known location and a sufficient proportion of heterozygotes in the population can act as marker in a mapping study. However, in practice, the marker has to be easy and inexpensive to genotype from the available biological material. The number of markers selected is based on the area scanned and the desired precision of the results. Generally, mapping requires that markers not be located farther away than 10-20 cM from each other to enable the genomic area to be scanned precisely and information from adjacent markers to be used in multipoint analysis.

Four types of DNA markers are typically used in mapping studies: restriction fragment length polymorphisms (RFLPs), minisatellites, macrosatellites, and single nucleotide polymorphisms (SNPs). RFLPs are nowadays rarely used, as they only have two alleles: present or absent. The minisatellites and microsatellites are variable number tandem repeats and have many alleles. However, minisatellites are too long to amplify well with standard PCR procedures, and thus, di-, tri-, or

tetranucleotide microsatellites are used more often. The use of the fourth type of polymorphisms, SNPs has become very popular in recent years due to the development of genome-wide association studies (GWAS), including hundreds of thousands of SNP markers. In linkage analysis, the disadvantage of SNPs is that with usually only two alleles they are less informative than microsatellites. However, with SNPs, a more dense marker map (<10 kB distances between markers) can be created (Strachan & Read, 2004), resulting in high information content for linkage analysis.

2.5.6 Linkage analysis to locate quantitative trait loci

The aim of linkage analysis is to locate genetic elements influencing a trait in the genome by testing whether two loci tend to be co-inherited. As noted before, the closer the two loci are to each other, the more often they are co-inherited. Linkage analysis tests whether the recombination fraction between a marker locus with a known position and a locus influencing the traits differs significantly from 0.5 (H_0: $\theta=0.5$); two loci with a recombination fraction <0.5 are said to be linked. The statistical estimate of probability used in the linkage analysis is the LOD score, standing for logarithm of odds score. In the linkage analysis, the likelihood of two alternative hypotheses, H_o ($\theta=0.5$) and H_1 (θ estimated from the data), is calculated. The LOD score (Z) is the logarithm base 10 of the ratio of these likelihoods:

$$Z = \log(L_\theta / L_{\theta=0.5})$$

where L is the likelihood and θ the recombination fraction. A LOD score of 3, corresponding roughly to a p-value of 0.0001, is most often regarded as significant. However, this only applies to monogenic traits; the significance of the LOD score for a complex trait is more ambiguous (Lander & Kruglyak, 1995).

Quantitative traits, which are inherited to some extent, such as the level of sweet taste preference, are often influenced by more than one genetic element residing in different genetic loci. These loci are called quantitative trait loci (QTL). As the name suggests, the phenotypes are quantitative and are not divided into nominal classes, e.g. into cases and controls. The variance component linkage analysis method was developed for QTL linkage analysis. Its advantages over other methods include the possibility to incorporate covariates, genotype-environment interactions, and other confounding factors, and the ability to handle large pedigrees. It is a nonparametric method, meaning that the mode of inheritance and other parameters do not need to be specified. The variance component linkage method is based on correlation between the expected allele sharing of family members (identity-by-descent, IBD allele sharing), a locus, and their phenotypic covariance. The analysis can either use the data of one marker at a time ("singlepoint" or "two-point" analysis) or the data of more markers located near each other ("multipoint" analysis). The multipoint

approach generally increases the power to detect true linkages and decreases the false-positive rate (Almasy & Blangero, 1998).

2.5.7 Linkage disequilibrium

Linkage disequilibrium (LD) refers to a condition in which two loci do not occur independently in the general population. Linkage disequilibrium is based on the assumption that the study subjects comprise parts of a very large pedigree without an explicitly known structure. The relationships between the individuals analyzed do not need to be known though they are considered as being distant relatives belonging to the same, large pedigree.

Going back far enough, large fractions of the current world population are ultimately related to each others. Thus, apparently unrelated people with a given allele (e.g. an allele modifying the susceptibility to a disease) inherited from their common ancestor, also share alleles at a small region around the allele. The more generations (meioses) to the common ancestor, the smaller the region shared. Thus, the population history influences the LD between two loci with a given distance. In addition, the ability of LD to identify genetic markers associated with a disease fails if the phenotype is not due to common ancestral allele but is instead due to repeated newer mutations (Strachan & Read, 2004). In an isolated population with a small number of original founders, such as the Finnish population, a founder mutation results in long LD intervals, facilitating the mapping of alleles of Finnish disease heritage (Peltonen et al., 1999).

2.5.8 Association analysis

Genetic association refers to a co-occurrence of phenotypes and alleles. In a simplest case, the association occurs because the allele directly influences the phenotype (e.g. modifies the susceptibility to a disease) and usually the goal of association studies is to discover associations due to LD between the marker and a phenotype. Other causes of an observed association include natural selection (a certain an allele improves the chances of a person with a given disease to survive and have children), population stratification (existence of genetically different subgroups of different ancestry in the study population), and false positives due to multiple testing.

Traditionally, association analysis has been used for case control analysis, in which allele frequencies or genotypes of a sample of unrelated affected individuals and matched controls are compared (Schulze & McMahon, 2002). Cases and controls must be well-matched in terms of racial and ethnic background, age, sex, and other factors to avoid false positives due to population stratification. Statistical methods that take into account the population stratification have been developed. Falk and Rubistein (1987) initially developed the haplotype relative risk (HRR) approach,

which combines the two alleles that were not transmitted to the affected offspring and creates a pseudocontrol group. However, the HRR method has been criticized for not completely eliminating population stratification bias.

Another method, transmission/disequilibrium test (TDT), is based on the assumption that in the absence of linkage and an association between a marker and a trait, the marker alleles are transmitted to the offspring randomly (Spielman et al., 1993). TDT is commonly used for tests of association in the presence of linkage in fine-mapping studies. In the test for linkage, the pedigree structure of study populations must naturally be known. TDT eliminates population stratification, but is less powerful than HHR, as it can only use heterozygous parents. The basic TDT compares the transmission and nontransmission of marker alleles of a biallelic marker to affected offspring by chi-square statistics, but methods to study multiple markers with more than two alleles and quantitative traits with covariates have been also developed (reviewed by Schulze & McMahon, 2002).

Often, linkage and association analyses are seen as complementary. Linkage analysis can be used to scan the entire genome with several hundred markers. Once the candidate region displaying evidence of linkage has been localized, the region may be narrowed down by association analysis (Strachan & Read, 2004). Recently, several studies of genome-wide association of quantitative traits have been published. The HapMap project (www.hapmap.org) is a "multi-country effort to identify and catalog genetic similarities and differences in human beings" which has enabled the studying of LD among common human SNPs online. Thus, "tag SNPs", SNPs residing in a region with high LD and thus providing information also from the surrounding region, can be chosen as markers for association studies. The development of modern microarray chips capable of assessing more than 500 000 SNPs per sample has produced an enormous increase in the resolution and amount of data in genome-wide scans, posing challenges for the data analysis and interpretation of the results. One of the biggest problems is the multiple testing; false-positive results may arise, as investigating of a single phenotype comprises more than 500 000 comparisons. A Bonferroni corrected p-value of $0.05^{500,000}=0.0000001$ has been proposed as the threshold for genome-wide significance. However, with a limited sample size, a small p-value is often difficult to achieve. Thus, the original finding often needs to be replicated in one or more studies to ascertain the primary association. However, this approach has allowed the discovery of several unexpected associations between genes of chromosomal regions and diseases (Hunter & Kraft, 2007).

3 AIMS OF THE STUDY

The aims of the study were the following:

- To examine whether food use patterns of young adults are influenced by genetic or common environmental factors, and whether the proportional contribution of these varies for different food items and between sexes (I).

- To estimate the heritability of sweet taste perception and behavioral traits concerning sweet taste preferences (II, III), and to locate the influencing genetic elements in the genome (II).

- To estimate whether the correlations among sweet taste preference measures are due to genetic or environmental influences (III).

- To analyze the relationships between body weight and aspects of dieting behavior and to determine whether these variables are linked to consumption of and liking for fatty foods that are either sweet or salty foods (IV).

4 MATERIALS AND METHODS

More detailed descriptions of the materials and methods used can be found in the original publications.

4.1 Study populations

Characteristics of the populations are presented in **Table 1**.

4.1.1 FinnTwin16 (I)

FinnTwin16 is a consecutive and complete cohort of Finnish twins born in 1975-1979 (Kaprio et al., 2002) and contains MZ and same-sex and opposite-sex DZ twins. The data analyzed were from the fourth wave of the study conducted in 2000-2002 and comprise replies to the food-frequency questionnaires of 4667 subjects aged 22-27 years.

4.1.2 Finnish family study (II)

The subjects of the Finnish family study were participants of a migraine family study (Wessman et al., 2002). The sample comprises 146 adults, 83.6% of whom are migraine patients and 16.4% their healthy family members. The subjects belong to 26 Finnish families, and the data contain 9 spousal, 63 parent-offspring, 137 sibling, 4 half-sibling, 127 avuncular, and 37 first-cousin pairs. Participants were genotyped genome-widely with more than 360 microsatellite markers.

4.1.3 TwinsUK (III & IV)

The UK Adult Twin Registry (TwinsUK, Spector & Williams, 2006) consists of MZ and same-sex DZ twins. The majority of the participants are female. The data were collected during 2005. In study III, data for females only and in study IV data for both sexes are used.

4.1.4 FinnTwin12 (IV)

FinnTwin12 comprises five complete year-cohorts of Finnish twins born in 1983-1987 (Kaprio et al., 2002) and contains MZ, and same-sex and opposite-sex DZ twins. The data in study IV were collected during 2006 and 2007, and at the time of the analysis, responses from 403 twin individuals were available.

Table 1. ***Characteristics of study populations.***

Original publication	I	II	III	IV	IV
Study population	FinnTwin16	Finnish family study	TwinsUK	TwinsUK	FinnTwin12
N	4667	146	663	1027	403
Sex distribution (%)					
males	44.7	31.5	-	10.5	41.9
females	55.3	68.5	100	89.5	58.8
Age (years)					
mean	24.4	49.0	55.6	55.6	22.9
SD	(0.82)	(14.8)	(12.4)	(12.7)	(0.5)
range	22 - 27	18 - 78	17 - 81	17 - 82	22 - 24
Zygosity of twins (%)					
MZ	32.1	-	45.7	46.2	43.9
same-sex DZ	33.5	-	54.3	53.8	31.1
opposite-sex DZ	34.4	-	-	-	25.1

4.1.5 Ethical aspects

The study protocols of each study were approved by the local ethics committees: the Finnish family study and the FinnTwin studies by the Ethics Committee of Helsinki University Central Hospital, Finland, and TwinsUK by Guy's and St. Thomas' Hospital Ethics Committee, United Kingdom.

4.1.6 Determination of zygosity and checking pedigrees

Zygosity of the twins was determined by questionnaire, in the TwinsUK study by the "Peas in the Pod" questionnaire (Martin & Martin, 1975; Peeters et al., 1998) and in FinnTwin studies by a deterministic algorithm using questions on physical similarity during school age; this method has been shown to have high validity in another Finnish twin cohort (Sarna et al., 1987). If zygosity remained uncertain, it was checked by genotyping.

In the family study (study II), the pedigrees and the genotype data were checked by the program PedCheck (O'Connell et al., 1998), and no pedigree structure errors (level 0 errors) or Mendelian inconsistencies (level 1 or 2 errors) were detected. In addition, the MERLIN program was used to screen for unlikely but Mendelian-

consistent genotypes, and these were erased by the Pedwipe program, provided by MERLIN (Abecasis et al., 2002).

4.2 Chemosensory measurements

Chemosensory measurements were conducted after overnight (12-h) fasting. The instructions were given both orally and in written form, and the test administrator was present throughout the testing procedure.

4.2.1 Tests for sweetness perception (II & III)

In the first study with chemosensory testing (II), three suprathreshold sucrose solutions (Finnsugar Ltd., Kantvik, Finland) plus plain water were included as samples. The sucrose concentrations of the solutions were 3.0%, 7.5%, and 18.75% (weight/volume), ranging from mildly to extremely sweet. The subjects rated the intensity and pleasantness of the sweet taste using a labeled magnitude scale (LMS, Green et al., 1993) and a labeled affective magnitude scale (LAM, Schutz & Cardello, 2001), respectively. Due to the lack an equivalent for the word "dislike", like/dislike was replaced by pleasant/unpleasant (miellyttävä/epämiellyttävä) in the Finnish translations of the LAM.

4.2.1.1 Development of a sweetness perception test

The use of several samples was impossible in the international studies, with limited resources for sample preparation and in time for testing of subjects. Thus, based on the results of the family study, a new test for sweet taste preference was developed. Requirements for the test were 1) being easy and fast to prepare, 2) being easy to perform and administer, 3) providing a measure of sweet taste preference comparable with the strongest (18.75%) solution of the sample series used in the study II, and 4) providing sufficient variation and a significant heritability estimate.

At first, we tested whether an 18.75% sucrose solution was perceived similarly in a single-stimulus condition and in a series of four samples. In addition, we tested whether a 9-point hedonic scale instead of labeled magnitude scales could be used in the ratings. A total of 31 participants, aged 20-58 years (mean age 29.3 years, SD 9.9) rated the intensity and pleasantness of the sample series with 0%, 3%, 7.5%, and 18.75% sucrose on water in a randomized order and the 18.75% sample alone. The ratings were done in two separate days with half of the participants rating one 20% sample on the first day and four samples on the second day, and vice versa. The scatter plots of liking and intensity ratings of the sample in series and in a single-stimulus condition are shown in **Figure 7**.

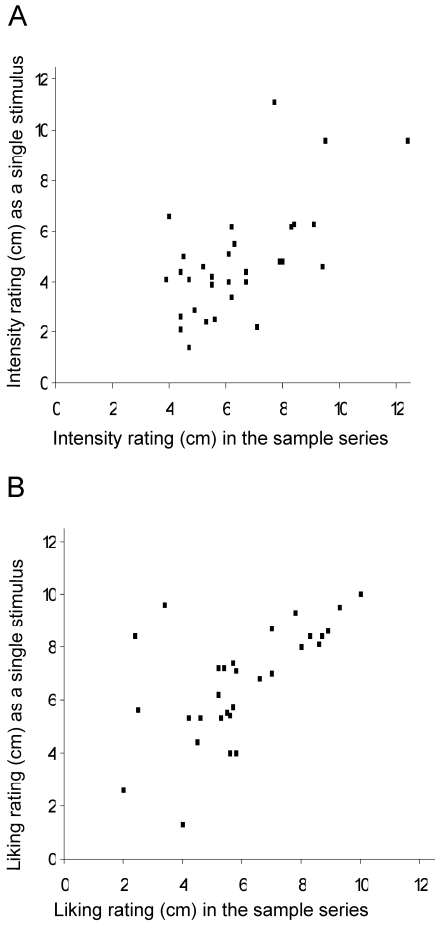


Figure 7. ***Scatter plots of the intensity (a) and liking (b) ratings of the 18.75% (w/v) sucrose solution in a series of four samples and as a single sample. The ratings expressed as cm on the LMS (intensity) and LAM (liking) scales.***

The sample was evaluated as less intense (mean 6.4 in series and 4.8 alone) and more pleasant (mean 6.0 in series and 6.7 alone) when presented alone compared with presentation in a series of three samples. The correlation between the ratings in the series and in the single-sample condition was for both intensity and liking ratings 0.62 (Pearson correlation coefficient). The 9-point intensity and hedonic scales were shown to inadequately separate between responses. We concluded that the condition in which the stimulus is presented has an effect on the rating, but the ratings still measure the same underlying variable. In further examination with a smaller group

(n=5), the 20% sample was shown not to be distinguishable from the 18.75% sample using paired comparison procedure. This was also expected from the Weber ratio (just noticeable difference/reference concentration) of sucrose in water (~0.17 in Laing et al., 1993).

We also tested whether the hedonic ratings of the aqueous solution predict the liking for a sweet food. The sample chosen was orange juice. The level of sucrose addition needed to obtain a sweetness level corresponding to that of the 20% water solution was tested with 15 subjects using a paired comparison. The subjects were presented a pair of 20% sucrose solution and orange juice with 7.5%, 20%, or 26% added sucrose. All judges tasted all three pairs and were asked to evaluate which of the samples was sweeter. The results of the test are shown in **Figure 8**.

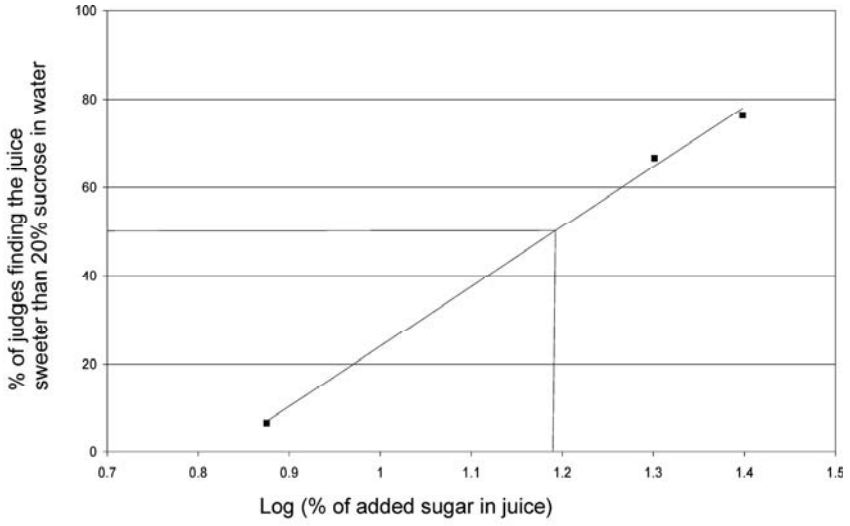


Figure 8. ***Results of the paired comparison between 20% sucrose in water and varying concentrations of sucrose added to orange juice.***

On the basis of the results, the sweetness of an orange juice corresponding to that of 20% sucrose solution (i.e. 50% of the judges' ratings were as sweeter than 20% sucrose solution) was 16%. The subsequent test with 34 subjects (2 men, 32 women, aged 18-43 years, mean age 23.9 years) included ratings for liking for and intensity of three samples: 20% sucrose in water, pure orange juice (with 10% sugar naturally), and orange juice with 16% added sugar. Each of the samples was rated on a separate day using 11.5-cm-long labeled magnitude scales, and the samples were presented in randomized order.

The correlations among the ratings for the pure orange juice, the orange juice with 16% added sugar, and the aqueous sucrose solution (20%) are presented in **Table 2**.

Table 2. ***Pearson correlation coefficients for the liking for and intensity ratings of samples***

n=34		Liking			Intensity		
		Pure juice	Juice with 16% added sucrose	20% sucrose in water	Pure juice	Juice with 16% added sucrose	20% sucrose in water
Liking	Pure juice	1					
	Juice with 16% added sucrose	0.06	1				
	20% sucrose in water	-0.25	0.29	1			
Intensity	Pure juice	-0.11	0.37*	0.44*	1		
	Juice with 16% added sucrose	0.20	0.29	0.07	0.45*	1	
	20% sucrose in water	0.26	0.17	0.10	0.05	0.23	1

* $p<0.05$

The results show that the liking for the sucrose solution poorly predicts liking for sweetness in juice ($r=0.29$, $p=0.091$). However, the correlation coefficient is nearly significant and can be interpreted as reflecting an underlying preference for sweet taste.

In addition, the subject rated the liking for and use-frequency of 16 sweet foods. The sweet foods were categorized by factor analysis with a maximum likelihood method and Varimax rotation. Three groups, fitting both liking and use-frequency data, were identified: sweet snacks, sweet fruits, and foods sweetened with artificial sweeteners. The internal consistency of the use-frequency and liking groups was evaluated with Cronbach's alpha values, which were between 0.604 and 0.851. The correlations between the food groups and the ratings for the samples were mostly very low. The only significant correlation was observed between the intensity rating for the orange juice with 16% added sugar and liking for sweet fruits ($r=0.36$, $p=0.04$).

Based on these tests, the liking and intensity ratings of the 20% (w/v) sugar solution were chosen as appropriate measures of sweetness perception and to correspond to the 18.75% sample used in Study I in a series of four samples. A single solution with a pre-packed sugar sachet (4 g) and a cup with a marked line for filling with water further enabled a wide use of the sweet taste perception test in genetic studies. Most importantly, the sample proved to be a good tool for research of genetic background of sweet taste preference, as in a subsequent large study (II) it showed sufficient variation and the liking for it using LAM provided a significant heritability estimate.

4.2.2 Test for saltiness perception (II)

In Study II, the heritability of salty taste perceptions was also determined. The salty taste series included three salty solutions (0.2%, 0.5%, and 1.25%) and plain water. The intensity and pleasantness of salty taste was rated using a 12.5-cm-long LMS and LAM, respectively.

4.2.3 Screening for PROP tasting ability (II, III)

The intensity rating of PROP (6-propyl-2-thiouracil, Sigma-Aldrich Chemie GmBH, 82460, Steinheim, Germany) was included in the study protocol as a positive control for heritability (II, III). The taste sensitivity for PROP was screened by a filter paper method (Zhao et al., 2003). The intensity of the filter paper containing 0.6 mg PROP was rated using the LMS.

4.3 Use-frequency and food liking/disliking questionnaires (I-IV)

Food-frequency questionnaires (FFQs) were used in all studies (I-IV), and liking ratings for the same foods were included in Studies II-IV. The questionnaires included slightly different foods in different study populations. The foods of the use-frequency and liking questionnaires were categorized using factor analysis with maximum likelihood extraction and orthogonal Varimax rotation. The food groups were named based on their essential content and the scores for the groups were calculated as factor loadings (I) or as mean for the items loading to the group (II, III, and IV).

4.4 Craving for sweet foods scale (II, III)

The "Craving for Sweet Foods" scale is a subscale of the Health and Taste Attitude questionnaires (Roininen et al., 1999), which measures the tendency to crave sweet foods with six statements. Each of the statements is evaluated using a 7-point Likert scale and the scale score is calculated as the mean of ratings given for the statements. The "Craving for Sweet Foods" scale was used in Studies II and III.

4.5 Three-factor eating questionnaire-R18 (IV)

The original "Three-Factor Eating Questionnaire" (TFEQ, Stunkard & Messick 1985) measures three dimensions of human eating behavior, namely “Restraint”, “Disinhibition”, and “Hunger”. Karlsson et al. (2000) evaluated the construct validity of the 51-item questionnaire in obese men and women, and the original factor structure was not replicated. Instead, they constructed a revised 18-item questionnaire (TFEQ-R18) based on the original items, and the new three factors

were now named "Cognitive Restraint" (6 items), "Uncontrolled Eating" (9 items), and "Emotional Eating" (3 items).

4.6 Quantitative genetic analysis (I-IV)

In the family study, the heritability and linkage analysis was performed using the variance component method of MERLIN (Abecasis et al., 2002). The inclusion of covariates in the linkage analysis was based on their significance in the heritability analysis of the SOLAR program (Almasy & Blangero, 1998) assuming a polygenic model. The linkage analysis was performed only for traits with a significant heritability estimate.

The quantitative genetic analysis of twin data was performed using an Mx program (Neale et al., 1995) version 1.5 (I) or 1.7 (III, IV). For multivariate analysis, Cholesky decomposition was used (III, IV).

5 RESULTS

5.1 Sweetness

5.1.1 Heritability of responses to sweetness (I-IV)

Heritability analysis of the intensity and liking ratings of three suprathreshold (3.0%, 7.5%, and 18.75%) sucrose solutions in the Finnish family study (II) revealed that the hedonic response to sweetness is more heritable than the intensity perception. Furthermore, the stronger the sweet taste in the solution, the higher the heritability estimate: the heritability of the liking for the two milder, 3% and 7.5%, sucrose solutions were 20% and 29%, respectively, whereas the liking for stronger solutions produced a heritability of over 40 % (**Table 3**). The heritability estimates (%) of variables measuring the degree of sweet taste preference in the four studies are provided in Table 3.

Table 3. ***Heritability estimates (% of variation) of variables measuring hedonic response to sweetness in the four studies.***

	I		II	III	IV	
Variable	males	females	combined	females	males	females
Strong sucrose solution[a]						
Intensity	-	-	0	33	-	-
Liking	-	-	41	49	-	-
Eating behaviors						
Use-frequency of sweet foods	42	43	50	53	47	43
Liking for sweet foods	-	-	40	54	39	46
Craving for sweet foods	-	-	31	38	-	-

[a] concentration of the solution 18.75% in Study II and 20% in Study III

As the heritability estimate in a family study includes the effects of both common family environment and additive genetic effects, the estimation of contribution of genetic factors to liking sweet taste was repeated with a twin study (III). A single 20% w/v sucrose solution was used, and the additive genetic effects and significant heritability estimates for both liking and intensity were obtained. However, the model in which within-pair correlation of the twins for the intensity rating was designated common environmental provided an equal fit.

Questionnaires measuring the degree of sweet taste preference included use-frequency of sweet foods (I-IV), liking for sweet foods (II-IV), and the Craving for Sweet Foods scale (II, III). The heritability estimates of the variables measuring responses to sweetness in different studies are presented in **Table 3**.

5.1.2 Linkage analysis (II)

Multipoint linkage peak with LOD score of 3.5 (empirical p-value 0.07) was identified on chromosome 16p11.2 (marker D16S753) for the use-frequency of sweet foods. In addition, the use-frequency of sweet foods was linked to loci on chromosomes 9q32.1 (LOD=2.0), 3p26.3 (LOD=1.9), and 20q13.2 (LOD=1.9) and liking for the 18.75% sucrose solution was linked to a marker on chromosome 1q41 (LOD=1.9).

5.1.3 Associations among measures of liking for sweetness (III)

The tetravariate modeling of measures of hedonic responses to sweetness revealed several significant correlations. The phenotypic correlation (r=0.23) between liking for the sweet solution and for sweet foods was explained by genetic factors (genetic correlation between traits=0.27). The three correlations among the questionnaire variables (r ranging from 0.30 to 0.55) were due to both genetic and environmental factors, the genetic factors explaining, on average, 62% of the trait correlations.

5.2 Other dietary and chemosensory variables

5.2.1 Heritability analysis (I-IV)

Intensity or liking ratings of salty solutions were not inherited (II). The only significant heritability of the NaCl solution ratings was obtained for the liking for the 0.2% solution (33.1%). Heritability of PROP tasting ability was estimated in Studies II and III using the filter paper method. The heritability estimates were 60% in the Finnish family study and 65.5% in the UK female twins.

Variance in the food use of young adults was explained by additive genetic and specific environmental factors. Four food groups were identified: “healthy” foods, high-fat foods, sweet foods, and meats. Common childhood environment had no significant influence on the use-frequency of 24 commonly used foods or on four food use patterns based on these. On average, 40% of the variation in the use of individual food items and 45% of the variation in the food use patterns was explained by additive genetic effects. The proportional variance components of the food groups are presented in **Figure 9**. In addition, sex differences were observed in

the magnitude of variance components (for chocolate, other sweets, fried foods, and meat) and in the genes underlying food use (for fresh vegetables, fruits, and cheeses).

Variation of the three dieting behaviors measured by the Three-Factor Eating Questionnaire-R18 (Cognitive Restraint, Uncontrolled Eating, and Emotional Eating) was explained by additive genetic and specific environmental factors, and the common environmental factors did not contribute to any variance in these. The heritability of Cognitive Restraint (29% for males and 57% for females) and Uncontrolled Eating (52% for males and 58% for females) was higher than that of Emotional Eating (heritability for 0% males and for 43% females). The heritability estimate obtained for BMI was 64% in females and 75% in males.

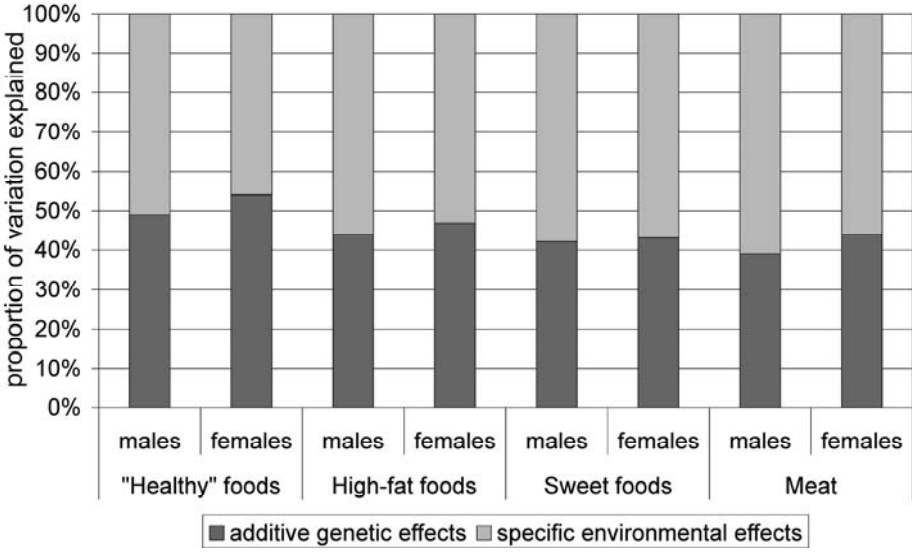


Figure 9. ***Proportional estimates of additive genetic effects and specific environmental effects on food use patterns in young adult twins (Study I).***

5.2.2 Associations among dietary measurements and body mass index (IV)

Closer consideration of the phenotypic correlations among BMI, dieting behaviors, and use and liking ratings for fatty foods revealed that BMI was genetically correlated with all three dieting behaviors, which, in turn, were correlated with fatty food use and liking ratings. However, no significant correlations between BMI and the dietary measures were observed.

6 DISCUSSION

6.1 Heritability of chemosensory measurements

6.1.1 Sweet taste perception

Studies II and III showed that the degree of liking for sweetness in an aqueous solution is partly genetically determined. However, the intensity perception appeared to be only weakly, if at all, inherited. These were the first studies to determine the heritability of responses to sweet taste with data from chemosensory measurements with suprathreshold concentrations. One earlier study, published in 1982 by Krondl et al., had estimated the genetic influences on recognition thresholds with 13 MZ and 10 DZ pairs. A nonsignificant heritability estimate of 0.52 using the Holzinger index of heritability:

$(Var_{DZ} - Var_{MZ})/Var_{DZ}$

where Var is the within-pair variance of the mean difference, was found for the recognition threshold of sucrose. The estimate strongly suggests that MZ twins resemble each other more than DZ twins, but the heritability estimate failed to reach significance, probably because the statistical method was insufficiently sophisticated to reveal genetic effects, the recognition threshold possibly was not a very relevant measure (not easy to determine accurately and weak predictive value of the perception of suprathreshold concentrations), or the sample size was too small.

As the hedonic response to sweetness varies between individuals due to genetic differences, the degree of liking for sweet taste in adulthood is not entirely learned. This finding is of major importance in understanding the background of human taste preferences. Despite living in similar environmental conditions, individuals may have differences in the liking for sweet taste. This should be considered when suggesting changes to the diet, e.g. in dietary counseling; for some, the preference for a variety of sweet foods may be learned and it may be easy to decrease their use or reduce the level of added sucrose, but for others products containing less sugar simply will not taste as palatable. Environmental factors, e.g. earlier experiences, also influence the degree of liking for sweetness. For instance, a person with an inherited low preference for sweet foods living within a family of sweet-likers probably learns to like sweet foods if they are offered and tried continuously.

6.1.2 Heritability of salty and bitter (PROP) taste perceptions

Earlier studies have shown that the familial correlation in the salty taste perception is due to common environmental effects. Beauchamp et al. (1985) determined the most preferred level of salt in soup (0.47-2.90%), the detection threshold of salt in water, and the use of salt added to foods with 20 MZ and 24 DZ young adult (17-23 years) same-sex twins. None of the measures provided evidence of genetic effects influencing salty taste perception. The pair-wise correlations of MZ twins were no higher than those of DZ twins, suggesting that the correlations were due to shared environmental factors. Similarly, a recent twin study (Wise et al., 2007) found that the recognition threshold for NaCl was not genetically determined, but partly influenced by common environmental effects (22% of variation). Thus, the heritability estimate obtained in our study is likely not due to shared genes, but to common environmental effects.

Heritability estimates obtained for the intensity rating of PROP filter paper (II, III) were similar to those reported earlier. Hansen et al. (2006a) found a heritability of 72% for 0.01% PROP solution in a sample of twins (102 MZ pairs, 210 DZ pairs) and their singleton siblings (n=229). The high sensitivity to PROP has been associated with reduced acceptance of some bitter foods (reviewed by Drewnowski & Rock, 1995), and the effect of PROP tasting on other dietary habits, body weight, and other variables is still under wide discussion (Prescott & Tepper, 2004). However, studies on sensitivity to PROP and its implications have encouraged additional research on taste genetics by suggesting that genetic differences between individuals can influence food habits.

6.2 Heritability of other dietary measurements

6.2.1 Reported responses to sweet foods

Small differences were present between heritability estimates of questionnaire measures of hedonic responses to sweet foods obtained in different studies. These differences are most probably due to variations in the questionnaires of use-frequency and liking (slightly different foods in different studies, slightly different meaning due to translation) and differences between the size, age, and sex distributions of the populations. However, all of the studies clearly indicate that these traits are partly genetically determined and that the heritability of the use-frequency is slightly greater than that of liking for sweet foods. The reason for this difference may be that the evaluation of a use-frequency of a food item is easier for subjects than the evaluation of liking. In addition, variation in liking scores may arise because in our questionnaires many of the food names refer to several types of foods. For example, sweet desserts, a variable often used, includes numerous

different dishes for which liking may vary greatly. However, by combining all sweet desserts, the use-frequency estimate may reflect reality.

The heritability of food preferences was also determined in the study of Krondl et al. (1982). Food preferences were measured by rating foods (stimuli presented as a list of food names) using a 5-point hedonic scale. The sweet foods included were honey, jam, ice cream, and doughnut. Using the Holzinger index, heritability estimates of 0.49, 0.44, and 0.16 were obtained for honey, ice cream, and doughnuts, respectively, but all three estimates were nonsignificant. For jam, DZ twins resembled each other more than MZ twins, and the heritability estimate was thus not calculated.

Other related studies have exploited slightly different instrument such as sugar intake or food intake diaries. The intake of macronutrients and of 13 food items, among these two sweet food items (candy and ice cream), was measured by de Castro (1998) using food intake diaries. Data from male and female twins provided results very similar to ours; the heritability estimate for intake of candy was 42% and for intake of ice cream 48%. Hur et al. (1998) studied macronutrient intakes of twins reared apart and their spouses, and thus, had an extraordinary study design. They reported a heritability of 24% for intake of simple carbohydrates. In general, they found that around 30% of the variance in diet is genetically determined, which is a strong confirmation of the results of ordinary twin studies with only twins reared together.

6.2.2 Age and sex differences in variance of food use patterns

Several earlier studies have evaluated the contribution of genetic and environmental factors to food (Heitmann et al., 1999; Krondl et al., 1983; van den Bree et al., 1999) and nutrient (Hur et al., 1998; de Castro, 1993; Heller et al., 1988) intake of adults, and all data have indicated that childhood family environment does not affect food use in adulthood. However, in 4- to 5-year-old children, family environment has been shown to influence food preferences (Breen et al., 2006). Family resemblance for food preferences is surprisingly low among adults. For example, Rozin (1991) found that whereas parent-child correlations for values (e.g. conservativeness, religiosity, homosexuality, and abortion) were high among 118 students and their parents (mean r=0.54), the correlations for food preferences were very low (mean r=0.17). A possible explanation of this "family paradox" is that the parents originally have different food preferences (due to genetic and environmental differences) and thus give "mixed messages" to the child on whether to like a food or not. Supposedly this also implies to the child that it is acceptable to disagree on taste preferences, allowing more variation within the family.

Based on previous studies, whether family environment plays a role in food use of young adults who have just recently left their parents' home was unknown. Study (I)

showed that the effect of family environment ceases after childhood, implying that proportional contributions of environmental and genetic effects change with age. If transition periods exist during which the determinants of variance in food use change within a couple of years, special attention should be paid to development or maintenance of healthy eating patterns during these phases. Otherwise, the resulting change in food habits may lead to worsening of dietary patterns. Comparing our results with those of Breen et al. (2006) suggests that such a transition period occurs between childhood and young adulthood. This hypothesis is supported by the increase in body weight that often takes place during young adulthood (Pietiläinen et al., 2004), although other factors, such as growth cessation and diminishing physical activity, may also contribute to the weight gain at this age. However, no longitudinal studies to confirm changes in variance components of food use along age have been performed.

Studies I, II, and IV all included both males and females, but the most similar proportions of the sexes were in Study I. In addition, special attention was focused on sex differences in the analyses. The sex-specific genetic effects, i.e. different genes influencing a trait in males and females (based on lower within-pair correlations among opposite-sex than same-sex DZ twins) were significant for three of four patterns of food use: "healthy foods", high-fat foods, and sweet foods. The only food use pattern unaffected by sex-specific genetic effects was that related to meat, which was instead influenced by common environmental factors. These results encourage consideration of sex effects whenever studying variation in dietary phenotypes. The identification of genetic elements influencing traits may be easier, if considering that different genes may influence the traits in males and females.

6.2.3 Dieting behaviors and body mass index

In Study IV, we considered three dieting behaviors measured by the TFEQ-R18. This was the first time that the heritability of these factors was determined. Tholin et al. (2005) had examined the heritability of the factors of a very similar instrument, the TFEQ-R21 (with 21 items), in male twins. Both our findings and their results suggest that the heritability of Cognitive Restraint and Uncontrolled Eating is higher than that of Emotional Eating. The proportional contribution of genetic effects to the variance (heritability) of Cognitive Restraint was significantly higher in females (57%) than in males (29%). Steinle et al. (2002), who applied the original TFEQ with 51 items and a different factor structure, observed a heritability of 28% for Restraint. It is noteworthy that although the name of the trait refers to a cognitive decision, the variance in the trait is to a large extent determined by genetic factors, especially in females.

Another interesting finding regarding sex differences was that while the heritability of Emotional Eating was 43% in females, it was nonsignificant in males. Females also rated higher on this scale. For Uncontrolled Eating, the heritability estimates for

the sexes were quite similar (52% for males, 58% for females), but males had higher mean on this trait than females. Thus, males appear to have higher tendency towards loss of control over eating, but disinhibition in emotional situations is more typical of females. The heritability obtained for BMI was very similar to findings in other European studies (Schousboe et al., 2003), namely that variation in obesity is largely genetically determined, and to lesser extent by environmental factors.

6.3 Genetic and environmental associations among variables

Several correlations were observed among the measures for hedonic responses to sweetness (III). The tetravariate Cholesky modeling showed that the correlation (r=0.23) between the liking rating for the 20% sucrose solution and the reported liking for sweet foods was due to genetic effects (genetic r=0.27). This result implies that these traits are influenced by the same genetic elements and further validates the sweet taste perception test. The variables are associated although the measurements were taken in different situations: the questionnaire was filled in at home and the sweet taste perception test was completed during the clinic visit.

The questionnaire phenotypes measuring sweet taste preference, namely use-frequency, liking, and craving for sweet foods, were all intercorrelated (r=0.30-0.50), and, on average, 62% of the phenotypic variation was explained by a genetic correlation. The rest of the trait correlations were explained by environmental factors specific to a member of the twin pair. However, the relatively low correlations imply that these commonly used measures of sweet taste preference may measure different aspects of the phenomenon. Due to limited resources, we were unable to measure twins' sugar intake by food intake diaries or wider food-frequency questionnaires, and thus, which of the measures predicts intake of sugar remains unknown. Most probably only one test would be insufficient to predict dietary choices (Mattes & Mela, 1986). The results warrant eludication of the relationships among the different measures.

The relationship of liking and use-frequency of sweet foods to dieting behaviors and BMI was further examined in Study IV. These results showed that BMI was associated with dieting behaviors, but not with use of or liking for salty or sweet fatty foods. However, the liking for sweet and fatty foods was associated with Emotional Eating. The correlation was mediated through genetic factors, and as genetic factors significantly explained the variation only in females, the results suggest that women's impulses to eat in emotional situations are directed towards sweet foods.

The relationship between BMI and use of fatty foods appears to be mediated through dieting behaviors (**Figure 10**). The finding is very significant in terms of background of obesity. Reported food intake as such is a poor predictor of BMI, an observed in many studies (Hill & Prentice, 1995). Our results imply that the

measurement of unhealthy dieting behaviors, instead of diet directly, may provide valuable information when examining the determinants of body weight.

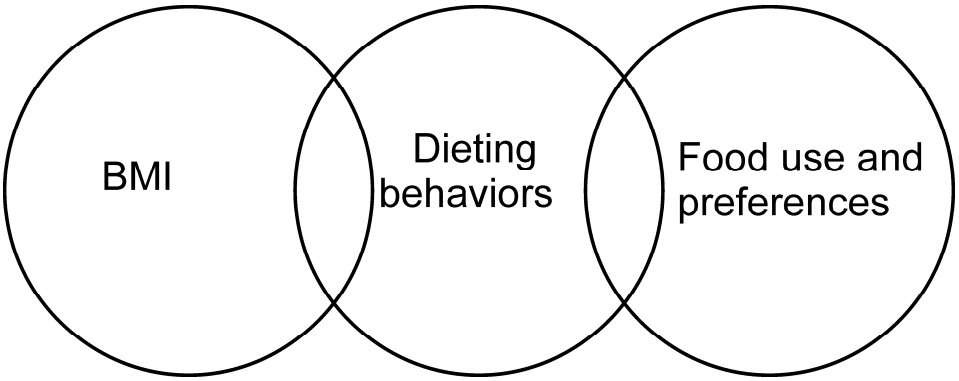


Figure 10. ***Schematic representation of the relationships between BMI, dieting behaviors, and diet.***

6.4 Location of genetic elements influencing traits

The strongest linkage result was obtained for use-frequency of sweet foods on chromosome 16p11.2. No obvious genes affecting sweet taste preferences were identified in the area. However, in the other chromosomal arm (Chr16q12.2), a variant predisposing to type 2 diabetes and affecting BMI has been identified in the *FTO* gene (Frayling et al., 2007). The area had earlier been linked to taste-related traits: Drayna et al. (2003) found a LOD score of 3.33 for PTC tasting ability at 14 cM in a scan conditional for QTL on chromosome 7. Fine-mapping and identification of influencing elements on chromosome 16 are underway.

None of the linkage peaks were found at the location of the sweet taste receptor genes. This, in addition to liking ratings but not intensity ratings of the sweet solution being inherited, implies that the hedonic and intensity responses of sweetness are separate.

The PROP filter paper intensity ratings yielded no significant or suggestive linkage results. Earlier studies have found linkages on chromosomes 5p15 (Reed et al., 1999), 7q (Drayna et al., 2003; Kim et al., 2003; Hansen et al., 2006b), and 16p (Drayna et al., 2003). The discordance between the earlier findings and ours is probably due to the method applied for the PROP sensitivity measurement. As shown by Hansen et al. (2006b), the measurement of tasting ability using the filter paper produces phenotypes that are only poorly associated with the *TAS2R38* gene on chromosome 7q.

6.5 Methodological considerations

6.5.1 Subjects

Subjects recruited to the studies were not selected in terms of sensory performance or food preferences. In twin studies, the selection criterion was being a twin of certain age without selecting for particular disease of trait. Studies I, III, and IV applied large, population based twin cohorts. FinnTwin16 and FinnTwin12 are longitudinal studies of five consecutive birth cohorts of all twin pairs born in Finland (Kaprio et al., 2002). With high response rates (>85%), they can be regarded as representative sample of Finnish population of the certain age. The UK twin adult registry consists of adult (16 to 85 years old) twins from all over the United Kingdom and Ireland (Spector & Williams, 2006). However, the registry predominantly consists of same sexed female twins, because the initial focus of the study group was on diseases more common on females than males. Thus, the data from this registry does not fully allow sex comparisons. In twin studies (I, III, IV) the sample sizes are large and thus the analyses have strong statistical power.

In the Finnish family study (Study II), the sample size was significantly lower. However, the heritability estimates obtained in this study can be regarded as highly accurate as they were replicated within the larger twin studies. The marker genotypes had high information content meaning that the data was well suited to linkage analysis.

6.5.2 Chemosensory measurements

The aqueous sucrose and NaCl solutions are very simple stimuli which as such are not part of a normal diet. However, presenting such stimuli prevented the effect of cultural differences on hedonic responses. Holt et al. (2000) demonstrated that liking for sweetness in a water solution is not strongly correlated with liking for sweetness in different media (foods). In their study, the most preferred level of sucrose in water did not predict the most preferred level of sucrose in foods. The correlation between hedonic responses to sweetness in water and orange juice was low (r=0.14). A similar result was obtained when we developed the test for sweetness perception (r between liking ratings for similar levels of sweetness in water and in orange juice=0.25)

However, as the goal was to develop a simple and easy measure that could be used in international studies with limited time resources, this test proved to be useful. In addition, the phenotype was required to measure the same underlying hedonic response to sweetness as the 18.75% sucrose solution in a series and provide sufficient variation in a general population (see Section 4.2.1.1). All of these

requirements were met, and thus, we concluded that 20% sucrose in water is a useful and rapid tool to screen for the degree of hedonic response to sweetness.

The decision to include PROP in the study protocols (II, III) was based PROP tasting ability being the only taste-related trait known to be partly genetically determined. Because time available for chemosensory testing of the subjects was limited, application of more time-consuming methods, such as determination of taste thresholds, was impossible. To quickly but reliably screen for individual differences in PROP sensitivity, the filter paper method was chosen. Hansen et al. (2006b) examined the relationship of this measure to intensity ratings of a PROP solution (0.1%). They observed that partly different genes are responsible for the genetic variation in the outcomes of the two methods. Thus, our measurement with the filter paper method is not fully comparable with the results of solution tests. However, PROP sensitivity screening was not the main focus of our studies, but instead acted as a positive control for heritability. The filter paper method was suitable for this purpose. In addition, high heritability estimate obtained for the PROP intensity rating confirmed that the reason for the low heritability estimate of intensity ratings of sweet or salty solutions was not difficulty in usage of the LMS instrument (Green et al., 1993).

6.5.3 Food behavior questionnaires

When using behavioral questionnaires, it is noteworthy that the outcomes are indirect measures of human behavior that can be affected by misreporting. The misreporting may be caused by several factors. First, the questionnaire should be validated carefully to ensure that it measures the desired trait. Often, the questionnaires are validated only in their original language, then translated by the researchers using them, without any further validation. The meaning of the items may remain similar, but differences in the nuances, such as in the seriousness of the situation, can be caused by language differences (Berkanovic, 1980).

For food items and dietary habits, differences between countries may be caused by cultural differences. For example, if studying the liking for sweet foods, which sweet foods are most frequently used in the population studied, should be considered. If some of the most popular food items are left out, an essential part of the variation among individuals may be missed. In our study, nearly the same food items were presented to British and Finnish respondents. However, the British collaborators thought that the sweet food items presented to Finnish respondents will apply for the British population. In the case of salty and fatty foods, some modifications to the questionnaires were made in each country to include specific popular food items.

6.5.4 Statistical methods

Heritability analysis in families is a measure of familial correlation. Thus, it may include environmental variance that makes family members more similar to each other. However, the heritability estimates obtained in our family study were very similar to those in our twin studies.

The assumptions of a classical twin design: equal environment, random mating, and the absence of gene-environment interactions, are the basis for the analysis and the fulfillment of these for the trait being studied should be ensured. The equal environment assumption means that a twin pair raised together experiences a similar environment regardless of zygosity. Although it is difficult to estimate whether MZ twins are treated more similarly than DZ twins, it has been suggested that the environment (e.g. parent's) does not cause the phenotypic similarity of MZ twins or the differences between DZ twins, but the environment merely responds to the similarity of the MZ twins by treating them similarly (Kendler et al., 1993). If MZ twins were treated more similarly than DZ twins, resulting in higher MZ pair-wise correlations, it would cause an increase in the heritability estimates. Another assumption of the classical twin design is random mating, meaning that individuals do not choose partners based on similarity on the trait in question. In the case of food habits, these two effects are highly unlikely to occur. However, the third assumption, an absence of gene-environment interactions, may be relevant when studying eating behavior. Gene-environment interactions mean that individuals with different genetic make-up respond to the environment differently. If the environmental factor is shared by the twin pair, the effect of gene-environment interactions is estimated as part of the additive genetic variance. Thus, the estimate of additive genetic effects may also include genetic differences in susceptibility to environmental factors. If the environmental factor interacting with genetic factors is not shared by the twins, it is included in the specific environmental variation (Neale & Cardon, 1992).

In twin studies, we first decomposed the variation to additive genetic (A), common environmental (C), and specific environmental effects (E). For each phenotype, it was noted that the common environmental effects were not significant. Persons living in the same household may consume the same foods, as they often sit down to the same meals, and thus, the common environment should have a major effect. This may have been the case had we been able to measure the family environment of the twins at the time of the study. Instead, what we measured was the correlation between co-twins. All of the twins in our studies were adults, and thus, often they had not been living together for years. The measurement of the current family environment of adult twins can be accomplished by measuring the traits in current family members. Most often, the spouses of the twins are included. One study of twins reared apart and nontwin individuals (mostly spouses) has concentrated on dietary variables (Hur et al., 1998). In general, they found that approximately 20-

30% of the variance in diet reported by a food-frequency questionnaire was attributable to genetic factors. Spousal correlations for nutrient intakes were moderate and positive (0.01–0.37, mean 0.22). However, the common environmental effects were not significant in the final models.

7 CONCLUSIONS AND FUTURE PROSPECTS

This study demonstrated that sweet taste preferences are partly inherited. This finding was replicated in two populations using both chemosensory measurements and behavioral questionnaires. When this study was initiated, investigations of taste genetics had mainly concentrated on bitter taste. Contrary to bitter compounds, the intensity perception of sucrose solution does not appear to be genetically determined. By contrast, however, almost half of the variation in the liking for sweet taste is inherited. If perceived intensity and liking/disliking are strongly associated, as expected in the case of bitter tastes, the taste threshold or intensity rating of a suprathreshold solution may be associated with food acceptance ratings. However, if the two perceptions are not very strongly linked, as in case of sweetness perception, the hedonic response to a stimulus likely provides a better aid than the intensity ratings for predicting food acceptance.

Findings also revealed that the intensity perception of sweetness is only weakly, if at all, inherited and that different measures of sweet taste preference partly reflect different aspects of the phenomenon. Thus, when investigating the genetic factors underlying sweet taste perception, the choice of the instrument(s) should be made carefully. It is crucial to measure the hedonic responses in addition to the intensity ratings or threshold determination, and even better measurement can be obtained by implementing behavioral questionnaires to assess responses to sweet foods. Inclusion of more than one instrument is recommended to obtain a more extensive view of the study population's response to sweetness.

A locus influencing the use-frequency of sweet foods was identified on chromosome 16. It is noteworthy that no linkage was found on chromosome 1, where the genes encoding the subunits of the sweet taste receptor are located. This finding also implies that factors apart from tasting ability determine behavior towards sweet foods in humans. Studies on rodents, mostly mice, have demonstrated that polymorphisms in the sweet taste receptor gene *Tas1r3* influence behaviors towards sweetness. As food choice in humans is more complicated than in mice, which are thought to eat any acceptable food, the extrapolation of findings in mice to human eating behavior may be of limited value. Although more laborious and expensive, the examination of human subjects is highly encouraged when studying food behavior.

When examining taste or food preferences in humans, the effect of cultural and attitudinal factors cannot be ignored. The role of social factors and dietary education in the development of healthy dietary habits is very important. Young adulthood often includes changes in dietary habits, unfortunately generally in an unhealthy direction, and is a period in which the liability for weight gain is increased. However, this study showed that the effect of family environment ceases soon after a child leaves the parental home. Changes in the variance components of food behavior with age should

be investigated in more detail in longitudinal studies. Identification of age-dependent alterations in factors influencing food behavior and weight gain may reveal vulnerable age groups to which dietary education should be targeted.

Although the link between diet and obesity is broadly recognized, many studies have failed to establish correlations between food intake as measured by dietary questionnaires and BMI. In a study examining the relationships between dieting behaviors, BMI, and liking for and use of fatty foods, we also did not observe correlation between BMI and responses to fatty foods. However, BMI was positively correlated with dieting behaviors, which, in turn, were correlated with fatty food traits. These results imply that the relationship between BMI and food use is not straightforward, and individuals' attitudes and conscious and unconscious behaviors play a major role in the development of obesity.

In conclusion, this study broadened our understanding of the factors underlying human eating behavior. Both genetic and environmental factors were shown to be important in determining the degree of liking for sweetness. This study thus provides information additional research on taste genetics as well as also for epidemiologic studies on human nutrition in general.

8 ACKNOWLEDGMENTS

This study was carried out at the Department of Food Technology, University of Helsinki and at the Department of Molecular Medicine, National Public Health Institute (KTL), Helsinki during 2004-2007. I wish to thank the director of KTL, Professor Pekka Puska, the heads of the Department of Molecular Medicine Professor Leena Palotie and Docent Anu Jalanko, and the head of the Department of Food Technology, Tapani Alatossava, for providing the research facilities for this multidisciplinary work. This project was financially supported by the Academy of Finland and the Biomedicum Helsinki Foundation.

I was supervised by two outstanding scientists, Professor Hely Tuorila and Docent Markus Perola. I thank both of you for the open-minded attitude needed in this project combining two scientific fields. Hely, thank you for your contagious enthusiasm for sensory science, for teaching me a lot, and for your help with big or small problems. You responded quickly to my emails and always found time in your busy schedule for discussions. Markus, thank you for introducing me to the world of genetics. I have enjoyed working with a person dedicated his research. Thank you also for taking care of our team spirit and for sharing some of your knowledge of enology.

Professor Mika Kähönen and Dr. Dennis Drayna are thanked for reviewing this thesis and for comments that improved the manuscript. Dr. Danielle R. Reed is thanked for accepting the role of opponent in the thesis defense.

I thank all personnel at the Department of Food Technology. Professor Lea Hyvönen, my kustos, is thanked for support during the last phases of this work. I am grateful to former and present members of the Sensory Food Science Group: Sari, Kaisu, Antti, Anna, Sanna-Maija, Kevin, Hanna, and Mari and all other members of our lunch and coffee group for creating an enjoyable working atmosphere. Sari has helped me numerous times with practical, bureaucratic, and statistical troubles, and Kaisu was there for me when I needed a hand in the lab (or wanted to hear our beautiful dialect). I have worked side by side with Antti over these four years and thank him for good company.

I worked in yet another inspiring lab, at the Department of Molecular Medicine, KTL. Leena, the head of the Genetic Epidemiology Research Unit infused us all with her scientific drive and positive attitude. I thank the senior scientists Iski, Samuli, Kaisa, and Marjo for their support. Marjo, thank you for your help during the writing and printing of the thesis. Members of the Quantitative Genetics Group: Kati, Mervi, Johannes, Sampo, Kirsi, Elina, Katja, Annina, Henna, and “hang-around” member Tero, are thanked for good company and for sharing their knowledge in genetics with me. I owe my deepest gratitude to Sampo, Tero, Johannes, and Kaisa for advising me regarding genetics software. I wonder how long all the analyses would have taken without you. Anna, Nora, Tiia, Mari, Jonas, Henna, Iita, Heli, Jonna, Taina, Tanja, Pia, Olli, Jussi, PP, Markus, Krista, and all other lab members are thanked for good company, encouragement, and loads of fun.

I'm very grateful to "my third group", the Twin Research Unit, "kaksari", for the welcoming and friendly atmosphere. I am indebted for Professor Jaakko Kaprio for explaining statistics to me and for helping with even the smallest practical issues of the sensory testing of the twins. Dr. Karri Silventoinen is warmly thanked for teaching me the basics of twin modeling, which was not an easy task, but he succeeded in making me very interested in the secrets of Mx. Professor Aila Rissanen and Dr. Kirsi Pietiläinen are thanked for generous support when writing my first manuscript. I also thank the students who collected the data: Johanna, Maarit, Laura, Kirsi, and Jenni.

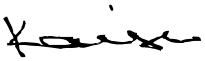
The Migraine Family Study Group, Professor Aarno Palotie, Dr. Maija Wessman, Dr. Mikko Kallela, Dr. Markus Färkkilä, and Tanja Moilanen are gratefully acknowledged for their collaboration and for allowing us to phenotype the participants of their study. In the Twin Research and Genetic Epidemiology Unit of St. Thomas' Hospital, London, more than one thousand British twins participated in our taste tests and filled in a bunch of questionnaires. Professor Tim Spector, Dr. Lynn Cherkas, Ursula Perks and all nurses are thanked for collecting this data. Kyllikki Kilpi from Finnsugar Ltd. is thanked for providing the pre-packed samples. Naturally, this study would not have been possible without the twins and family members participating in the studies, thank you!

The supportive and always stimulating company of my friends has been invaluable. I'm grateful to Tiina H, Ulla, Paula, Anna G, and Tiina P for all the enjoyable moments before, during, and after this thesis. The friends I got to know in Kuopio: Katariina, Miia, Minna, Johanna, Marika, Kirsi, Anna K, Carme, Mareike, and Silvia, I thank for being there for me, despite not living nearby. Hanna, our horseback riding trips were really therapeutic. Anne, thank you for being the perfect flat mate and for your friendship.

Äiti, Ana, and Saana are thanked for bringing me up as an autonomous person with the courage to work for the things she wants. Saana, I am truly blessed to have an energetic sister like you who understands that dogs and horses can be an important part of life. All of my relatives and Jussi's family are thanked for their support and for the interest they have shown towards my work. Pertti and Raili, the possibility to relax at the mökki has surely helped me to finish this thesis.

Most importantly, I would like to thank Jussi for all the wonderful adventures abroad and in our everyday life. I'm really lucky to share the ups and downs of life with you. Thank you for your endless support, humor, love, and enthusiasm for the things that really matter (like cooking and gardening).

Helsinki, February 4th, 2008



Kaisu Keskitalo

9 REFERENCES

Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 2002;30:97-101.

Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198-211.

Bachmanov AA, Li X, Reed DR, Ohmen JD, Li S, Chen Z, Tordoff MG, de Jong PJ, Wu C, West DB, Chatterjee A, Ross DA, Beauchamp GK. Positional cloning of the mouse saccharin preference (Sac) locus. Chem Senses 2001a;26:925-33.

Bachmanov AA, Tordoff MG, Beauchamp GK. Sweetener preference of C57BL/6ByJ and 129P3/J mice. Chem Senses 2001b;26:905-13.

Barkeling B, Andersson I, Lindroos AK, Birkhed D, Rössner S. Intake of sweet foods and counts of cariogenic microorganisms in obese and normal-weight subjects. Eur J Clin Nutr 2001;55:580-5.

Beauchamp GK, Moran M. Dietary experience and sweet taste preference in human infants. Appetite 1982;3:139-52.

Beauchamp GK, Moran M. Acceptance of sweet and salty tastes in 2-year-old children. Appetite 1984;5:291-305.

Beauchamp GK, Bertino M, Engelman K. Sensory basis for human salt consumption. In: Horan MJ, Blaustein MP, Dunbar JB, Kachadorina W, Kaplan NM, Simpolous AP (eds.), NIH Workshop on Nutrition and Hypertension. New York, USA: Biomedical Information Corporation, 1985:113-24.

Bejerano G, Pheasant M, Makunin I, Stephen S, Kent WJ, Mattick JS, Haussler D. Ultraconserved elements in the human genome. Science 2004;304:1321-5.

Benton D, Greenfield K, Morgan M. The development of the attitudes to chocolate questionnaire. Person Individ Diff 1998;24:513-20.

Benton D. Role of parents in the determination of the food preferences of children and the development of obesity. Int J Obes 2004;28:858-69.

Berkanovic E. The effect of inadequate language translation on Hispanic's responses to health surveys. Am J Public Health 1980;70:1273-81.

Bertino M, Beauchamp GK, Jen KC. Rated taste perception in two cultural groups. Chem Senses 1983;8:3-15.

Bertino M, Chan MM. Taste perception and diet in individuals with Chinese and European ethnic backgrounds. Chem Senses 1986;11:229-41.

Bowen DJ, Grunberg NE. Variations in food preference and consumption across menstrual cycle. Physiol Behav 1990;47:287-91.

Breen FM, Plomin R, Wardle J. Heritability of food preferences in young children. Physiol Behav 2006;88:443-7.

Cai G, Cole SA. Bastarrachea RA, MacCluer JW, Blangero J, Comuzzie AG. Quatitative trait locus determining dietary macronutrient intakes is located on human chromosome 2p22. Am J Clin Nutr 2004;80:1410-4.

Cardello AV, Schutz HG. Numerical scale-point locations for constructing the LAM (labeled affective magnitude) scale. J Sens Stud 2004;19:341-6.

Chandrashekar J, Hoon MA, Ryba NJP, Zuker CS. The receptors and cells for mammalian taste. Nature 2006;444:288-94.

Collaku A, Rankinen T, Rice T, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. A genome-wide linkage scan for dietary energy and nutrient intakes: the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study. Am J Clin Nutr 2004;79:881-6.

Conner MT, Booth DA. Preferred sweetness of a lime drink and preference for sweet over non-sweet foods, related to sex and reported age and body weight. Appetite 1988;10:25-35.

Conner MT, Haddon AV, Pickering ES, Booth DA. Sweet tooth demonstrated: individual differences in preferences for both sweet foods and foods highly sweetened. J Appl Psychol 1988;73:275-80.

Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Varadarajan V, Zou S, Jiang P, Ninomiya Y, Margolskee RF. Detection of sweet and umami taste in the absence of taste receptor T1r3. Science 2003;301:850-3.

de Castro JM. Genes and environment have gender-independent influences on the eating and drinking of free-living humans. Physiol Behav 1998;63:385-95.

de Graaf C, Zandstra EH. Sweetness intensity and pleasantness in children, adolescents, and adults. Physiol Behav 1999;67:513-20.

Delay ER, Hernandez NP, Bromley K, Margolskee RF. Sucrose and monosodium glutamate taste thresholds and discrimination ability of T1R3 knockout mice. Chem Senses 2006;31:351-7.

Desor JA, Maller O, Turner E. Taste in acceptance of sugars by human infants. J Comp Physiol Psychol 1973;84:496-501.

Desor J, Greene L, Maller O. Preferences for sweet and salty in 9- to 15-year-old and adult humans. Science 1975;190:686-7.

Desor JA, Beauchamp GK. Longitudinal changes in sweet preferences in humans. Physiol Behav 1987;39:639-41.

Dietary Guidelines for Americans, 2005. The Department of Health and Human Services and the Department of Agriculture. Released Janury 12th, 2005. Internet: http://www.health.gov/dietaryguidelines (accesssed November 9th, 2007).

Drayna D, Coon H, Kim UK, Elsner T, Cromer K, Otterud B, Baird L, Peiffer AP, Leppert M, Utah Genetic Reference Project. Genetic analysis of a complex trait in the Utah Genetic Reference Project: a major locus for PTC tasting ability on chromosome 7q and a secondary locus on chromosome 16 p. Hum Genet 2003;112:567-72.

Drewnowksi A, Rock CL. The influence of genetic taste markers on food acceptance. Am J Clin Nutr 1995;62:506-11.

Drewnowski A. Taste preferences and food intake. Annu Rev Nutr 1997a;17:237-53.

Drewnowski A, Henderson SA, Shore AB. Genetic sensitivity to 6-n-propylthiouracil (PROP) and hedonic responses to bitter and sweet tastes. Chem Senses 1997b;22:27-37.

Drewnowski A, Henderson SA, Shore AB, Barratt-Fornell A. Nontasters, tasters, and supertasters 6-*n*-propylthiouracil (PROP) and hedonic response to sweet. Physiol Behav 1997c;62:649-55.

Drewnowski A, Henderson SA, Levine A, Hann C. Taste and food preferences as predictors of dietary practices in young women. Public Health Nutr 1999:2;513-9.

Drewnowski A, Hann C. Food preferences and reported frequencies of food consumption as predictors of current diet in young women. Am J Clin Nutr 1999;70:28-36.

Falk CT, Rubinstein P. Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. Ann Hum Genet 1987;51:227-33.

Finnish Nutrition Recommendations. National Nutrition Council, Nutrition Recommendation Section. Ministry of Agriculture and Forestry, Edita Publishing Oy, Helsinki, Finland, 2005.

Foster MW, Sharp RR. Beyond race: towards a whole-genome perspective on human populations and genetic variation. Nat Rev Genet 2004;5:790-6.

Frayling TM, Timpson NJ, Weedon MN et al. A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889-94.

Frye CA, Crystal S, Ward K, Kanarek RB. Menstrual cycle and dietary restraint influence taste preferences in young women. Physiol Behav 1994;55:561-7.

Ganchrow JR, Mennella JA. Ontogeny of human flavor perception. In: Doty RL (ed.), Handbook of olfaction and gustation. 2nd Ed. Philadelphia, USA: Marcel Dekker, 2003;823-46.

Gilmore MM, Murphy C. Aging is associated with increased Weber ratios for caffeine, but not for sucrose. Percept Psychophys 1989;46:555-9.

Green BG, Shaffer GS, Gilmore MM. Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties. Chem Senses 1993;18:683-702.

Grogan SC, Bell R, Conner M. Eating sweet snacks: gender differences in attitudes and behaviour. Appetite 1997;28:19-31.

Hansen JL, Reed DR, Wright MJ, Martin NG, Breslin PAS. Heritability and genetic covariation of sensitivity to PROP, SOA, quinine, HCl, and caffeine. Chem Senses 2006a;31:403-13.

Hansen JL, Reed DR, Wright MJ, Martin NG, Breslin PA. Replication of linkage and association of PROP perception to chromosome 7 and suggestion of novel loci on chromosome 6. Abstract in AChemS meeting 2006. Chem Senses 2006b;31:479.

Harel-Raviv M, Laskaris M, Chu KS. Dental caries and sugar consumption into the 21st century. Am J Dent 1996;9:184-90.

Heitmann BL, Harris JR, Lissner L, Pedersen NL. Genetic effects on weight change and food intake in Swedish adult twins. Am J Clin Nutr 1999;69:597-602.

Henkin RI, Shallenberger RS. Aglycogeusia: the inability to recognize sweetness and its possible molecular basis. Nature 1970;227:965-6.

Hill JO, Prentice AM. Sugar and body weight regulation. Am J Clin Nutr 1995;62:264S-74S.

Holt SHA, Cobiac L, Beaumont-Smith NE, Easton K, Best DJ. Dietary habits and the perception and liking of sweetness among Australian and Malaysian students: a cross-cultural study. Food Qual Pref 2000;11:299-312.

Hunter DJ, Kraft P. Drinking from the fire hose – statistical issues in genomewide association studies. N Engl J Med 2007;357;436-9.

Hur Y-M, Bouchard TJ Jr., Eckert E. Genetic and environmental influences on self-reported diet: a reared-apart twin study. Physiol Behav 1998;64:629-36.

Inoue M, McCaughey SA, Bachmanov AA, Beauchamp GK. Whole nerve chorda tympani responses to sweeteners in C57BL/6ByJ and 129P3/J mice. Chem Senses 2001;26:915-23.

International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature 2004;431:931-45

Issanchou S, Nicklaus S. Measuring consumers' perception of sweet taste. In: Spillane (ed.), Optimising sweet taste in foods. Boca Raton, FL, USA: Woodhead Publishing, 2006:97-131.

Jamel HA, Sheiham A, Cowell CR, Watt RG. Taste preference for sweetness in urban and rural populations in Iraq. J Dent Res 1996;75:1879-1884.

Jorde LB, Wooding SP. Genetic variation, classification, and ´race´. Nat Genet 2004;36:S28-S33.

Kampov-Polevoy AB, Alterman A, Khalitov E. Garbutt JC. Sweet preference predicts mood altering effect of and impaired control over eating sweet foods. Eat Behav 2006;7:181-7.

Kaprio J, Pulkkinen L, Rose R J. Genetic and environmental factors in health-related behaviors: studies on Finnish twins and twin families. Twin Res 2002;5:366-71.

Kaprio J. Differences in smoking habits of MZ and DZ twins: a commentary on Tishler and Carey. Twin Res Hum Genet 2007;10:718-20.

Karlsson J, Persson L-O, Sjöström L, Sullivan M. Psychometric properties and factor structure of the Three-Factor Eating Questionnaire (TFEQ) in obese men and women. Results from the Swedish Obese Subjects (SOS) study. Int J Obes 2000;24:1715-25.

Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. A test of the equal-environment assumption in twin studies of psychiatric illness. Behav Genet 1993;23:21-27.

Kim U-K, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phynylthiocarbamide. Science 2003;299:1221-5.

Kim U-K, Wooding S, Riaz N, Jorde LB, Drayna D. Variation in the Human TAS1R taste receptor genes. Chem Senses 2006;31:599-611.

Koskinen S, Kälviäinen N, Tuorila H. Perception of chemosensory stimuli and related responses to flavored yogurts in the young and elderly. Food Qual Pref 2003;14:623-35.

Krondl M, Coleman P, Wade J, Milner J. A twin study examining the genetic influence on food selection. Hum Nutr Appl Nutr 1093;37:189-98.

Laeng B, Berridge KC, Butter CM. Pleasantness of a sweet taste during hunger and satiety: effects of gender and "sweet tooth". Appetite 1994;21:247-54.

Laing DG, Prescott J, Bell GA, Gillmore R, James C, Best DJ, Allen S, Yoshida M, Yamazaki K. A cross-cultural study of taste discrimination with Australians and Japanese. Chem Senses 1993;18:161-8.

Lander E, Kruglyak L, Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995;11:241-7.

Li X, Li W, Wang H, Cao J, Maehashi K, Huang L, Bachmanov AA, Reed DR, Legrand-Defretin V, Beauchamp GK, Brand JG. Pseudogenization of a sweet-receptor gene accounts for cats' indifference towards sugar. PLoS Genet 2005;1:e3.

Liao J, Schultz PG. Three sweet receptor genes are clustered in human chromosome 1. Mamm Genome 2003;14:291-301.

Liem DG, Mennella JA. Sweet and sour preferences during childhood: role of early experiences. Dev Psychobiol 2002;41:388-95.

Looy H, Weingarten HP. Facial expressions and genetic sensitivity to 6-*n*-propylthiouracil predict hedonic response to sweet. Physiol Behav 1992;52:75-82.

Ly A, Drewnowski A. PORP (6-*n*-propylthiouracil) tasting and sensory responses to caffeine, sucrose, neohesperdin dihydrochalcone and chocolate. Chem Senses 2001;26:41-7.

Lähteenmäki L, Tuorila H. Attitudes towards sweetness as predictors of liking and use of various foods. Ecol Fodd Nutr 1994;31:161-70.

Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. Am J Clin Nutr 2006;84:274-88.

Mattes RD, Mela DJ. Relationship between and among selected measures of sweet-taste preference and dietary intake. Chem Senses 1986;11:523-39.

Martin NG, Martin PG. The inheritance of scholastic abilities in a sample of twins. Ascertainments of the sample and diagnosis of zygosity. Annals Human Genetics 1975;39:213-8.

Martin CK, O'Neil PM, Pawlow L. Changes in food cravings during low-calorie and very-low-calorie diets. Obesity 2006;14:115-121.

McCaughey SA. Taste-evoked response to sweeteners in the nucleus of the solitary tract between C57BL/6ByJ and 129P3/J mice. J Neurosci 2007;27:35-45.

Mennella JA, Pepino YM, Reed DR. Genetic and environmental determinants of bitter perception and sweet preferences. Pediatrics 2005;115:216-22.

Miller IJ Jr, Reedy FE Jr. Variations in human taste bud density and taste intensity perception. Physiol Behav 1990;47:1213-1219.

Murphy C. Nutrition and chemosensory perception in the elderly. Crit Rev Food Sci Nutr 1993;33:3-15.

Naim M, Huang L, Spielman AI, Shaul ME, Aliluiko A. Stimulation of taste cells by sweet compounds. In: Spillane JW (ed.), Optimising sweet taste in foods. Boca Raton, FL, USA: Woodhead Publishing, 2006:4-29.

Neale MC, Cardon LR. Methodology for genetic studies of twins and families. Dordrecht, Germany, Kluwer Academic Publishers BV, 1992.

Neale MC, Boker SM, Xie G, Maes HH. Mx: statistical modeling. 5th ed. Richmond, VA, USA, Department of Psychiatry, Virginia Commonwealth University, 1999.

O'Connell JR, Weeks DE. PedCheck: A Program for identification of genotype incompabilities in linkage analysis. Am J Hum Gen 1998;63:259-266.

Pangborn RM. Influence of hunger on sweetness preferences and taste thresholds. Am J Clin Nutr 1959;7:280-7.

Pangborn R. Individualigy in responses to sensory stimuli. In: Solms J, Hall RL (eds.), Criteria of food acceptance. 6th Ed. Zürich, Switzerland: Foster Publishing, 1981;177-219.

Pasquet P, Monneuse M-O, Simme B, Marez A, Hladik C-M. Relationship between taste thresholds and hunger under debate. Appetite 2006;46:63-66.

Peltonen L, Jalanko A, Varilo T. Molecular genetics of the Finnish disease heritage. Hum Mol Genet 1999;8:1913-23.

Peeters H, Van Gestel S, Vlietinck R, Derom C, Derom R. Validation of a telephone questionnaire in twins of known zygosity. Behav Genet 1998;28:159-63.

Peryam DR, Pilgrim FJ. Hedonic scale method of measuring food preferences. Food Technol 1957;11:S9-S14.

Pfaffman C, Bartoshuk LM, Mc Burney DH. Taste psychophysics. In: Beidler LM (ed.), Handbook of sensory physiology, Vol 4, Part 2: Gustation. New York, USA: Springer-Verlag, 1971:87-88.

Pietiläinen KH, Rissanen A, Laamanen M, Lindholm AK, Markkula H, Yki-Järvinen H, Kaprio J. Growth patterns in young adult twin pairs discordant and concordant for obesity. Twin Res 2004;7:421-9.

Posthuma D, Beem AL, de Geus EJC, van Baal GCM, von Hjelmborg JB, Iachine I, Boomsma DI. Theory and practice in quantitative genetics. Twin Res 2003;6:361-76.

Prescott J. Comparisons of taste perceptions and preferences of Japanese and Australian consumers: overview and implications for cross-cultural sensory research. Food Qual Pref 1998;9:393-402.

Prescott J, Tepper B (eds). Genetic variation in taste sensitivity. Marcel Dekker cop. New York, USA, 2004.

Reed DR, Nanthakumar E, North M, Bell C, Bartoshuk LM, Price RA. Localization of a gene for bitter-taste perception to human chromosome 5p15. Am J Hum Genet 1999;64:1478-80.

Reed DR, Li S, Li X, Huang L, Tordoff MG, Starling-Roney R, Taniguchi K, West DB, Ohmen JD, Beauchamp GK, Bachmanov AA. Polymorphisms in the taste receptor gene (*Tas1r3*) region are associated with saccharin preference in 30 mouse strains. J Neurosci 2004;24(4):938-46.

Reid M, Hammersley R. Effect of carbohydrate intake on subsequent food intake and mood state. Physiol Behav 1995;58:421-7.

Roininen K, Lähteenmäki L, Tuorila H. Quantifications of consumer attitudes to health and hedonic characteristics of foods. Appetite 1999;33:71-88.

Roininen K, Tuorila H, Zandstra EH, de Graaf C, Vehkalahti K, Stubenitsky K, Mela DJ. Differences in health and taste attitudes and reported behavior among Finnish, Dutch and British consumers: a cross-national validation of the Healh and Taste Attitude Scales (HTAS). Appetite 2001;37:33-45.

Rolls ET, Scott TR. Central Taste Anatomy and Neurophysiology. In Doty RL (ed.) Handbook of olfaction and gustation. 2nd Ed. Marcel Dekker, Philadelphia, USA, 2003;651-77.

Roper SD. Signal transduction and information processing in mammalian taste buds. Pflügens Arch – Eur J Physiol 2007;454:759-76.

Rozin P. Preference and affect in food selection. In Kroeze JHA (ed.) Preference behaviour and chemoreception. Information Retrieval Ltd, London, 1979:389-302.

Rozin P, Vollmecke TA. Food likes and dislikes. Ann Rev Nutr 1986;6:433-56.

Rozin P. Family resembelance in food and other domains: the family paradox and the role of parental congruence. Appetite 1991;16:93-102.

Salbe AD, DelParigi A, Pratley RE, Drewnowski A, Tataranni PA. Taste preferences and body weight changes in an obesity-prone population. Am J Clin Nutr 2004;79:372-8.

Sarna S, Kaprio J, Sistonen P, Koskenvuo M. Diagnosis of twin zygosity by mailed questionnaire. Hum Hered 1978, 28:241-54.

Schiffman SS, Graham BG, Sattely-Miller EA, Peterson-Dancy M. Elevated and sustained desire for sweet taste in African-Americans: a potential factor in the development of obesity. Nutrition 2000;16:886-93.

Schousboe K, Willemsen G, Kyvik KO, Mortensen K, Boomsma DI, Cornes BK, Davis CJ, Fagnani C, Hejlmborg J, Kaprio J, De Lange M, Luciano M, Martin NG, Pedersen N, Pietiläinen KH, Rissanen A, Saarni S, Sørensen TI, Van Baal GC, Harris JR. Sex differences in heritability of BMI: a comparative study of results from twin studies in eight countries. Twin Res 2003;6:409-21.

Schulze TG, McMahon FJ. Genetic association mapping at the crossroads: which test and why? Overview and practical guidelines. Am J Med Genet 2002;114:1-11.

Schutz HG, Cardello AV. A labeled affective magnitude (LAM) scale for assessing food liking/disliking. J Sens Stud 2001;16:117-59.

Scinska A, Sienkiewicz-Jarosz H, Kuran W, Ryglewicz D, Rogowski A, Wrobel E, Korkosz A, Kukwa A, Kostowski W, Przemyslaw B. Depressive symptons and taste reactivity in humans. Physiol Behav 2004;82:899-904.

Sclafani A. Sucrose motivation in sweet "sensitive" (C57BL/6J) and "subsensitive" (129P3/J) mice measured by progressive ratio licking. Physiol Behav 2006;87:734-44.

Sham P. Statistics in human genetics. Oxford UniversityPress, New York, USA, 1998.

Smith Richards BK, Belton BN, Poole AC, Mancuso JJ, Churchill GA, Li R, Volaufova J, Zuberi A, York B. QTL analysis of self-selected macronutrient diet intake: fat, carbohydrate, and total kilocalories. Physiol Genomics 2002;11:205-17.

Spector TD, Williams FM. The UK Adult Twin Registry. Twin Res Hum Genet 2006;9:899-906.

Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin independent diabetes mellitus (IDDM). Am J Hum Genet 1993;52:506-16.

Steiner JE. Facial expressions of the neonate infant indicating the hedonics of food-related stimuli. In: Weiffenbach JM (ed.): Taste and development: genesis of sweet preference. U.S. Dept. H. E. W. Publications, Betsheda, MD, USA, 1977:173-88.

Steiner JE, Sgan-Cohen HD, Nahas J. Sweet preferences and dental caries among Bedouin youth in Israel. Community Dent Oral Epidemiol 1984;12:386-9.

Steinle NI, Hsueh W-C, Snitker S, Pollin TI, Sakul H, St Jean PL, Bell CJ, Mitchell BD, Shuldiner AR. Eating behavior in the Old Order Amish: heritability analysis and a genome-wide linkage analysis. Am J Clin Nutr 2002;75:1098-106.

Strachan T, Read AP. Human Molecular Genetics, 3rd Ed. Garland Press, New York, USA, 2004.

Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res 1985;29:71-83.

Terwilliger JD, Göring, HHH. Gene mapping in the 20th and 21st centuries: statistical methods, data analysis, and experimental design. Hum Biol 2000;72:63-132.

Than TT, Dealy ER, Maier ME. Sucrose threshold variation during the menstrual cycle. Physiol Behav 1994;56:237-9.

Tholin S, Rasmussen F, Tynelius P, Karlsson J. Genetic and environmental influences on eating behavior: the Swedish Young Male Twins Study. Am J Clin Nutr 2005;81:564-9.

Tishler PV, Carey VJ. Can comparison of MZ- and DZ-twin individuals concordance rates be used invariably to estimate heritability? Twin Res Hum Genet 2007;10:712-7.

Tuorila H, Huotilainen A, Lähteenmäki L, Ollila S, Tuomi-Nurmi S, Urala N. Comparison of affective rating scales and their relationship to variables reflecting food consumption. Food Qual Pref 2008;19:51-61.

Tuorila-Ollikainen H, Mahlamäki-Kultanen S. The relationship of attitudes and experiences of Finnish youths to their hedonic responses to sweetness in soft drinks. Appetite 1985;6:115-24.

Tuschl RJ. From dietary restraint to binge eating: some theoretical considerations. Appetite 1990;14:105-9.

van den Bree MBM, Eaves LJ, Dwyer JT. Genetic and environmental influences on eating patterns of twin aged ≥50 y. Am J Clin Nutr 1999;70:456-65.

Wessman M, Kallela M, Kaunisto MA, Marttila P, Sobel E, Hartiala K, Oswell G, Leal SM, Papp JC, Hämäläinen E, Broas P, Joslyn G, Hovatta I, Hiekkalinna T, Kaprio J, Ott J, Cantor RM, Zwart JA, Ilmavirta M, Havanka H, Färikkilä M, Peltonen L, Palotie A. A susceptibility locus for migraine with aura, on chromosome 4q24. Am J Hum Genet 2002;70:652-662.

Wise PM, Hansen JL, Reed DR, Breslin PAS. Twin study of heritability of recognition thresholds for sour and salty taste. Chem Senses 2007;32:749-54.

Witt M, Reutter K, Miller IJ Jr. Morphology of the peripheral taste system. In Doty RL (ed.) Handbook of olfaction and gustation. 2nd Ed. Marcel Dekker, Philadelphia, USA, 2003;651-77.

Xu H, Staszewski L, Tang H, Adler E, Zoller M, Li X. Different functional roles of T1R subunits in the heterodimeric taste receptor. Proc Natl Acad Sci U S A 2004;101:14258-63.

Yeomans MR, Tepper BJ, Rietzschel J, Prescott J. Human hedonic responses to sweetness: role of taste genetics and anatomy. Physiol Behav 2007;91:264-73.

Zhao L, Kirkmeyer SV, Tepper CJ. A paper screening test to assess genetic taste sensitivity to 6-*n*-propylthiouracil. Phys Behav 2003;78:625-33.

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NI, Zuker CS. The receptors for mammalian sweet and umami taste. Cell 2003;115:255-66.

Zandstra EH, de Graaf C. Sensory perception and pleasantness of orange beverages from childhood to old age. Food Qual Pref 1998;9:5-12.

Zverev YP. Effects of caloric deprivation and satiety on sensitivity of the gustatory system. BMC Neuroscience 2004;5:5.