UNIVERSITY OF BURGUNDY

Ecole Doctorale Environnement, Santé, STIC n°490

PhD THESIS

To obtain the degree of
Doctor of the University of Burgundy

Discipline: Food Sciences

Presented by

Sofia BOUHLAL

Consequence of salt, sugar and fat content modifications in foods on children's preference and intake

PhD defence scheduled on December 21st, 2011, in presence of a jury composed of:

Pr. Marion Hetherington	University of Leeds, United Kingdom	Reviewer
Dr. Remco Havermans	Maastricht University, The Netherlands	Reviewer
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UNIVERSITE DE BOURGOGNE

Ecole Doctorale Environnement, Santé, STIC n°490

THÈSE DE DOCTORAT

Pour obtenir le grade de
Docteur de l'Université de Bourgogne

Discipline : Sciences de l'Alimentation

Présentée par

Sofia BOUHLAL

Conséquences des variations de teneur en sel, sucre et matière grasse sur les préférences et les consommations alimentaires des enfants

Soutenance de thèse prévue le 21 décembre 2011, devant un jury composé de:

Pr. Marion Hetherington	Université de Leeds, Royaume Uni	Rapporteur
Dr. Remco Havermans	Université de Maastricht, Pays-Bas	Rapporteur
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Remerciements / Acknowledgments

Comme toute bonne recette, la préparation d'une thèse est un processus très délicat. La parfaire requiert beaucoup d'affection, d'attention, de rigueur et de patience. A l'image de l'importance que peut avoir la qualité et la nature des ingrédients qui composent une recette, la nature et la valeur des expériences vécues pendant une thèse contribuent à la rendre unique. Ces expériences sont souvent le fruit de rencontres avec des personnes exceptionnelles. Tout comme le secret d'une recette réside dans la liste des ingrédients qui y sont incorporés, qu'ils soient ajoutés au début, pendant ou à la fin de la recette, plusieurs acteurs entrent en scène à différents moment de cette aventure. Cette liste de personnes qui jouent un rôle majeur dans la réussite d'une expérience de thèse est différent d'un thésard à l'autre; voici la mienne…

Tout d'abord, je vais remercie les personnes qui ont participés à la dernière -mais néanmoins peu négligeable- étape de cette thèse: *with all my affection and respect, I would like to thank Marion Hetherington and Remco Havermans who kindly accepted to discuss and judge my work.* J'aimerais également remercier Catherine Dacremont et Sandrine Péneau d'avoir accepté de faire partie de mon jury de thèse, ainsi que Vincent Boggio, Natalie Rigal et Kees De Graaf pour m'avoir conseillés et guidés dans ce travail. Merci également au Conseil Régional de Bourgogne pour avoir financé ce travail.

Ma thèse n'aurait jamais été l'agréable expérience qu'elle fut sans Sophie Nicklaus et Sylvie Issanchou. Merci pour votre disponibilité, votre patience et pour l'inspiration que vous avez été pour moi et pour mon travail. Merci de m'avoir aidé à murir mes réflexions scientifiques. Grâce à votre ouverture d'esprit, votre clairvoyance et votre grande rigueur scientifique, vous avez grandement contribué à façonner le chercheur en moi. Je n'oublierais jamais nos discussions passionnées autours de théories un peu capillo-tractées, et les nombreux fous rires pendant les différents 'points' de corrections *en stéréo*. MERCI!

Une mention très spéciale pour Claire Chabanet, merci de m'avoir si patiemment et efficacement guidée à travers les ruelles sombres de statisticville. Grâce a toi les statistiques n'auront plus de mystères pour moi (ou presque!!).

Ce travail n'aurait jamais vu le jour sans l'implication et l'enthousiasme des membres du service de la petite enfance de la Ville de Dijon: Mmes Tenembaume, Barney et Kreitter. Sans oublier les chefs, équipes et directrices des crèches Gaffarel, Montchapet, Voltaire, Gresille, Fontaine d'Ouche, Roosevelt et Junot. Je n'oublie pas Mme Catherine Laurent et toute l'équipe de l'école Saint Dominique. Merci à tous de m'avoir fait confiance et de nous avoir accueillis à bras ouverts et aidés lors des expériences. Un très très grand merci aux parents et aux enfants, j'espère vous avoir régalés avoir tous ces bons mets.

Je n'oublie pas de remercier chaleureusement les 'brigades en cuisine' qui ont aidées aux différentes étapes des études. A commencer par remercier Valérie et Emilie, sans qui les premières manips n'auraient pas été ce qu'elles étaient. Je n'oublie pas Françoise, Vincent, Jennifer, Géraldine, Céline, Judith, Charlotte B., Caro. et Jenny… Et Christophe pour la conduite des profils sensoriels.

Merci à toutes les personnes croisées aux détours des couloirs pour votre soutien, votre sourire et pour avoir contribué à faire de cette thèse une aventure humaine extraordinaire. Un clin d'œil aux habitants du 050 pour leur bonne humeur et surtout à Thierry pour ses conseils avisés et pour les discussions passionnantes (pas toujours qu'en sciences d'ailleurs!).

Je n'oublie pas de remercier les 'Castors' [a.k.a. Chloé, Chacha, Juju, Pierre, Eric et mon Charly], pour les merveilleux moments qu'on a partagés. Je me rappellerais avec le sourire et beaucoup de nostalgie de nos soirées 'restaurant gastronomique' suivi du visionnage de films historiques ! Bon courage à vous tous …

Je ne peux pas finir ma liste sans remercier ma famille. En commençant par mes « parents » d'adoption Nicole et Jean Louvet. Je vous remercie du fond du cœur d'avoir enrichi mon expérience Dijonnaise et de m'avoir fait découvrir la Bourgogne, ses bons petits plats et ses châteaux.
Un énorme merci a ma sœur et a mes parents pour avoir toujours cru en moi, en mes décisions et avoir validé mes choix sans poser de question. En somme merci de m'avoir permis de vivre ma vie pleinement, je vous adore !
And last but not least, je remercie ma meilleure moitié et mon meilleur ami, Tony, d'avoir été là tout au long de ces années intenses. Merci de m'avoir soutenu malgré la distance, de m'avoir parlé et réconforté quand j'en avais besoin et de m'avoir écouté et conseillé quand j'en avais besoin.

Merci à tous de m'avoir supporté et supporté pendant ces belles années.

Avec une Dream-Team comme celle-ci, l'expérience de thèse ne pouvait être qu'une réussite.
Don't you think ?
…

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List of Abbreviations

AFSSA	Agence Française de Sécurité Sanitaire des Aliments - French Food Safety Agency
ANC/RDA	Apports Nutritionnels Conseillés – Recommended Dietary Allowance
ANSES[1]	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail - French Agency for Food, Environmental and Occupational Health and Safety
BP	Blood Pressure
COMA	Committee on Medical Aspects of Foods (USA)
CS	Conditioned Stimulus
CVD	Cardiovascular diseases
CWC	Creamy White Cheese
ED	Energy density
EFA	Essential Fatty Acids
EI	Energy intake
FFL	Flavor-Flavor Learning
FNL	Flavor-Nutrient Learning
FSAI	Food Safety Authority of Ireland
GB	Green bean
IHF	Irish Heart Foundation
INCA	Enquête Individuelle et nationale de Consommation Alimentaire – National Individual survey of eating habits (France)
Inserm	Institut national de la santé et de la recherche médicale – National Institute for Health and Medical Research (France)
IOM	Institute Of Medicine (USA)
ME	Mere Exposure
MUFA	Monounsaturated Fatty Acids
PNNS	National Nutrition and Health Program - Programme National Nutrition Santé- (France)
PUFA	Polyunsaturated Fatty Acids
RSM	Response Surface Method
SACN	Scientific Advisory Committee on Nutrition (UK)
SFA	Saturated Fatty Acids
US	Unconditioned Stimulus
WHO	World Health Organization

[1] The ANSES was founded on the 1st July 2010 following the merger of two French health agencies: the French Food Safety Agency (AFSSA) and the French Agency for Environmental and Occupational Health Safety (AFSSET).

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General introduction

General introduction

"To improve your health, the quality of your life and your lifespan, cut down on your consumption of salt, sugar and fat". This general idea is relayed by several public health authorities around the world.

Attractive foods are generally those containing fat, sugar and salt or their combination (Drewnowski, 1995). Salt (NaCl), sugar (sucrose) and fat (dietary fat) are key ingredients with a great contribution to food structure, food taste and food intake and consequently to body composition. They are believed to be related to many health problems, so called 'diet-related diseases'; with the most known ones being overweight and obesity, leading in particular to cardiovascular diseases (CVD). This association led the governments to give guidelines and policies in order to lower energy density intake and improve the global health (WHO, 2003). These advices are in form of 1) qualitative targets, as to reducing the levels of salty, sweet and fatty foods' intake, and 2) quantitative targets (in terms of percent of energy or numbers of grams per day), by advising a reduction of salt, sugar and fat quantities in food preparations for the general population and for children in particular. In France, to reach these objectives, the National Nutrition and Health Program ("Programme National Nutrition Santé" or "PNNS"; Table 1) was set in 2001 and is still on-going (Hercberg, Chat-Yung & Chauliac, 2008).

Few studies to date have evaluated the impact of the advised reductions on food preferences and intake, in particular in young children. Scientific evidences highlighted the influence of salt, sugar and fat in young children's eating behavior (Drewnowski, 1997a), and the importance of early childhood in the development, establishment and long standing of later food habits (Nicklaus, Boggio, Chabanet & Issanchou, 2005a). Therefore, studying the impact of recommendations such as decreasing salt, sugar and fat content in foods on children's food intake, seems necessary in order to understand better their role as primary determinants of food preferences and intake.

Table 1. French dietary guidelines (PNNS). Source: Hercberg et al., 2008.

PNNS DIETARY RECOMMENDATIONS THAT CORRESPOND TO THE NUTRITIONAL GOALS OF THE PNNS FOR CHILDREN AGED 3 AND OVER AS WELL AS TEENAGERS. FORMS AND QUANTITIES MUST BE ADAPTED ACCORDING TO AGE.

Fruits and/ or vegetables 	**At least 5 a day**	• At each meal and as snacks •Raw, cooked, in natural state or prepared •Fresh, frozen or tinned. •One freshly squeezed fruit or a half-glass of fruit juice with no added sugar, at breakfast or as a snack
Bread and other cereal products, potatoes and pulses 	**At each meal and according to appetite**	• Favour variety: bread, rice, pasta, semolina/couscous, wheat, potatoes, lentils, dried beans, etc. [including brown bread and other whole grains] •Choose breakfast cereals that are low in sugar, limit very sugary varieties [chocolate cereals, with honey] or particularly fatty and sugary or filled cereals
Milk and dairy products 	**3 a day [or 4 according to the portion size and calcium concentration]**	•Vary the products •Favour natural products and those richest in calcium, as well as products that are low in fat and salt: milk, yogurt, cottage cheese, etc.
Meat Fish and seafood Eggs 	**Once or twice a day**	•The quantity should be inferior to the side dish made of vegetables and starches •If twice a day, reduce portions size at each meal •Vary types and choose the least fatty pieces [veal, chicken breast without skin, minced beef with 5% fat, etc]. •Limit fried and breaded preparations •Fish: at least twice a week, fresh, frozen or tinned.
Added fat 	**Limit intake**	•Favour fats of plant origin [olive oil, rapeseed oil, etc.] •Give preference to variety •Limit consumption of products of animal origin [butter, cream, etc.].
Sugary products 	**Limit intake**	• Limit sugary drinks [soda, fizzy drinks, fruit squashes and nectars] and sweets •Limit fatty and sugary products [cakes and pastries, desserts containing cream, ice cream, chocolate bars, etc.]
Drinks 	**Drink as much water as you like**	• Water is the only recommended beverage during and apart from meals. •Limit sugary drinks. •No alcoholic drinks and premix [mixture between soda and alcohol]
Salt 	**Limit intake**	• Choose iodised salt. •Do not add salt before tasting. •Reduce the amount of salt to cooking water. •Limit consumption of salty and fatty products: deli meats, crisps, other salty prepared foodsE
Physical activity 	**A minimum of 30 minutes of brisk walking per day [or equivalent]**	• Integrate any type of physical activity in your daily life: walking, cycling, gardening, gym, yoga, swimming... •Limit inactivity and sedentary activities [television, computer or video games...]

During the present thesis, we aimed at understanding the role of salt, sugar and fat content on food preference, liking and intake. The goal was to experiment the impact of subtle -but well perceived- modifications of these ingredients on commonly eaten foods 1) among children in natural settings such as nursery or school canteens 2) and to describe their sensory properties by adults.

In the first chapter of the present manuscript, we will present a literature review explaining the basis of our interest on salt, sugar and fat in early childhood. This five-section chapter will start with a section including a brief overview of the various roles of these three ingredients as technological/hygienic, sensory and physiological contributors; followed by a description of the role they might play on the prevalence of diet-related diseases and especially on overweight and obesity; and by a presentation of the governmental policies and recommendations taken, on the light of intake data collected via national surveys. In the second section, we will present an outline of the development of the perception of these ingredients from birth to early adulthood. A third section will deal with the impact of these ingredients on hedonic responses and food intake. The fourth section of the first chapter will discuss and present the contribution of these ingredients to learning to prefer and consume foods by children. This literature review will be concluded by the research questions raised during the thesis work and its objectives. Chapters 2 to 5 will present the experimental works conducted during the thesis, and will be followed by a last chapter presenting a summary and a general discussion of the thesis findings, and ruled off by suggestions for future researches.

Before starting, few terms that will be used in the present manuscript should be defined:

- *Salt* refers to sodium chloride (NaCl or table salt). Many kinds of other salts exist (e.g. KCl, LiCl, L-arginine, Na-acetate…). We used NaCl as it represents the major salt used while preparing foods and the one pointed out by public health authorities and nutritional recommendations.
- *Sugars,* referring to the chemical definition, are the compounds resulting from the combination of carbon, hydrogen and oxygen atoms. They are classified as mono-, di- or oligosaccharides, and they provide 4 kcal / g (15.7 kJ / g). In the present work, we focused on sucrose or table sugar, a disaccharide composed of a combination of glucose and fructose, and we will talk about 'sugar'.

- *Fat.* In most of the text, fat will refer to dietary fat in general and not to a specific fat quality, unless specified. Most dietary fats found in foods are in the form of triglycerides (a glycerol and three attached fatty acids). Fatty acids are classified into three groups: saturated (SFA; abundant for example in butter), monounsaturated (MUFA; abundant for example in olive oil) and polyunsaturated (PUFA; abundant for example in sunflower oil). Dietary fats provide the body with 9 kcal / g (35.3 kJ / g), representing its most concentrated source of energy. During this work, fat variations concerned the saturated fatty acid form; we used butter and dairy products (mainly composed of SFA) where the percent of fat varied.

List of publication and presentation arising from thesis

Publications:

- **Bouhlal S**., Issanchou S. & Nicklaus S. (2011). The impact of salt, fat and sugar levels on toddler food intake. *British Journal of Nutrition*, 105, pp 645-653 doi: 10.1017/S0007114510003752.
- Nicklaus S., **Bouhlal S.**, et al. (2010). Development of fat preferences in children (Développement des préférences pour les lipides chez l'enfant). *Innovations Agronomiques* 10: 115-124.
- **Bouhlal S**., Chabanet C., Issanchou S., & Nicklaus S. (Submitted). Salt content: effect on preferences and consequences on intake in children. *Journal of the American Dietetic Association.*
- **Bouhlal S.,** Issanchou S., & Nicklaus S. (Submitted). The different impact of fat content on toddlers' and adults' food intake. *Food Quality and Preference.*
- **Bouhlal S**., Issanchou S., & Nicklaus S. (In preparation). 'Just a pinch of salt': conditioning vegetable acceptance in toddlers with salt or spice.

Oral presentations:

- **Bouhlal S.**, Issanchou S. & Nicklaus S. (2011). "Mysterious fat. The different impact of fat on toddlers' and adults' food intake" 19th meeting of the Society for the Study of Ingestive Behavior, 12-16 July, 2011, Clearwater, Florida, USA.
- **Bouhlal S**. (2010). Impact de la teneur en sel sur les consommations et les préférences alimentaires d'enfants de 8 à 11 ans. Young Investigators' Forum. University of Franche-Comté, Besançon, France.
- **Bouhlal S.** (2010). Le sel dans l'alimentation du jeune enfant : effet sur les préférences, conséquences sur les consommations. Internal PhD candidate workshop, Centre des Science du Goût et de l'Alimentation, Dijon, France.
- **Bouhlal S**., Chabanet, C., Issanchou S. & Nicklaus S. (2010). Le sel dans l'alimentation du jeune enfant: effet sur les préférences, conséquences sur les consommations. 8èmes Journées Francophones de Nutrition. Lille, France.

***Poster presentations*:**

- **Bouhlal S.,** Bernard C., Issanchou S. & Nicklaus S. (2011). "Sugar content impacts food intake in toddlers, but could be reduced". 19th meeting of the Society for the Study of Ingestive Behavior, 12-16 July, 2011, Clearwater, Florida, USA.
- **Bouhlal S.**, Issanchou S. & Nicklaus S. (2010). The impact of salt level on children's food intake and preferences. Conference on Feeding and Eating in Infancy and Early Childhood. The Institute of Child Health, London, UK.
- **Bouhlal S**., Issanchou S. et Nicklaus S. (2009). Does toddlers' food intake differ according to variations in fat, salt or sugar in foods? Young Investigators' Forum (Forum des jeunes chercheurs). Dijon, France. Award for the best poster.
- **Bouhlal S.**, Issanchou S. & Nicklaus S. (2009). How does fat content of a snack food impact toddlers' and adults' intake? 8th Pangborn Sensory Science Symposium, Florence, Italy.

***Abstracts published in scientific journals*:**

- **Bouhlal S.**, Issanchou S. & Nicklaus S. (2011). "Mysterious fat. The different impact of fat on toddlers' and adults' food intake" *Appetite* 57(1): S6-S7. doi:10.1016/j.appet.2011.05.131.
- **Bouhlal S.,** Bernard C., Issanchou S., Nicklaus S. (2011). "Sugar content impacts food intake in toddlers, but could be reduced" *Appetite 57 (1): S7.* doi:10.1016/j.appet.2011.05.132.
- **Bouhlal S.**, Issanchou S. et Nicklaus S. (2008). "Does toddlers' food intake differ according to variations in fat, salt or sugar in foods?" *Appetite 51 (2): 355. doi:10.1016/j.appet.2008.04.044*

Chapter 1. Literature review

Part 1. Relevance of research on salt, sugar and fat

Part 2. Development of taste perceptions

Part 3. Tastes: from detection to behaviour

Part 4. Learning mechanisms involved in eating behaviour

Part 5. Research questions and thesis outline

Part 1. Relevance of research on salt, sugar and fat

1.1 Functions of these ingredients

1.1.1. The technological and the hygienic roles of salt, sugar and fat

The role of salt. During the manufacturing process of a large number of products, manufacturers add salt for hygienic and/or technical reasons. Since prehistoric times, salt has always been used to preserve foods for extended periods, due to its bacteriostatic function; it lowers water activity below the threshold needed for bacterial growth; e.g. *Pseudomonas, Escherichia, Bacillus …* (Hergarty, 1995 in Lee, 2011). In the case of meat products for example, salt appears to be an important technical ingredient, as one of its roles is to give the typical coloring of meat (Durack, Alonso-Gomez & Wilkinson, 2008). In the bread-making sector salt is added as it contributes to enhance the bread volume, it improves its appearance and it also promotes a good color crust (AFSSA, 2002). In the cheese sector salt is added during the 'maturation', a key stage in the manufacturing process that insures a balance of moisture and solids. Adding salt to cheese insures its adequate evolution during ripening, the development of the identifying characteristics of the finished product and it also insures safety during aging (AFSSA, 2002).

The role of sugars. Depending on their type, and beyond their perceived sweet taste, sugars influence safety or quality in many different ways. They increase the boiling point and decrease the freezing point of certain products; which is the case of candies for example. Sugars such as fructose have the ability to reduce water and microbiological activities as well as mold formation; making it for instance useless to refrigerate foods with high content of sugars, and provides an extensive shelf life. In baked products, sugars such as glucose, fructose, galactose, sucrose, lactose and maltose, play a role as substrate for fermentation (helps dough rising). Reducing sugars (e.g. monosaccharides) provide the brown coloring of products like some desserts or bread, thanks to the nonenzymatic browning reactions that control food quality, caramelization, Maillard and Strecker reactions. Finally, invert sugars[2] play a textural role by producing a smoother product; e.g. candies (Davis, 1995; Sigman-Grant & Morita, 2003).

The role of fat. Fat plays essential functional properties in foods. By trapping the air during the creaming process in baked products, fat provides their structure. It is also responsible for the crisping character of fries or chips, by rapidly and uniformly transmitting the heat for example. Likewise, the chewiness and softness of foods are provided by their fat component melting points. Furthermore, the given fat content of a product determines its storage stability (Hahn, 1997). The moistness of cakes and the juiciness of hamburgers are determined by the water-holding quality of fats. Moreover, crispy and crunchy textures can be achieved by cooking in fats at temperatures above that of boiling water (Drewnowski, 1997b).

1.1.2. The organoleptic functions of salt, sugar and fat

Organoleptic[3] functions refer to the properties of a food that are perceived by sense organs and are partially responsible for its palatability. Such properties include visual appearance, sound, smell, taste, texture, temperature, and trigeminal inputs of a food (Sørensen, Møller, Flint, Martens & Raben, 2003). During the present work, we are focusing on the orosensory qualities of foods. Taste qualities are known to be a primary driver of ingestion, especially in children (Birch, 1999; Drewnowski, 1997a). Although all sensory properties of a food, such as its appearance and/or smell might impact its intake (Drewnowski, 1997a; Rolls, Rowe & Rolls, 1982; Shepherd, 1988), the gustatory sensations from foods and beverages rank first among the drivers of an individual's food choice (Glanz, Basil, Maibach, Goldberg & Snyder, 1998). Salt, sugar and fat for instance are known to enhance the palatability of foods, thanks to their flavoring properties, and their capacity to enhance other flavors within a food (Hegarty, 1995 in Lee, 2011). We will discuss in this section the taste, flavor and texture contribution of salt, sugar and fat in foods.

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[2] *Invert sugars* are defined by the Oxford dictionary as 'a mixture of glucose and fructose obtained by the hydrolysis of sucrose'.

[3] *Organoleptic* is defined by the Oxford dictionary as the 'acting on, or involving the use of, the sense organs'.

Salt (NaCl) is responsible for one of the five basic tastes, the salty taste (Chandrashekar, Hoon, Ryba & Zuker, 2006). Humans' avidity for salty taste is not due to a 'sodium appetite' (a desire for sodium) as it is the case for animals, but is most probably related to a 'salt appetite' (seeking the taste of NaCl), an attraction to the taste of salt independently from the subject's sodium status (Leshem, 2009). Salt is a flavor enhancer (Shepherd, 1988), as it reinforces the flavor of other ingredients present in a food, due to its "salting out" effect. This effect is a result of '*an increase in headspace concentration of aroma compounds presumably related to the reduction in available solvent in the liquid phase resulting from the presence of salt*' (Salles et al., 2011). In other respects, salt 'selectively filter flavors': unpleasant ones -i.e. bitterness- are suppressed, while palatable ones -i.e. sweetness- are not, thereby 'increasing the salience and/or intensity of the latter' (Breslin & Beauchamp, 1997). Salt is also known to help controlling texture and consistence of certain preparations by interacting with other components (Doyle & Glass, 2010): in baked products an optimal amount of salt ensures loaves of bread with adequate texture; and in meat products, salt enables the binding between proteins and water thus enhancing tenderness.

Sugars, another of the five basic tastes, generally taste sweet (Chandrashekar et al., 2006). In the case of sweet, the color of the sample might influence its organoleptic judgment. Indeed, studies cited by Rose Marie Pangborn demonstrated that an unflavored solution with a red color will be judged as sweeter than the same but less red solution, and pear nectar with a green coloring will be judged as less sweet than the same nectar without coloring. In 1987, R. M. Pangborn described a salting-out mechanism by which sugars increase the concentration of volatile aroma compounds in the headspace (Pangborn, 1987). In foods, sugars impart texture and provide the viscosity and the density of products such as in dairy products; they also responsible for other functions such as the crispiness and the brittleness of cookies for example (Davis, 1995).

Studies have shown that fat contributes to several organoleptic properties such as the aroma, flavor and texture of foods (Drewnowski, 1997b; Drewnowski, 2000). Fats carry, enhance and release the flavors of other food ingredients. Fat is a carrier of aroma compounds, most of which are fat-soluble and thus are associated with the lipid phase (Yackinous & Guinard, 2000). The first sensory impact of fat is the perception of fat-soluble aroma compounds, first through the nose -orthonasal- or through the mouth -retronasal- (Drewnowski, 1997b). In fact, reduction of fat negatively impacts aroma release, as fat affects the partition of volatile compounds between the food and the air phase in the mouth (Bayarri, Taylor & Hort, 2006). That explains the greater perception of fat in some products such as potato chips when the olfactory dimension was present, while wearing nose-clips (suppressing the olfactory dimension) lowered the fatty ratings (Yackinous & Guinard, 2000). The second impact of fat is on texture, as it is a 'critical part of the physical food matrix' (Yackinous & Guinard, 2000). Fat contributes to many textural properties such as viscosity, mouth-coating, cohesiveness, crispiness and hardness, etc. (Mela, 1992). In dairy products, fats provide smoothness, thickness and viscosity; whereas the cooling feel of fats in the mouth is the characteristic feature of butter and cream (Drewnowski, 1997b). Another textural characteristic of importance in dairy products is their 'creamy' or 'unctuous' attribute. As described by Tournier and co-workers, when it concerns dairy products, French consumers use the term 'crémeux' the French for 'creamy' as a synonym of 'unctuous' the term widely used in non-French based studies. Moreover, '*creaminess appears to be an integrated concept, which is related to textural properties, fattiness, flavour and even the pleasantness of food products'* (Tournier, Martin, Guichard, Issanchou & Sulmont-Rossé, 2007).

1.1.3. The nutritional and physiological functions of salt, sugar and fat

Salt. Sodium is a vital and essential nutrient for the body. Several studies cited by Durack and colleagues (2008) estimate that for an adult, average physiological requirements for salt do not exceed 4 g per day (1.6 g of sodium), and is often estimated around 1 or 2 g per day (AFSSA, 2002; Durack et al., 2008). The WHO estimates physiological needs of sodium to be around 10-20 mmol per day (230-460 mg of sodium or 0.575-1.15 g of salt); (Eliott & Brown, 2006). Children's physiological needs for salt were not yet studied and thus need to be established (He & MacGregor, 2006).

Sodium maintains water balance in body cells and extracellular fluid volume. It is also essential for the transmission of nerve impulses, for muscle and nerve activities for instance. It helps maintaining water homeostasis and constant biological parameters with regard to changes of external environment (Durack et al., 2008; Garcia-Bailo, Toguri, Eny & El-Sohemy, 2009; Spetter, Smeets, de Graaf & Viergever, 2010). So, low salt intake is likely to cause muscle cramps, weakness and fatigue, and might have adverse effects on thermoregulation in high environmental temperatures for example. Extreme depletions can even be fatal (SACN, 2003). When dietary sodium exceeds physiological needs, plasma sodium level is maintained constant in the body thanks to the excretion in urine and sweat to a certain upper limit (Durack et al., 2008). As it will be discussed in section 1.3., due to the important role of salt in maintaining electrolyte balance, in regulating blood pressure and blood volume and in water homeostasis, its intake recommendations are more precise than those for sucrose.

Sugar. Glucose resulting from the digestion of sugars (mono- and disaccharides) is one of the energetic substrates for the body and for the brain. When it is not used, glucose is stored as glycogen in the liver and in the muscles; and if the body is lacking energy, the glucose is released -or remains- into the bloodstream to be delivered and metabolized into the brain, kidney, muscle cells and adipocytes (Sigman-Grant & Morita, 2003). Moreover, a strong physiological role of sweet solutions (glucose or sucrose) is observed in neonates. In fact, when a sweet solution is orally administered, it exerts a pain-relief effect; this effect is specifically attributed to the taste of sweet and not to its post-ingestive consequences (Nicklaus & Schwartz, 2008). This analgesic effect is observed when 1 ml of a 30% glucose solution is administered, and the effect was not seen to induce tolerance, even if administered three times a day for 3 to 5 days (Eriksson & Finnstrom, 2004).

Fat. Fat is present in the body as cell lipid droplets in adipose tissue and FAs are gathered in there and are stored as TGs. That makes fat the macronutrient providing the most concentrated source of energy to the body. For example, 55% of the energy that newborns get from breast milk comes from fat; that insures a rapid growth, as well as brain and nervous system development. When it concerns total FAs, physiological needs[4] are equivalent to 30% of energy intake (EI) in healthy adults with an energy intake equivalent to

[4] The physiological needs for lipids established by the report of the French ANSES are in accordance with international data.

2000 kcal per day, whereas no precision in given for children (ANSES, 2011). Before going forwards, few definitions and distinctions must be made.

SFAs are synthesized by the human body (in the brain, liver and adipose tissue) and their physiological functions differ according to their type (ANSES, 2011). Butyric acid (C4:0) has a positive action in apoptosis of tumor cells. Medium-chain SFA such as caproic (C6:0), caprylic (C8:0) and capric (C10:0) acids, are a rapid source of energy and have a protective effect against obesity in humans, as they are rapidly absorbed and oxidized in the liver; they have an antiviral effect; and they also have no hypercholesterolemic effect and are not associated to CVD risk contrarily to long-chain SFAs. Indeed, long-chain SFAs such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) and stearic (C18:0) acids, are a great source of energy, and have functional roles in cell membranes. Although, some of these especially lauric, myristic and palmitic acids may have deleterious effects on health in case of excess, due to their atherogenic effects (ANSES, 2011).

Part of the MUFAs is synthetized by the body, and the other part is provided by the diet. They also constitute cell membranes and are of importance for enzyme, transporter and receptor activities.

PUFA play a major role in cell walls, as they insure cell communication and hormone production. This family includes essential fatty acids (EFA) as they cannot be synthesized by the body. Those are the linoleic acid (LA, C18:2 n-6) the precursor of the n-6 or ω6 FA family, and the alpha linolenic acids (ALA, C18:3 n-3) the precursor of the n-3 or ω3 FA family. They are precursors for other fatty acids such as long chain-PUFAs: arachidonic (ARA, n-6), eicosapentaenoic (EPA, n-3) and docosahexaenoic (DHA, n-3) acids, so called 'conditionally essentials' or 'non-essential FA'. Physiological needs for French healthy adults are equivalent to 2 (4.4 g/d), 0.8 (1.8 g/d) and 0.1% (250 mg/d) of EI respectively for LA, ALA and DHA (ANSES, 2011). Besides being essential for insuring the functions of the central nervous system, LA and ALA (and their metabolites) are also important in the early development of visual acuity and cognitive functions (Macé, Shahkhalili, Aprikian & Stan, 2006). These long-chain-PUFAs can be synthesized by humans and animals, providing that their precursors are delivered in sufficient quantities through the diet. Some FAs contribute to the structure of phospholipids (PL), the major constituents of all body cell membranes in particular in neural cells, as they modulate their fluidity and their activity. Other FAs contribute to the structure of sphingolipids, also present in cell membranes and responsible for cell recognition and

signaling. PUFA are of great importance for hemostasis, platelet aggregation, immune reactions, cell differentiation, reproduction and also gene regulation (ANSES, 2011).
Fat is also important if not necessary to vehicle and facilitate the absorption of fat-soluble vitamins such as vitamin A, D, E and K (Reed, Mela & Friedman, 1992). Suboptimal fat intake might impart health problems such as the supply of minerals, fat-soluble vitamins and caloric insufficiency (Rogers & Emmett, 2001).

1.2. Public health concerns related to salt, sugar and fat intake

1.2.1. Diet-related health problems in relation to salt, sugar and fat intake

Scientific evidences highlight the link between diet quality and several chronic diseases. Some of these diet-related diseases are obesity, diabetes, cardiovascular diseases, cancer, osteoporosis and dental diseases (WHO, 2003). In addition to the costly burden to health care services, these diet-related diseases represent a major public health preoccupation worldwide, as they were responsible for approximately 60% of the reported deaths in the world in 2001. Although deaths due to these diseases have, for a long time, been identified only in adults, they start to appear in childhood and represent a source of concern. Recent literature brings evidence that the diet during early childhood might predispose an individual to develop diet-related health problems in later adult life (Bateson et al., 2004). It may be possible to reverse this tendency by acting on diet improvement in order to prevent rather than heal. Several diet-related diseases were linked to the intake of some nutrients such as salt, sugar and fat, as reported below.

1.2.1.1. Salt

Although salt has for long been thought to be a health preservative (by killing/limiting food-borne pathogens), it is nowadays considered as a health threat in response to an excessive consumption (Doyle & Glass, 2010). Excess dietary salt intake was shown to be responsible for several health problems and is strongly presumed to influence the rise in blood pressure (BP) or hypertension with age (Simons-Morton & Obarzanek, 1997). BP subsequently pre-disposes individuals to CVD, a leading cause of death in developed countries (He & MacGregor, 2004; WHO, 2003), as BP is responsible for 62% of strokes and 49% of coronary heart diseases (He & MacGregor, 2010).

The most debated effect of salt on health is probably its effect on the early pathogenesis of hypertension. Neither the observational studies, nor the randomized experimental studies (of short- or long-term) reviewed by Geleijnse and Grobbee could establish a direct association between blood pressure and early sodium intake (Geleijnse & Grobbee, 2002). Besides the biological plausibility exposed by the authors, they state that it is not clear to date if children's hypertension "tracks" into adulthood. A more recent meta-analyses conducted by He and MacGregor, concluded that in order to prevent the rise of blood pressure in adulthood, a reduction in dietary salt intake during childhood would be beneficial (He & MacGregor, 2006). The results of this later meta-analysis are in accordance with earlier ones, in that higher sodium intake is related to higher BP in children and adolescents (Simons-Morton & Obarzanek, 1997). Thus, the importance of diet during childhood in the prevalence of later health concerns has to be taken into account. The inconsistency of the results obtained by different studies investigating the link between BP and salt intake in children or adolescents, could be due to several factors, such as the use of different methodologies to investigate dietary salt intake (reviewed in 1.4.), the adjustment or not for confounders such as total energy intake, physiological activity etc. (Geleijnse & Grobbee, 2002), or due to the short term of the considered studies He & MacGregor, 2010.

Concerning the epidemiological approach, a large international study aiming at evaluating the link between BP and salt intake called INTERSALT study (1985-87), includes 52 communities of 10 079 adults aged 20-59 years, from 32 countries, with variable salt intakes (from 6 to 25 g/day), found an association between the rise in BP with age and salt intake (Intersalt Cooperative Research Group, 1988 cited by He & MacGregor, 2010); a high sodium intake during life is partially responsible for BP increase with age. Moreover, other epidemiological studies such as the INTERMAP and the Norfolk Cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk) studies underline the importance of salt intake in determining the levels of population BP (Zhou *et al.*, 2003 and Khaw *et al.*, 2004 cited by He & MacGregor, 2010)

The report of the AFSSA (France) draws the reader's attention to the fact that literature data do not allow a generalization to the whole population of the potential harmful effect of salt intake for health especially hypertension (AFSSA, 2002). This observation is shared by other scientists, pointing the uncertainty about the value of low-salt diets for the management of hypertension (Alderman, Cohen & Madhavan, 1998). Besides, the effect of dietary sodium on blood pressure may only be detectable in populations in which the range of sodium intake is sufficiently large, or in population subgroups that have a specific sensitivity for the effects of sodium (Watt et Foy, 1982; Grobbee 1991 and Weiberger 2000 in Geleijinse et Grobbee, 2002). However, He and MacGregor maintain the necessity to reduce salt intake in the whole population in order to efficiently lower BP. This effect on BP would be more efficient if salt intake reduction is included in a global well balanced diet (rich in fruits, vegetable, low-fat dairy products) as it was proven by the DASH study (Dietary Approach to Stop Hypertension); He & MacGregor, 2010.

A review by Durack and colleagues, listed other illnesses caused by excessive salt intake, such as the development of kidney diseases as well as early kidney damages, and the aggravation of asthma conditions. Moreover, an increased intake of salt favors sodium and calcium excretion in urine, leading to a decrease in bone density and to the onset of osteoporosis (Durack et al., 2008). Salt and salt-preserved foods were also shown to be linked to the prevalence of certain cancers (e.g. colon, stomach or kidney cancers); WHO, 2003.

1.2.1.2. Sugar

It was widely advanced that the etiology of dental caries is linked to dietary sugars, a report by the WHO/FAO experts sets that "a low free sugars consumption by a population will translate into a low level of dental caries" WHO, 2003. However, a recent review conducted by Ruxton and colleagues, on publications available from 1995 to 2006, is not affirmative about a systematic relationship (Ruxton, Gardner & McNnulty, 2010). The 46 studies reviewed included experimental and epidemiological studies, found the relationship between sugar and caries to be either null, positive or complex (with significance observed only in some subgroups where tough brushing was infrequent for example). The reason of this inconsistency may be due to the use of invalidated tools to record intake, or to the lack of consideration in some studies of some confounders such as oral hygiene, intake of sugar *per se* or of sugar-rich foods, consideration of the level of oral bacteria, food oral clearance etc. (Ruxton et al., 2010); thus, highlighting the complexity of demonstrating and affirming such a simple causal relationship. However, the importance of lowering sugar intake and brushing teeth with fluoride in order to stop or at least delay the prevalence of dental caries remains valid (Reed & McDaniel, 2006). The report edited by the WHO/FAO experts also concluded that "evidence for a relationship between dental caries and both amount and frequency of free sugars was described as 'convincing'" WHO, 2003.

In other respects, the link between sugar intake and the occurrence of some cancers (breast, colorectal or lung), or between sugar intake and metabolic syndrome[5] is not yet fully established and conclusions vary from a study to another. Although the link between intake of sugars and attention-deficit hyperactivity disorders (ADHD) was suggested in children, all reviews of the subject conducted between 1995 and 2003 failed to bring any scientific proof (Ruxton et al., 2010). However, the link between high carbohydrate, high sweet food intake and mood is more likely; for instance depressed persons prefer this kind of foods (Christensen and Somers, 1996 cited by Ruxton et al., 2010). A similar positive effect of sugar on mood was described by other authors (Reed & McDaniel, 2006); some even considered carbohydrate snacks as 'self-medications' due to their positive effects to improve mood state, relieve depression and fatigue (Lieberman, Wurtman & Chew, 1986).

1.2.1.3. Fat

In the case of fat, both an excessive and a poor intake are linked to health problems in humans. On the one hand, excess dietary intake of fats was shown to be strongly influencing the risk of cardiovascular diseases, as it affects blood lipids, blood pressure and arterial functions among other things (WHO, 2003). The PNNS group in France is in charge of

[5] The metabolic syndrome consists of a cluster of disease states, including 3 of the following 5 criterion: central obesity, hypertriglyceridemia, low levels of high-density lipoproteins (HDL), elevated BP, impaired glucose tolerance; ANSES, 2011.

evaluating and advising for lipid use. It stated that health problems due to fat might be caused by the excess of fatty acid intake in relation to needs (PNNS, 2009); keeping in mind their qualitative effects. A recent review by Brown and his colleagues goes around that issue and provides an overview of the impact of fats on some diseases such as the metabolic syndrome and the importance of the diet's fat profiles on health (Brown et al. -chapter 21- in Montmayeur & Le Coutre, 2010). Moreover, the intake of saturated fatty acids in particular was linked to the increased concentration of cholesterol, a factor known to be linked to the prevalence of heart diseases (Montmayeur & Le Coutre, 2010). Thus, restricting the intake of SFAs, such as those found in dairy products or butter, appears of importance for ensuring cardiovascular health. On the other hand, a poor intake of PUFA (especially long-chain n-3) was linked to psychopathological and cognitive disorders such as depression, schizophrenia, ADHA and autism (ANSES, 2011).

1.2.2. Body weight and nutrient intakes

1.2.2.1. Definition of overweight and obesity

Overweight and obesity are defined by the WHO as an "*abnormal or excessive fat accumulation that may impair health".* Obesity was recognized as a disease in 1997 by the WHO. The prevalence of overweight and obesity has become a threat for all public health services worldwide (World Health Organisation, 2000).

Overweight and obesity can be assessed objectively by using a universal index named Body Mass Index (BMI) or Quetelet index (Thibault & Rolland-Cachera, 2003). Researchers, clinicians and health professionals, defined this index using the subject's weight (in kilograms) and height (in meters): $BMI_{(kg/m^2)} = weight / height^2$. It is a useful proxy measure of adiposity (Cole, Faith, Pietrobelli & Heo, 2005). Even if it does not measure body fat directly, it correlates to other direct measures such as underwater weighing and dual x-ray absorptiometry (DXA). This measure has the advantage for being inexpensive and easy to perform.

1.2.2.2. Cut-offs and prevalence of overweight and obesity in adults

The WHO defines overweight as a BMI greater than or equal to 25 kg/m² and lower than 29.9 Kg/m², and obesity as a BMI greater than or equal to 30 kg/m² respectively (World Health Organisation, 2011).

In 2008, one in ten of the world's adult population was obese and in 2010, overweight and obesity were responsible for more deaths around the world than do underweight (World Health Organisation, 2011). In France as in other countries, the prevalence of obesity increased in adult populations, as it evolved from 8.5% in 1997 to 14.5% in 2009 (ObEpi-Roche, 2009). The French INCA2 study conducted between 2006 and 2007, identified 36 % and 50 % of women and men, to be overweight or obese (AFSSA, 2009).

1.2.2.3. Cut-offs and prevalence of overweight and obesity in children

Due to the variations of body composition during growth, children and adolescents' BMI is age and sex specific (Cole, 1990). Therefore, another measure of adiposity is the standard deviation (or z-score[6]) of BMI, calculated using the LMS method (Cole, 1990). It is "the deviation of the value for an individual from the mean value of the reference population divided by the standard deviation of the reference population".

BMI-for-age curves were developed by several countries, with their own population references (Rolland-Cachera & Péneau, 2011). The French reference curves, established in 1982 and revised in 1991, use data from a sample of French subjects who participated to the International Growth Study conducted by the International Children's Center, from 1953 to 1955 (Rolland-Cachera, Cole, Sempe, Tichet, Rossignol & Charraud, 1991; Rolland-Cachera, Sempé, Guilloud-Bataille, Patois, Péquignot-Guggenbuhl & Fautrad, 1982). In order to define overweight and obesity the thresholds defined for the French reference population are used along with those established by the International Obesity Task Force (IOTF) (Cole, Bellizzi, Flegal & Dietz, 2000; Thibault & Rolland-Cachera, 2003); these new curves, as depicted in figure 1, established under the PNNS program, are included in individuals' health book.

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[6] http://www.cdc.gov/growthcharts/growthchart_faq.htm. Last access August 4th, 2011.

In order to define overweight and obesity, cut-offs during childhood differ according to the source and thus to the reference data used. The IOTF references provide cut-off values based on percentiles passing through BMI 25 (IOTF-25 in the curve) and 30 kg/m² (IOTF-30 in the curve) at age 18, defining overweight and obesity respectively. Concerning French references they use all ranges from the 3rd to the 97th percentile. overweight is defined as a BMI >97th percentile, with two levels of obesity: level 1 when the value is between the 97th percentile of the French references and the IOTF-30, and level 2 when the value is above the IOTF-30 value as shown in Figure 1 (Thibault & Rolland-Cachera, 2003). Besides, the Center for Disease Control (CDC), defines children and adolescents as overweight when their BMI is greater than the 85th but lower than the 95th percentile; whereas pediatric obesity is defined as a BMI above the 95th percentile (Yanovski, 2001). These various references and growth parameters used by scientists across the literature, made it difficult to compare data from different studies and/or different countries, and to reach comparable conclusions on the prevalence of children's overweight and obesity (Rolland-Cachera & Péneau, 2011; Salanave, Péneau, Rolland-Cachera, Hercberg & Castetbon, 2009).

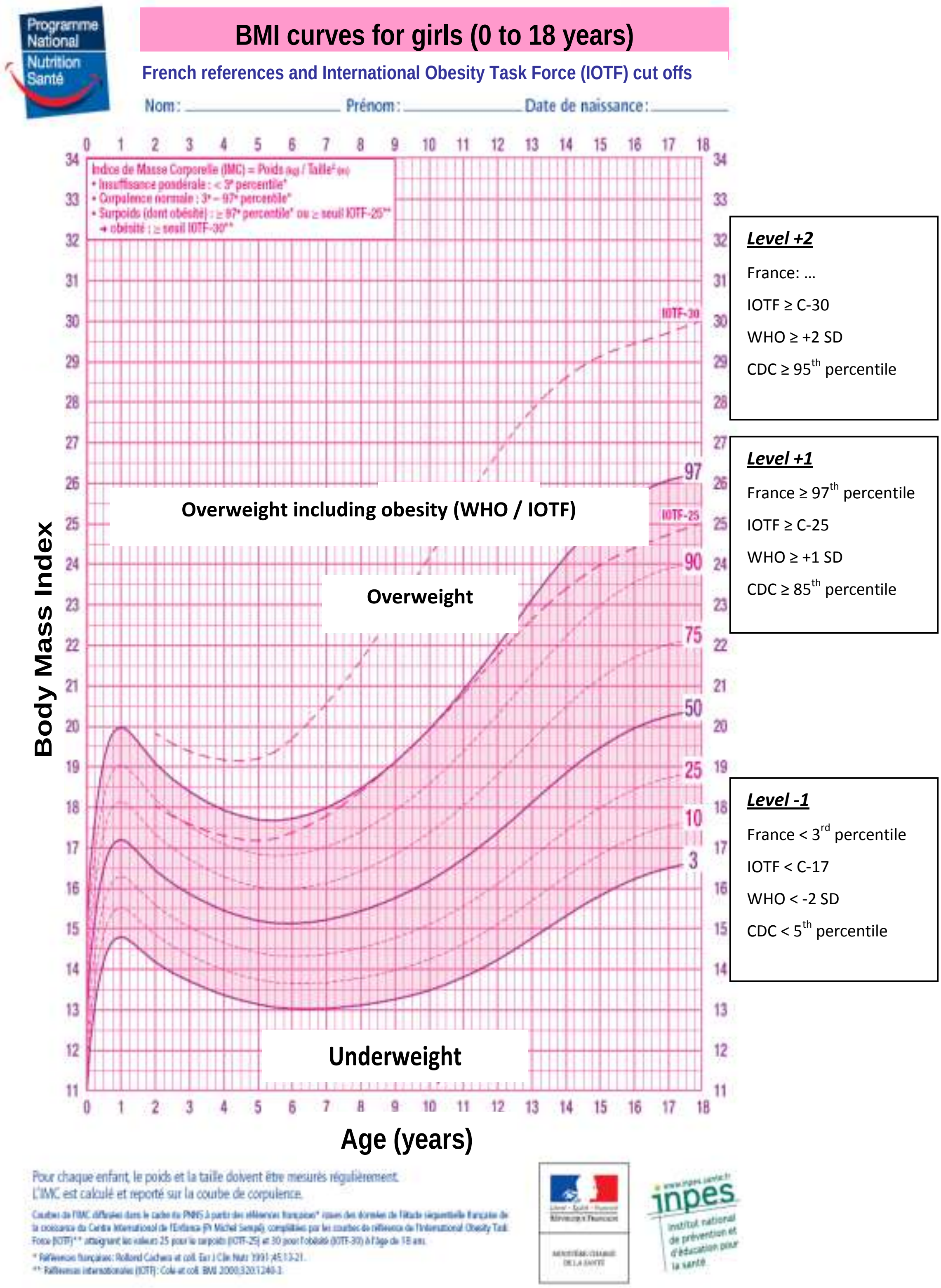

Figure 1. French Body Mass Index curve.

Body Mass Index curves for Girls adapted for the PNNS[7] (Thibault & Rolland-Cachera, 2003), with cut offs and terminologies (Rolland-Cachera et al., 2002).

[7] http://www.inpes.sante.fr/CFESBases/catalogue/pdf/IMC/courbes_enfants.pdf. Access date August 2011.

During the work conducted in this thesis, the French reference data for children were used to calculate z-BMI, and international cut-offs for adults were used in studies implicating adults.

A persistent obesity at 6-8 years old is likely to track until adolescence and adulthood. The WHO identified 43 million children under five to be overweight (World Health Organisation, 2000). In France, before the 90s, no national data on the prevalence of overweight and obesity were available. The only available data were conducted at the regional level, and the definition of obesity was based on the French references established in 1960 and published by Roland-Cachera and co-workers. A collective expertise conducted by Inserm (France), highlighted the fact that the prevalence of obesity (>97th percentile) in 5 to 12 year-old children, is equal to 3 % in 1960, rose in a 15 year period from 6 % in 1980 to reach 10-12 % in 1996 (Expertise collective Inserm, 2000; Rolland-Cachera et al., 1991). Since then, health authorities in France recognized the necessity to monitor children's weight, and conducted several studies in order to follow its evolution. Thus, overweight concerns 10.9 to 14.3 % of the pediatric population and obesity 2.4 to 3.8 % as shown on Table 2.

Table 2. Evolution of the prevalence of overweight and obesity in French children from 1998 to 2007.

	Year	Population (n; age)	Overweight (%)	Obesity (%)	Ref.
INCA 1	1998-1999	1016 ; 3-14 years	11.7	3.5	[1]
National Study ECOG protocol	2000	1582 ; 7-9 years	14.3	3.8	[2]
ObÉpi	2000	6084 ; 2-17 years	10.9	2.4	[3]
INCA 2	2006-2007	1146 ; 3-17 years	11.2	2.8	[4]

Cut-off references were those of the IOTF definition from Cole et al., 2000.

[1] Lioret, Maire, Volatier & Charles, 2007; [2] Rolland-Cachera et al., 2002; [3] Charles, Eschwege & Basdevant, 2008; [4] AFSSA, 2009. Adapted from the AFSSA report: AFSSA, 2009.

The prevalence of overweight and obesity seems to reach a statistical stability in France observed between 2000 and 2008, and also in other countries (Salanave et al., 2009). In a recent review published in 2011, data of 467.294 children aged 2 - 19 years, belonging to 9 countries (Australia, China, England, France, Netherlands, New Zealand, Sweden, Switzerland and USA) were compared. As a result of this cross-country comparison done on the basis of the IOTF cut-offs, the authors found that although it remains high, the prevalence of overweight and obesity is leveling off in these countries (Olds et al., 2011). Far from been exclusive and/or definitive, several hypotheses were suggested to explain this plateauing effect. One of them is the '*intervention hypothesis'*, suggesting that nutritional guidelines such as the one edited by the PNNS in France since 2001, and targeting children among other populations, might have positive consequences on this stabilization. Another hypothesis would be '*the saturation equilibrium hypothesis'*, which suggests that the maximum obesogenicity of our societies has been reached within a given community, leaving no more place to a further increase in overweight and obesity (Olds et al., 2011; Salanave et al., 2009).

1.2.2.4. Dietary factors involved in the etiology of overweight and obesity

Up to 33 % of adult obesity may have their origins in childhood obesity (see figure 2; Olstad & McCargar, 2009; Power, Lake & Cole, 1997). Even if the methods used to evaluate overweight and obesity are comparable, the importance of genetic and environmental factors is not to be neglected. For example, northern European countries have a lower prevalence of overweight (including obesity) compared to southern ones (Lobstein & Frelut, 2003).

The 6 % increase in obesity observed across 12 years (between 1997 and 2009), triggers serious questioning about a better comprehension of the multiple causes of weight gain, in order to stop or reduce the observed prevalence (ObEpi-Roche, 2009). Scientists agree that the causes of obesity are multifactorial (Olstad & McCargar, 2009; World Health Organisation, 2000). However, some of its causes are difficult to change, such as genetic predispositions considered as a vulnerability factor in the occurrence and development of obesity, but environmental ones such as diet composition can be modified (Birch, 2002). For example, taste preferences appear to be one factor in the complex causes of obesity (Donaldson, Bennett, Baic & Melichar, 2009). It is believed that overeating and obesity are somehow linked to individuals' diet. In the present context of the universal prevalence of pediatric obesity (Yanovski, 2001) and of the wide availability of palatable processed foods, it is essential to take into account sensory drivers of eating (Drewnowski, 1997a; Nasser, 2001).

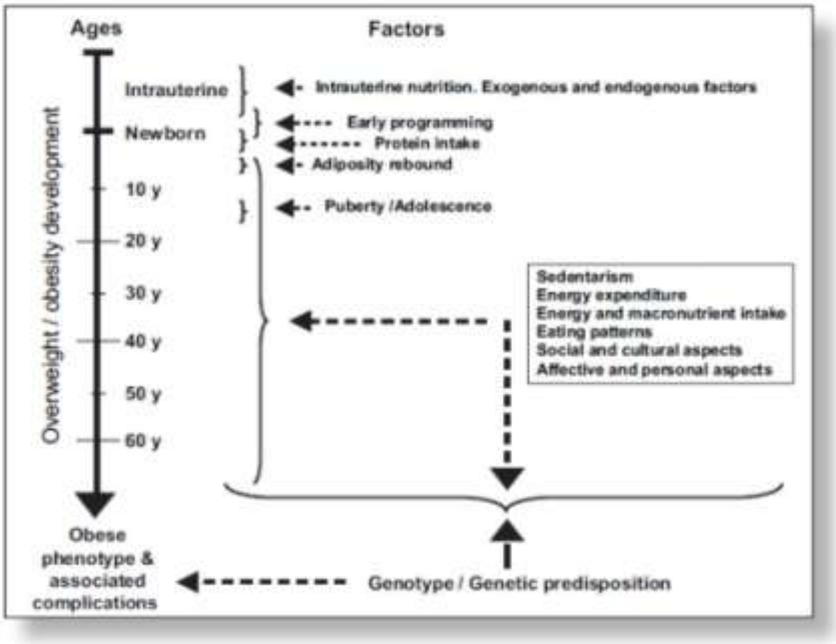

Figure 2. Obesity development and its early origins. Source: Rodriguez & Moreno, 2006.

Obesity is due to an imbalance between energy intake and energy expenditure, resulting in an excess weight (Lioret et al., 2009). This imbalance over a long time period might be one of the possible implications for the role of taste on the development of obesity (Nasser, 2001). In order to explain the role of dietary factors on the etiology of overweight and obesity, although the list is not exhaustive, many points should be kept in mind: humans have a tendency to consume a similar amount of foods whatever their energy density (Rolls & Bell, 1999); the world's nutrition transition, gives access for all to energy dense foods with high salt, sugar and fat contents available at low costs (Darmon, Briend & Drewnowski, 2004); and the increasing sedentarity and decreasing physical activity (Poskitt, 2009). The interaction of some or all of these factors makes such an association between energy dense foods' intake and obesity more than probable. Moreover, the report of the WHO/FAO expert group, classified high intake of energy-dense micronutrient-poor foods (e.g. processed foods, high in fat and/or sugars), as a "convincing" factor promoting the increase of weight gain and obesity (WHO, 2003).

A link between salt intake, sugar intake and the prevalence of obesity was highlighted (Durack et al., 2008). In fact, high dietary salt intake was shown to be related to a higher consumption of fluids in adults. In children and adolescents, most consumed fluids are sugar-sweetened beverages which are suspected to be related to the higher prevalence of obesity. Hence, children and adolescents eating less amounts of salt had a lower consumption of sugar-sweetened drinks, which lowers their chances of developing obesity, high blood pressure, heart attack and strokes later in life; UK data cited by He & MacGregor, 2008. Moreover, higher salt intake was correlated to a higher BMI in French adult population; moreover, 54% of high-salt consumers (> 12 g/d) are overweight (AFSSA, 2002).

Fat- and sugar-rich foods are frequently associated with the high prevalence of overweight and obesity as they provide dietary energy at low cost (Drewnowski, 2003; Drewnowski & Darmon, 2005). Indeed, the majority of foods preferred by obese subjects are high in fat and sugar (Mela & Sacchetti, 1991; for further details see also section 2.4). The implication of high fat diet consumption on the development of obesity was clearly established (Bray, Paeratakul & Popkin, 2004). In recent works, researchers have found heavier (Stewart, Feinle-Bisset, Golding, Delahunty, Clifton & Keast, 2010) and obese (Stewart, Seimon, Otto, Keast, Clifton & Feinle-Bisset, 2011b) subjects to have a reduced ability to detect fatty acids. That might explain the higher craving for high-fat diet more frequently observed in obese than in lean subjects (Bray et al., 2004).

In children, no strong evidence of the contribution of dietary energy from fat on children's overweight or obesity was shown in reviews of the question based on epidemiological analysis (Rodriguez & Moreno, 2006). Findings from studies involving toddlers and children are not consistent on the significant relationship between fat intake and weight gain or BMI, with some observational studies finding no significant link, whereas other do (see discussion in Rogers & Emmett, 2001). Besides, children's 'fat appetite' –i.e. their consumption of a high percentage of total energy from fat - was linked to their parents' and to their own body mass index (Fisher & Birch, 1995).

High levels of dietary sugars contribute to the overconsumption of hidden calories (Elliott, 2010). Other studies have found that increasing intake of calories from soft drinks is significantly linked to the epidemics of obesity and type 2 diabetes (Apovian, 2004). Public health authorities do advice a decrease in the intake of sugar-rich foods and beverages, in order to decrease the prevalence of overweight and obesity (WHO, 2003). However, with regard to evidences from the scientific literature on the link between sugar intake and weight status, a relationship is far from being demonstrated. Moreover, the absence of such a causal link observed in cross-sectional studies (simple point on time) is not surprising; if we consider the dynamics of weight change (Drewnowski & Bellisle, 2007). An epidemiological study conducted in France between 1992 and 1993, including 501 children aged 5-11 years, found an inverse relationship between sucrose intake and body weight (Maillard et al., 2000). On the contrary, as far as fat in concerned epidemiological studies found the positive link between fat intake and indices of obesity to be more likely than that between high intake of carbohydrates (simple and/or complex) and indices of obesity (Benton, 2004). This absence of association was also described in children and young adults participating in other studies, and could be explained by their greater physical activity, lower weight or simply by underreporting of their real intakes (Drewnowski & Bellisle, 2007).

Beyond the intake from added sugar, it may be the form of presentation that matters; the intake of sugars in liquid or solid form might have a differential effect on food intake regulation and weight. Liquid sugar calories are brought by sugar sweetened beverages (SSB) such as carbohydrate soft drinks, juice-based beverages, 100% juices and flavored milks (Drewnowski & Bellisle, 2007). Some authors propose that liquid sugar calories are not as well perceived by the body as solid sugar calories, as they have a rapid oral and gastrointestinal transit and absorption, as well as a unique pattern of satiety hormone response, which might promote a positive energy balance and weight gain (Mattes, 2006). Thus, a parallel was made between the prevalence of overweight, the obesity epidemic and the rising levels of sugar-sweetened beverages (SSB) consumption (Drewnowski & Bellisle, 2007; Hu & Malik, 2010). In fact, some studies found that a regular high intake of SSB was associated to weight gain (James, Thomas, Cavan & Kerr, 2004). However, the scientific reviews to date did not reach a final conclusion. For example, a meta-analysis conducted by Ruxton and co-workers on studies from 1995-2006, cited two reviews published in 2006 that reached different conclusions. According to Bachman and colleagues the findings were still inconsistent and studies should focus on populations at risk for obesity, whereas Malik and colleagues conclude to a demonstrated relationship; the later review was further criticized by other authors due to the absence of an objective interpretation of the studies (Ruxton et al., 2010).

The question of whether SSB contribute to weight gain needs further investigation, as a great discrepancy of scientific evidences either from cross-sectional, cohort, case-control or even from intervention studies does exist (Drewnowski & Bellisle, 2007). The report of the WHO/FAO experts concluded that "*overall, the evidence implicating a high intake of sugars-sweetened drinks in promoting weight gain was considered moderately strong*". Thus SSB are considered as "a probable etiological factor" of overweight and obesity (WHO, 2003).

1.3. Public health recommendations and actions

A large number of human studies show a high dietary intake of salt, sugar and fat. Based on their results, worldwide initiatives were taken in order to reduce current intake of salt, sugar and fat, especially by reducing their levels in foods. In order to reduce and prevent overweight and obesity, several national (PNNS in France) and international (WHO) organizations advocate some diet-related recommendations, on the one hand by encouraging food industry and caterings to reduce quantities used while preparing foods, and on the other hand, by encouraging consumers to limit energy intake from total fats and to limit intake of salt and sugar.

1.3.1. General recommendations

Concerning salt, current goals of a number of public health agencies worldwide are to reduce, or at least to restrict daily salt intake to less than 5-6 g / day (or < 2 g of sodium per day) as a population nutrient intake goal (World Health Organization, 2007). From a public health perspective, it is important to remind that 1 g of NaCl is equivalent to 400 mg of sodium; as it is especially the consumption of sodium that should be reduced. Unfortunately, dietary intake exceeds this recommendation in many countries (Brown, Tzoulaki, Candeias & Elliott, 2009). The WHO recommends that salt intake must not exceed 5 g / day in adults (WHO, 2003). In the USA, the Health and Human services (HHS) as well as the US Department of Agriculture (USDA), recommend the daily salt intake of adults not to exceed 5.75 g / day, whereas at risk populations (black people, individuals with hypertension, middle-age people and older adults) are required to consume less than 3.75 g of salt per day (Durack et al., 2008). In French adults, the public health low of August 2004 stipulates that salt intake must be reduced to less than 8 g / person / day Hercberg et al., 2008. In France, the AFSSA aimed in 2002 at reducing salt intake by 20 % in 5 years, so that the average intake will fall from 10 g salt / day to 8 g of salt /day within these five years (AFSSA, 2002). Experimental studies revealed that a 30 % and even up to a 50 % gradual reduction of sodium levels in foods over a long-term period (5 months or even no longer than 6 consecutive weeks) might be reached without negatively affecting the consumers' acceptability of the reduced salt foods (Bertino, Beauchamp & Engelman, 1982; Girgis et al., 2003).

In France, the recommended intake ranges from 90 to 170 g of sodium / day (0.225 and 0.425 g of salt / day) for 0-6 month-olds, and from 189 to 212 g of sodium / day (0.47 and 0.53 g of salt / day for 7-12 month-olds) Martin, 2001b. Anyway, the intake must not be above 40 mmol of sodium / day (2.33 g of salt / day) for children under one year (Martin, 2001b; Mosser, 2005). The Scientific Advisory Committee on Nutrition[8] (SACN; UK) limits the target average salt intake (representing the achievable population goal) for children, to <1, 1, 2, 3, 5 and 6 g / day respectively for 0-6 month-olds, 7-12 month-olds, 1-3, 4-6, 7-10 and 11-14 year olds (SACN, 2003). These values are those recommended for European children by the European Food Information Council (EUFIC). The Institute Of Medicine (IOM) recommends that 7-12 month-olds' salt intake should 0.225 g / day, and that of 12-24 year-olds should not exceed 2.5 g / day (Elliott, 2010).

[8] The SACN is an advisory Committee of independent experts that provides advice to the Food Standards Agency and Department of Health as well as other Government Agencies and Departments.

Concerning the baby and infant food sector, the AFSSA recalls the Order amended on July 1976, according to which sodium content of cereal-based products (for 0-3 years) must not exceed 100 mg /100 kcal (0.25 g of salt), that the final sodium content of jars and ready meals must not exceed 200 mg / 100 g or kcal (0.5 g of salt) AFSSA, 2002. These French recommendations are in line with those at the European level, as the sodium content of processed cereal-based foods, shall not exceed 100 mg / 100 kcal (The Commission of the European Communities, 2006). These two sources also advise an avoidance of sodium additions to products based on fruit, desserts, puddings etc. (except for technological purposes).

Concerning sugar, recommendations are less precise than those for salt. As it was discussed earlier (see 1.1.3) 'sucrose intake is tolerable for the body in far greater amounts' than that of salt for example (Spetter *et al.*, 2010). The WHO established total carbohydrate intake goals at 55-75 % of daily energy intake (unless otherwise stated), and that the intake of simple or free sugars must be less than 10 % of daily energy intake (WHO, 2003). Regarding its major physiological contributions for energy in the body, especially for the brain, the American Institute Of Medicine (IOM) estimates the average requirements of carbohydrate in children and adult diet, to be around 130 g / day (Sigman-Grant & Morita, 2003). In other respects, the American Heart Association (AHA) recommendations released in 2009, are that added sugars do not have to exceed 100 and 150 calories per day respectively for women and men (Johnson et al., 2009). The AHA recommends a maximum added sugar intake of 6 and 9 teaspoons a day, respectively for women and men (Elliott, 2010). The French recommended nutrient intake of carbohydrates is in the range of the WHO recommendations; a nutritional recommendation for carbohydrate intake of 50-55 % of total energy intake was set in 2001, and revised in 2004 by an expert group of the AFSSA on the light of the scientific evidences, and was kept unchanged (Ancelin, 2004; Martin, 2001a). Thus, one of the priority objectives of the PNNS is to increase complex carbohydrate intake while reducing simple sugar intake by 25 % in 5 years (Ancelin, 2004; Hercberg et al., 2008). Concerning manufactured baby foods, recommendations for total carbohydrates (in general without specifying which ones), set by the European Union range from 5 to 25 g / 100 g depending on the product (The Commission of the European Communities, 2006). For added sugar, the above recommendations were set with a focus on adults, whereas for children these recommendations are scares or vague. The AHA recommends to 'reduce the intake' for children under 2, whereas the UK National Diet and Nutrition Survey recommended added sugar should not contribute to more than 10 % of total daily calories in 1-3 year-olds (Elliott, 2010).

Concerning fat, the WHO recommends a reduction of fat intake, with regard to their quality and quantity, while keeping in mind the need to meet energy requirements (WHO, 2003). For children under 2 years, no clear 'appropriate' or necessary level of fat in the diet has been defined so far, thus their fat intake should not be limited, or should even be high due to their growth needs (for review see Uauy & Dangour, 2009; and table 3 for recommendations concerning children 0 to 3 years). The daily fat intake should not exceed 30 to 35 % of energy for adults and children over 2 years, depending on the physical activity (Uauy & Dangour, 2009). French population reference intakes or recommended dietary allowances (ANC) for fat are slightly above this limit (see table 3; ANSES, 2011). For processed baby foods, the European Union states that the total fat from all sources shall not exceed 4.5 g / 100 kcal, except if meat or cheese are the only ingredients then the limit is fixed at 6 g / 100 kcal (The Commission of the European Communities, 2006).

Table 3. Comparison of recommendations regarding total fat dietary intake (% of energy intake), for children 0 to 3 years. Source: Uauy & Dangour, 2009.

	Age range, months			
	0–4/6	6–12	12–24	24–36
AAP, 1986				30–40%
AAP, 1992				30%
Canadian Paediatric Society, 1993[a]	no restriction	no restriction	no restriction	no restriction
European Union, 1996	≥40–58.5%	≥32–58.5%[b]		
ESPGHAN, 1991 and 1994	≥40–58.5%	≥32–58.5%[a]	no restriction	30–35%
WHO/FAO, 1994	50–60%		30–40%	30–40%

AAP = American Academy of Pediatrics Committee on Nutrition; ESPGHAN = European Society for Pediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition.
[a] Roy et al. [1993]. [b] Recommendation for follow-on formulae only, not for total diet.

Moreover, a qualitative recommendation for fat intake was established (table 4), with regard to the differential effects of FAs on health (as discussed in section 1.2). It is also recommended to replace, wherever possible, animal fats by vegetable fats, particularly those rich in monounsaturated fatty acids.

Table 4. French population references intakes or recommended dietary allowances (ANCs) for fats. Adapted from: ANSES, 2011.

	Total dietary fat intake	**EFAs**			**SFA**
		LA	ALA	DHA‡	
Newborn/infant (first 6 months)				0.32 % TFA	---
Infants (6 months to 1 year)	45 – 50 % EI	2.7 % EI	0.45 % EI	70 mg	
Toddlers (1 to 3 years)					¥ ≤ 8 %EI ‼ ≤ 12 %EI
Children (3 to 9 years)				125 mg	
Adolescents (10 to 18 years)	35 - 40 % EI	4 % EI	1 % EI	250 mg	
Adults					¥ ≤ 8 %EI ‼ ≤ 12 %EI

Values are expressed as a % of energy intake (EI)
TFA; Total Fat intake
‡ The variability of the daily energy intake amount does not enable expression of ANCs as a % of energy
‼ Total Saturated Fatty Acids
¥ Lauric, myristic and palmitic fatty acids

1.3.2. Recommendations targeting the food industry and catering

Due to the ubiquitous presence of salt, sugar and fat in manufactured foods, public health authorities engage food manufacturers to reduce these ingredients in the foods they process. This involvement consists in a reformulation of low salt, low sugar and low fat products in order to improve the nutritional quality of foods (European Union, 2009; Hercberg et al., 2008).

Reformulating foods might result in an increase of the product's cost for the food companies, due to the use of new ingredients, replacers, flavors or new processes, which might limit their implication and willingness to act (European Union, 2009).

Moreover, an important insight should be given to the implications for food safety and structure that would be due to reformulation (European Union, 2009). Concerning salt for example, the AFSSA concluded that for the majority of foods where salt is added, there are no major technological or health constraints against reducing its levels, and that reductions in salt content in processed foods would have no impact on certain products' safety (AFSSA, 2002; Doyle & Glass, 2010).

Another important factor that might refrain food manufacturers from reducing salt, sugar and fat in their products, might be the fear that the consumer would not purchase, accept and consume the reformulated products due to taste or other qualities' modifications (European Union, 2009; Wansink, van Ittersum & Painter, 2004). A study conducted by Girgis and collaborators has shown that progressive reductions of salt in bread (from 2 to 1.5 g/ 100 g of flour), can be made without impacting the consumers' acceptance, even though they perceived the bread as less salty over time (more detailed aspects of the consumers' responses to salt, sugar and fat contents in food will be discussed in part 3 of this manuscript).

Food manufacturers can reduce the content of salt in foods by applying moderate and sustained reductions. This strategy was even shown to induce a preference for a lower salt diet (Bertino et al., 1982). In the United Kingdom, reductions in salt levels were performed in cereals (- 33 %; in a 7 year period), in Kraft processed cheese (- 33 %) and in Heinz products (- 11 to - 18 %), and according to the authors, these reductions were well tolerated by the consumers and the reformulated products were even better liked than the original ones (from kilcast & den Ridder, 2007 cited by Doyle & Glass, 2010). Thanks to the Consensus Action on Salt and Health (CASH), that aimed at changing the British national policy of health in relation to salt, as well as the consumers' awareness, the WHO recognized UK as the first country to have a "coherent, systematic, ongoing reduction of the salt content of nearly all foods to which salt has been added by the food industry […] without any consumer rejection as this process has introduced slowly over the course of a few years"; World Health Organization, 2007. In France, from 2003 to 2005 several significant reductions of salt content in some commercialized food categories were observed. For example, the maximum drop out was observed for breakfast cereals (- 23%) and the least one was observed for pastries (-3%). Moreover, according to the INCA2 study, the average dietary salt intake decreased in the French adult population by - 5.2 % in 8 years (from 8.1 to 7.7 g / day); AFSSA, 2009. In addition to France and UK, many countries managed to reduce sodium by 10 to 30 % (with exceptions up to 60 %) in a wide range of processed foods (Dötsch et al., 2009; World Health Organization, 2007).

Although acknowledging the potential consequences of reformulation on consumers' willingness to consume foods, the European Union report points to the fact that food manufacturers have two possibilities in order to face the consumers' reaction (European Union, 2009). The first approach would be to introduce it by "stealth", without informing the consumer about the reduction. Studies have shown this method to be efficient when salt (Girgis et al., 2003). The second approach would be, a marketing one, based on informing the consumers about the modifications brought, as for some consumers the choice of a food can be based on label information about the healthiness of the food they are purchasing (Hoefkens, Verbeke & Van Camp, 2011).

The Irish FSAI aimed for example at reducing the average population intake of salt from 10 to 6 g a day by 2010 through a partnership approach with the food industry (Durack et al., 2008). In France, the government encourages food manufacturers to perform reductions in salt, sugar and fat in foods and they can sign, with the governments, so called 'charters of commitments to nutritional improvement' (Hercberg et al., 2008). Since 2008, 27 firms in France have signed these charters (CCNI, 2008-2011). Moreover, in 2008, a nutritional composition databank of branded French products (Oqali: The Observatory of food QuALIty), has been developed in order to collect and centralize informations on the nutrient composition of commercial foods (Combris, Goglia, Henini, Soler & Spiteri, 2011). Besides centralizing data, this databank will also serve to monitor the efforts of food industry professionals and verify their compliance with the PNNS charters of commitments (Hercberg et al., 2008). By matching the information of this databank with those of the INCA2 consumption databank, the authors simulated scenarios of food consumption modifications, and proposed a minimum quality standards (MQS) approach, so that reformulated products will be still acceptable by the consumers -as the products' familiar tastes do not change- and feasible for the food industry -as it does not imply technological changes- (Combris et al., 2011).

Concerning caterings in France, the circular of June 25th, 2001 recommends "getting children used to eating only lightly salted food". Thus in all dining facilities (for children and adults) available table salt shakers are restricted, or if they are not, their size is smaller than before, the volume of sachets of salt was reduced from 1 to 0.5 g (AFSSA, 2002). Moreover, catering staffs follow training courses where they are informed, among other things, about the effects of salt, sugar and fat, and how to reduce their quantities in food. In a report targeting French caterings, informations relative to salt, sugar and fat usage are vague. The report reminds about their health effects, the nutritional recommendations of the PNNS, the prevalence of overweight and obesity, the findings of the INCA2 study etc. (Groupe d'Etude des Marchés de Restauration Collective et de Nutrition, 2011). While coming to the development of menus, recommendations given are as it follows:

- For children under 3 years, and for adolescents, *free access to salt should not be given*, and dressings are available depending on the dish, and set near to the food service counter so that staff can monitor their service use.
- *Decrease the consumption of simple carbohydrates added* for infants and young children in kindergarten, nurseries or health facilities. It is recommended to *restrict* the consumption of desserts or dairy products containing more than 20 g of total carbohydrates per serving, and less than 15 % fat.
- Butter and cream can be used to flavor some recipes (pasta, mashed potatoes, for example) but *in small quantities*. In all other recipes, the use of vegetable oils is recommended.

Thus, no clear recommendations on the quantities to be used while preparing foods for children are given.

1.3.3. Recommendations targeting the consumers

When it comes to the consumer, many strategies were established in France, in order to decrease the amount of salt, sugar and fat they consume. Most of the strategies on-going in France are summarized in table 5.

Table 5. The PNNS1 (2001-06) actions targeting the consumer. Adapted from: Hercberg et al., 2008.

Actions	2001	2002	2003	2004	2005	2006	2007
1. Provide and promote comprehensive nutrition communication for all consumers.							
PNNS logo created	↔						
Dietary intake guidelines established	↔						
Nutrition guide for the general population		↔					
Specific population nutrition guides				←	—	—	→
Media campaigns	←	—	—	—	—	—	→
Websites created	↔			↔			
2004 Public health law enacted (processed food ads)				↔			

Beside a range of specific dietary guides for children and adolescents, distributed largely in France, the PNNS group set other measures in order to promote a healthy eating and to increase the consumers' awareness about what is on their plate. The more important concerning salt, sugar and fat, are media campaigns targeting both children and adults, and health advantages of their reductions (PNNS2; 2007-2010). Moreover, one famous catchword now known by French children and adults: "For your health, avoid eating too much fat, too sweet, too salty", is broadcasted on French television during commercials, especially those for children, and also when advertising for snacks. Other campaigns aim at 'teaching' the consumer how to read and understand food labeling, now mandatory on foods. Consumers are encouraged to pay attention to information on the quantities of salt, sugar and fat on products and for a standard portion, and to compare products before purchasing them. Moreover, recommendations also integrate advices on- and warnings about 'hidden' salt /sugars/ fats found in some commercialized products, frequently consumed by children, such as pastries, snacks, meats and sausage etc. (see: www.manger-bouger.fr, www.sante.gouv.fr, and Hercberg et al., 2008).

1.4. Intake data: national and international surveys

1.4.1. Salt intake

Several sources of sodium exist and account for variable levels of dietary intake (%) such as natural sodium in foods (10 %), and that present in processed and catering foods (75 %); Doyle & Glass, 2010. In Europe and North America, 5-10 % of salt intake is due to the addition of salt by consumers at table and/or during cooking (Mattes & Donnelly, 1991 cited by Doyle & Glass, 2010).

To investigate salt intakes in populations different methods are used: dietary survey methods, 24-h urinary collection over X days, overnight and spot urine collections, markers of sodium intake etc. (For a full review see Brown et al., 2009).

In the INCA1 study, sodium intake was assessed using a 7-day record of foods and drinks, as well as an estimation of portion sizes with a validated tool. The results revealed that the mean salt intake (excluding added salt) of 8 g / day in adults (n=1474; > 15 years). If we consider an additional 1 to 2 g added during cooking or at the table, the total salt intake would reach 9 to 10 g / day; 8 % of adults have an intake above 12 g / day, these were identified as 'heavy consumers' and some even reach 25 g / day (AFSSA, 2002; Meneton et al., 2009). Moreover, sodium intake in the French adult population was higher in men than in women.

Current daily consumption figures can be as high as 10-12 g of salt per day (see table 6).

Table 6. Average adults' salt intake by country.

Country	Salt intake (g/day)	Data source / year	Reference
Ireland	10	FSAI / 2003	Durack et al., 2008
Europe	12	IHF / 2008	
UK	10	SACN / 2003	
France	6.9	ASPCC survey / 1994	AFSSA, 2002
	7.9	Afssa / 2000	Meneton et al., 2009
	7.4	Afsaa / 2006	AFSSA, 2009

In children and adolescents (< 15 year-olds), mean salt intake was 6 g / day (in 1999 and in 2006), with 50 % of them having an intake equal to 4.5 – 7 g / day, and 10 % having an intake of 8.8 g / day (AFSSA, 2009). Data on French children and adolescents, from the INCA1 study revealed that sodium intake was higher in boys than it was in girls. Another observation concerned an age-related effect: mean salt intake is equivalent to 0.22, 0.25 and 0.27 g / day respectively for children 2-5 (n=243), 6-10 (n=442) and 11-14 (n=333) years. Moreover, a progressive increase was observed between 2-5 and 15-24 years of age, underlying the greater exposition to salt-rich foods since early childhood (Meneton et al., 2009).

1.4.2. Sugar and fat intake

In the adult French population, the energy intake was found to be around 2066 kcal/day (without alcohol), with carbohydrates, lipids and proteins contributing respectively to 44, 39 and 17 % of total daily energy intake (AFSSA, 2009); whereas the recommended dietary allowance (ANC) should be respectively of 50-55 %, 30-35 % and 11-15 % of total energy intake (Martin, 2001a). Thus, the French adult population has a diet too rich in lipids and proteins, with a predominance of SFA (44%), which intake should be lowered in order to prevent health problems (AFSSA, 2009).

Table 7 summarizes the current nutrient intake for French children (3-17 years) and adults (18-79 years), and their adequacy to nutritional recommendations. Carbohydrates seems to be the major contributors on French children's energy intake compared to adults (47 vs. 44 %), with simple carbohydrates bringing more energy for children than for adults (23 vs. 19 %); AFSSA, 2009. In children, the major contributors for simple carbohydrates or sugars are drinks with a 19 % of intake (AFSSA, 2009). Lipids are on average consumed in excess by both adults and children in France, with a greater intake of SFA in children compared to adults (AFSSA, 2009). Foods considered as major vectors of fat in French children's diet are oils (9%), butter (8%) and pastries and cakes (8%). Butter is also found to be the major source of SFA (13%); AFSSA, 2009.

Table 7. Compliance of the French population with the nutritional recommendations.

Comparisons between the current nutrient and energy intake of the French population based on the INCA2 study conducted in 2006-2007: AFSSA, 2009, and the recommended daily allowance: Martin, 2001a.

	Adults (n=1918; 18-79 y.)	Adult's RDA (% EI)	Children (n=1444; 3-17 ans)	Children's RDA
EI[†] (kcal/d)	2066	1800 - 2200	1770	1219 - 3190
Total lipids (% EI)	39	30-35	38	30-35
SFA / MUPA / PUFA (% total fatty acids)	44 / 39 / 16	8 / 20 /…	47 / 38 / 15	8-12 /… / …
Proteins (% EI)	17	11-15	15	11-15
Carbohydrates [‡] (% EI)	44	50-55	47	50-55
Complex carbohydrates (% carbohydrates)	57	----	51	----
Simple carbohydrates (% carbohydrates/% EI)	43 / 19	< 10% of EI	49 / 23	----
Sodium (mg/d)	2968		2146	
Salt (g/d)	7.42		5.36	

[†] *Energy Intake without alcohol (EI)*

[‡] *Carbohydrates = starch + simple carbohydrates*

1.4.3. Exposure to salt, sugar and fat, and their intake during early feeding experiences

Although the majority of national and international surveys describe salt, sugar and fat intake data for children most frequently those over 2 years, intakes do not start at 2 years.

As depicted in table 8, the proportion of sodium in mature human milk is approximately the same as that in formulas (15 and 18 mg / 100 g respectively) at least in developed countries (Emmett & Rogers, 1997; He & MacGregor, 2010). During the first months of life, when babies are breast-fed, sodium intake is equivalent to 230 mg / day or 0.58 g of salt per day (Yeung et al., 1982 and Singhal et al ; 2001 cited by Geleijnse & Grobbee, 2002). In France, a cross-sectional study was conducted in a sample of 706 non breastfed infants aged 1 to 36 months. Sodium intake ranged from 179 to 332 mg / day in 1 to 5 month old infants (Fantino & Gourmet, 2008). While starting weaning, around 6-9 months and while growing, infants and toddlers are exposed to greater amounts of salt as they move from a milk regimen to solid foods. In France, the data collected in 2005 show that sodium intake increases from 468 mg /day in 6 month-olds, to reach 1303 mg / day in 36 month-olds (Fantino & Gourmet, 2008). In fact, when solid foods are introduced, salt intake increases in infants and toddlers, then the daily intake can even reach 5.5 g in children aged 5 years. Salt intake in developed countries increases gradually during childhood and adolescence, to reach a value of 9 g at adult age (Heino et al., 2000 and Anonymous 2000 in Geleijnse & Grobbee, 2002). That could be due in part to the high quantities of sodium in processed products, as a profile of Canadian baby and toddler food products (n=186), showed that in the top 20 highest sodium content products, sodium contents ranges from 180 to 550 mg (Elliott, 2010). In this study products with < 130 mg of sodium were classified as 'acceptable' and those with a sodium content > 260 mg were considered as high in sodium.

Table 8. Composition of human Caucasian women's, bovine and infant formula milk.
Adapted from: Emmett & Rogers, 1997.

Nutrient	**Human milk[‼]**			**Cow's milk**	**Formula milk**
(/100 g milk)	**Colostrum**	**Transitional**	**Mature**		
Fat (g)	2.6	3.7	4.1	3.9	3.6
SFA (g)	*1.1*	*1.5*	*1.8*	*2.4*	*1.4*
MUFA (g)	*1.1*	*1.5*	*1.6*	*1.1*	*1.3*
PUFA (g)	*0.3*	*0.5*	*0.5*	*0.1*	*0.6*
Carbohydrates (g)	6.6	6.9	7.2	4.8	7.1
Sodium (mg)	47	30	15	55	18
Energy (kcal)	56	67	69	66	64

[‼] Colostrum (also named Yellowish) is produced 5-7 days after delivery; Transitional milk is produced from day 6 to day 14

Due to its composition, the mother's milk, the first food experienced by the baby, has distinguishable taste qualitiesMcDaniel, 1980. For instance, in a study conducted in 1980 by E. Barker, a trained adult panel provided a description of sensory properties of the human milk; the main taste quality found was sweetness, it was described as being as sweet as a 7-8 % lactose or a 2 % sucrose solution (McDaniel, 1980).

As depicted in table 8, the proportion of carbohydrates in mature human milk is approximately the same as that in formulas (7.2 and 7.1 g / 100 g respectively). The cross-sectional study conducted in France in 2005, reveals that total carbohydrate intake ranges from 67.4 to 89.6 g per day, respectively in 1-3 and 5 month-olds. Total carbohydrate intake increases then from 92.3 g /day in 6 month-olds, to reach 140 g / day in 36 month-olds. Concerning mono- and disaccharides, their intake ranges from 43.7 g/ day in 1-3 month-olds, and reaches 81.7 g/ day in 31-36 month-olds. Besides they contribute to 31 and 29 % of total energy intake, respectively in 1-3 and 31-36 month-olds (Fantino & Gourmet, 2008).
As it is the case for salt, the profile of the Canadian baby and toddler food products (n=186), showed that the percentage of calories from sugar ranges from 58 to 80 % considering the top 20 products with the highest percentage of calories from sugar. In this study, the authors considered products providing more than 20 % of energy from sugar were considered as nutritionally poor, while previous studies classified foods with more than 10 or 15 g of sugar per portion or per 100 g as having a high level of sugar (Elliott, 2010).

Thus the infant is exposed to salty, sweet and fatty tastes since early experiences with milk, and it continuous at weaning, when solid and semi-solid foods are introduced. A recent description of the taste intensity of foods served French infants during the first year of life was conducted in our laboratory. The parents of 76 infants of Dijon area recorded the diet of their infant ones a week during the first year of his/her life, and a list of 2902 foods was generated. As shown in figure 3, the major taste that infants are exposed to during the first year is sweet, followed by sour taste, and some infants are exposed to great quantities of salty taste (Schwartz, Chabanet, Boggio, Lange, Issanchou & Nicklaus, 2010). In fact, an study conducted with 139 infants aged 4-9 months and leaving in Dijon, revealed that 64 mothers preparing foods to their infant at home, and that 59 of these add extra ingredients such as salt (Maier, Chabanet, Schaal, Leathwood & Issanchou, 2007b). Similar practices induce a high exposure to taste since early childhood, what would explain, high intakes in adulthood.

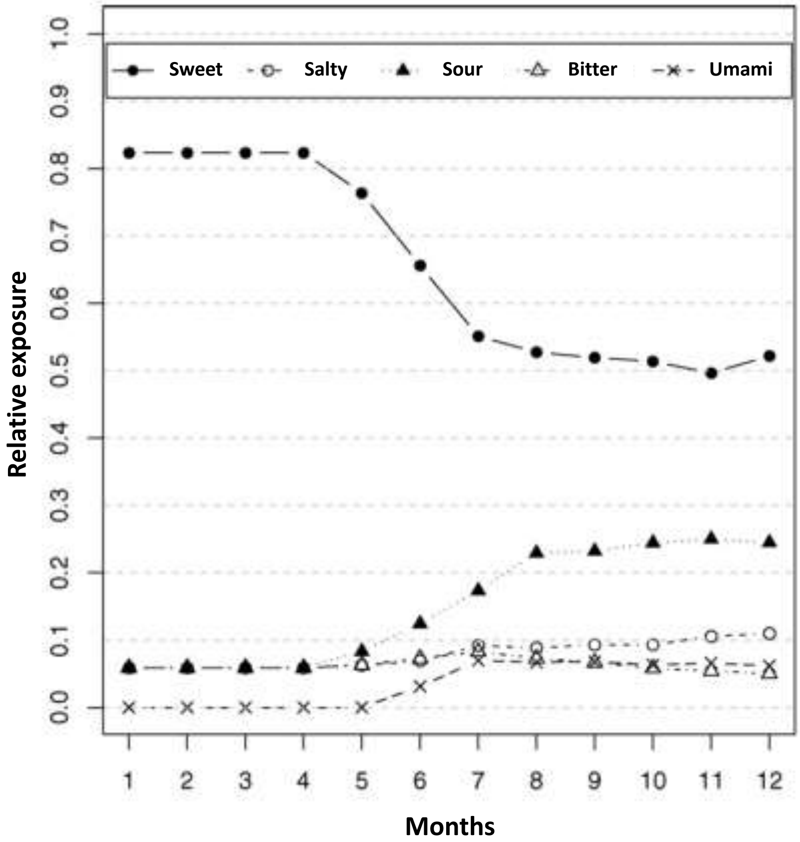

Figure 3. Monthly distribution of French infants' relative exposure to basic tastes. Adapted from: Schwartz et al., 2010.

Part 2. Development of taste perceptions

2.1. Taste: what are we talking about?

Frequently, the term taste and flavor are often confounded and confused. In fact, the perceptual conjunction of three anatomically distinct but complementary sensory systems, defines the term of 'flavor' of a food or a beverage. These sensory systems are smell (1st cranial nerve), oral chemical somatosensory stimulation, chemesthesis or so called chemical irritancy (5th cranial nerve), and finally taste (7th, 9th and 10th cranial nerves); Beauchamp & Mennella, 2011. Taste is used as a technical term (one of the five basic tastes) or as a more common term (frequently used as a synonym of flavor).

The sense of taste has early evolutionary origins. Although considered by some as one of the minor senses due to its 'limited repertoire', taste is the only sense that conditions nutrient intake (Mattes, 2010). It is sensed thanks to the stimulation, by molecules present in foods and beverages, of specific structures on the tongue, grouped in small clusters called taste buds (Lawless, 1985). Taste helps to solve one dilemma: identifying nutrients and avoiding poisons (Chandrashekar et al., 2006). Thus, taste detection and perception are key determinants of body health and safety. With regard to the tastes we are interested in, sweet and fatty detections in foods allow the identification of energy sources; whereas in the case of salt, our 'appetite' for it allows to ensure the needs of sodium for the body (Leshem, 2009).

To date, five basic tastes have been identified: sweet (e.g. sucrose), sour (e.g. citric acid), salt (e.g. table salt), bitter (e.g. coffee) and Umami or savory (e.g. soy sauce). However, it has not always been the case. In his recent review, Richard Mattes reminds of the history of tastes(Mattes, 2010). It starts at the time of Aristotle, when he suggested many modalities or stimuli as taste qualities, such as salty, sweet, sour, bitter, metallic, astringent, pungent, harsh, water/insipid and fatty etc. At that time, only four 'primaries' were agreed for: sweet, salty, sour and bitter. A century ago, another sensation elicited by a compound found in food, was proposed to be the fifth basic taste. Thus, sensation elicited by amino acids, and especially its prototypical stimulus monosodium glutamate (MSG), is the now well-known umami taste. Recently, researchers wondered about the existence of a sixth taste i.e. fatty taste (Mattes, 2010). Besides, qualities such as alkaline, metallic, astringent, pungent were attributed since then either to chemesthetic or to olfactory origins.

It requires many criteria for a stimulus brought by a food or a beverage, eliciting a specific perception, to be considered as a ‘taste’. In fact, it requires a specific receptor located within a taste receptor cell, ensuring an interaction with the stimulus, a transduction mechanism and a perception of the so supposed taste must be identified (Mattes, 2010; Stewart, Feinle-Bisset & Keast, 2011a). Thus, although fat was proposed to be part of the basic tastes, experimental evidences that fat ‘taste’ exists in humans is still controversial. However, concerning fat, the majority of criteria it requires to be recognized as the sixth basic taste are already evidenced in animal models, and is accumulating in humans Galindo et al., 2011; Stewart et al., 2011a.

2.2. How do we perceive tastes?

2.2.1. From the reception to the perception of the gustatory information

When a food, a beverage or a solution are in the mouth, the taste molecules or tastants are released and are detected by Taste Receptors Cell (TRCs); TCRs are located primarily on the oral cavity in several places: all edges of the tongue including its anterior dorsal surface, on the soft palate, in the pharyngeal and the laryngeal regions of the throat (Breslin & Huang, 2006). Sixty to 120 TCRs form a taste bud, and a group of taste buds is gathered in a gustatory papillae: circumvallate and foliate papillae containing hundreds of taste buds, and fungiform papillae with 0 to 15 taste buds; as depicted on **Figure 4**.

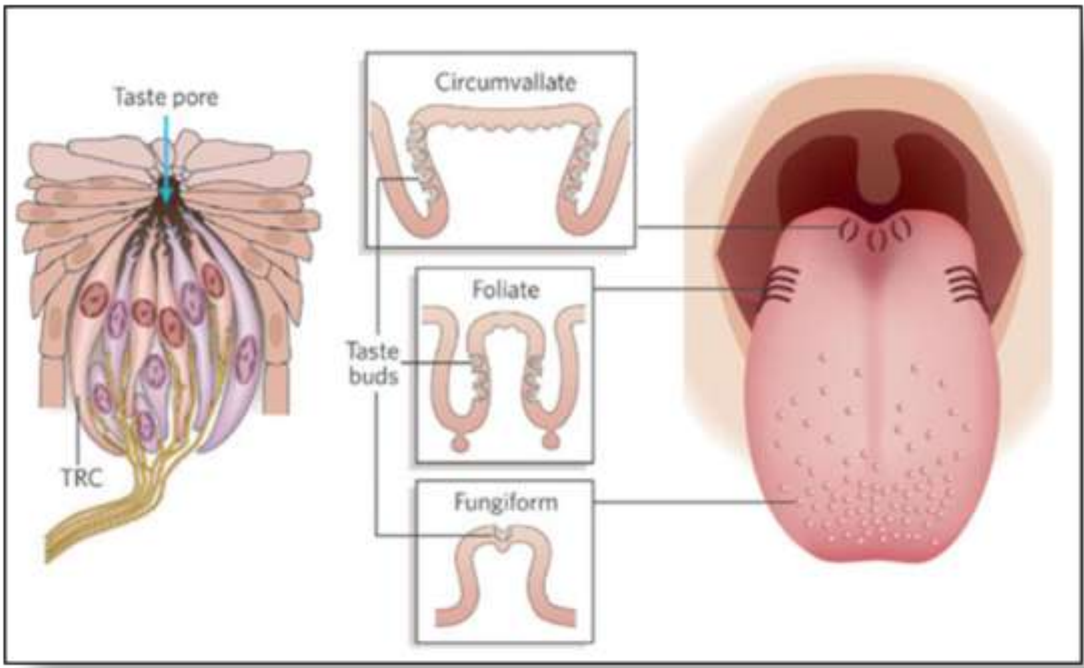

Figure 4. Taste-receptor cells, buds and papillae.

Taste buds (left) and their localisation in the tongue (right). Adapted from: Chandrashekar et al., 2006.

The apical end of receptor cells have microvilli that interact with the tastants present in the oral cavity, via their taste pore lying at the tip of each bud (Breslin & Huang, 2006). Receptor cells within a taste bud exist in four types, type I, II, III and IV, albeit the exact function of each type is not yet clear. Type II or sensory receptor cell, has microvilli with taste receptors that detect tastants (Figure 5). When it is stimulated, it secretes ATP (for Adenosine TriPhosphate) a neurotransmitter that has two functions: first, it excites gustatory primary afferent fibers; second, it acts as a cell-cell mediator between receptor (type II) and presynaptic cells (type III). The flux of ATP from receptor to presynaptic cells is thought to be regulated by type I cells (Frings, 2009). Another supposed role of type I cell would be their action as secretory structure and/or as a glia-like for the bud (Breslin & Huang, 2006). Type III cells have synapses with afferent axons, and release serotonin (5-HT); see Figure 5. Although the role of the released serotonin is still to be defined, it is thought to play a role in determining the human threshold of sensory receptor cells for sweet and bitter for example (Roper, 2007). Type IV cells were identified as basal cells, and are thought to give rise to the three other cell types, given the fact that cells within taste bud have a 10 day life Breslin & Huang, 2006.

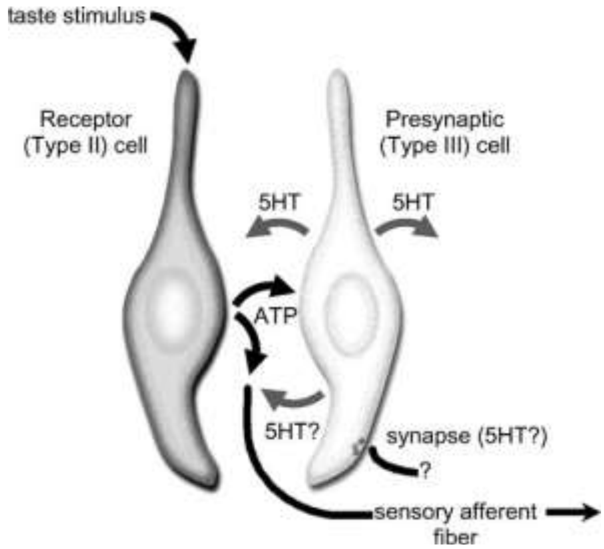

Figure 5. Cell to cell communication in the taste buds'. Source: Roper, 2007.

Thus, each taste bud contains a number of receptors that allow individuals to perceive the different tastes, and each taste has its own specific receptor(s). Each taste bud is innervated by branches of three cranial nerves and allows the transmission of the information (sensory influx) from the taste molecule to the specific brain areas. The extensions of these sensory cells are nerve fibers that are gathered in the lingual or glossopharyngeal nerve, responsible for the transmission of nerve messages to the brain areas responsible for the taste, where these messages are interpreted.

Sweet taste is due to a range of monosaccharides (e.g. glucose, fructose and others) and disaccharides (e.g. sucrose, lactose and others), but also to various amino acids (e.g. glycine, alanine and others), peptides (e.g. methyl ester of the dipeptide L-aspartyl-L-phenylalanine or aspartame) and proteins (e.g. brazzein) as well as to artificial sweeteners (e.g. sucralose, saccharine and others) and certain salts (e.g. diluted solutions of NaCl, LiCl and KCl); Frings, 2009; Roper, 2007. Sweet stimuli are recognized and transduced by dimer belonging to the family of G-protein-coupled receptors (GPCRs): T1R2 and T1R3, two closely related 7-transmembrane receptors (Chandrashekar et al., 2006).

T1 receptors are involved in the detection of generally attractive stimuli such as sweet and umami ones (Frings, 2009), whereas the T2 receptors are involved in the detection of generally aversive stimuli such as bitter (Breslin & Huang, 2006), The transduction of gustatory informations is complex and depends on the taste studied (see Figure 6 for a more detailed view of the transduction mechanisms of sweet and salty taste).

Salty taste is primarily due to the perception of sodium chloride, and its detection is not well understood to date and is still under investigation. Salty taste is thought to be transduced thanks to an epithelial sodium channel ENaC (for epithelial-type Na^+ Channel) located on the apical end of the taste cell, which is amiloride-sensitive (Frings, 2009; Roper, 2007). Thus, salty taste is not due to the binding of a molecule to its specific receptors such as it is the case for sweet taste)Bartoshuk & Beauchamp, 1994.)

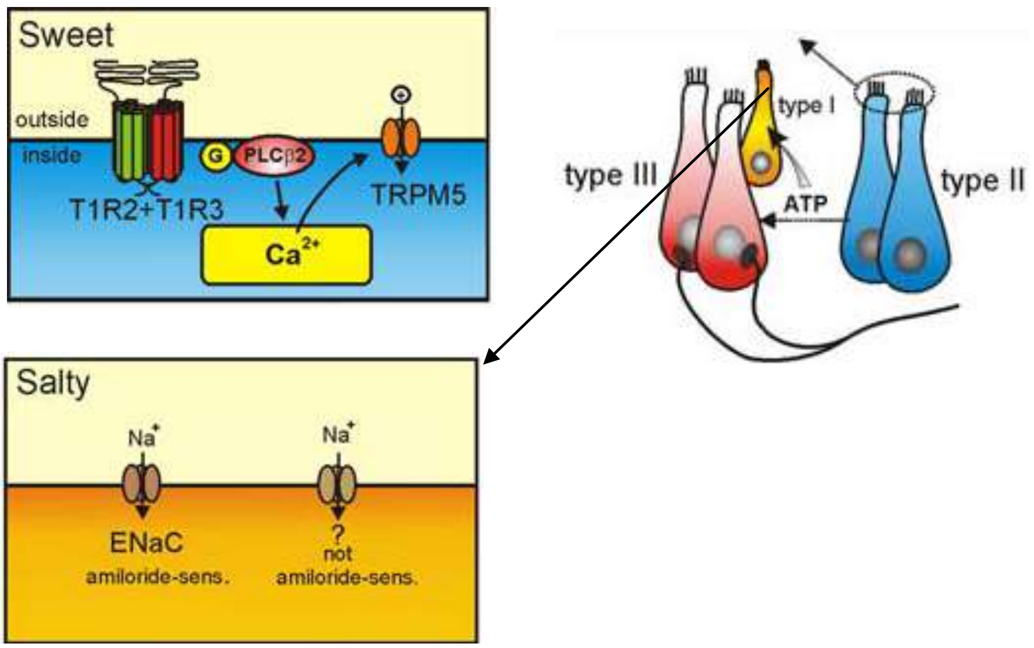

Figure 6. Primary processes of taste cells and transduction models.

Sweet taste resides in type II cells (blue) which release ATP as paracrine transmitter. Salt taste is mediated by type I cells (yellow). Adapted from: Frings, 2009.

Concerning the processing of sweet and salty taste informations, two theories were proposed to explain the coding of the gustatory information: the “labeled line theory”, according to which TRC innervated by individually tuner nerve fibers are set to respond to single taste modalities; and the "across-fibre model" according to which individual TRCs express different families of taste receptors and that the nervous fibers conduct the whole messages received whatever the concerned taste quality (Chandrashekar et al., 2006). According to present knowledge, it seems that the "labelled-line" theory of coding is the most likely occurring.

The gustatory information, now present at the level of the type III cells, is treated by the central nervous system (CNS). Three afferent cranial nerves, innervating taste buds, insure the transmission of the action potentials generated by the TRC in response to the presence of tastants (Breslin & Huang, 2006):

- the 7th cranial or facial nerve through its two branches, the chorda tympani (innervating the whole anterior 2/3 of the tongue, fungiform and anterior foliate papillae) and the greater superficial petrosal nerve branch (innervating the soft palate),
- the 9th cranial or glossopharyngeal nerve (innervating the foliate and circumvallate papillae),
- and the 10th cranial or vagus nerve (innervating taste buds in the pharynx and larynx).

The chemosensory afferent information is then sent to the first gustatory relay –the solitary tract nucleus (NTS) of the CNS medulla-, and then relayed into the upstream brain areas where taste processing and taste signal is decoded in order to elicit a perception of the taste sensation.

As depicted in figure 7, taste perception pathway is partly 'shared' by other sense pathways, which might explain the complexity of the understanding of the orosensory perception, and the frequent difficulties underlying its study. Indeed, this explains the confusions between taste and olfaction for instance. This system results in finely tuned detection abilities.

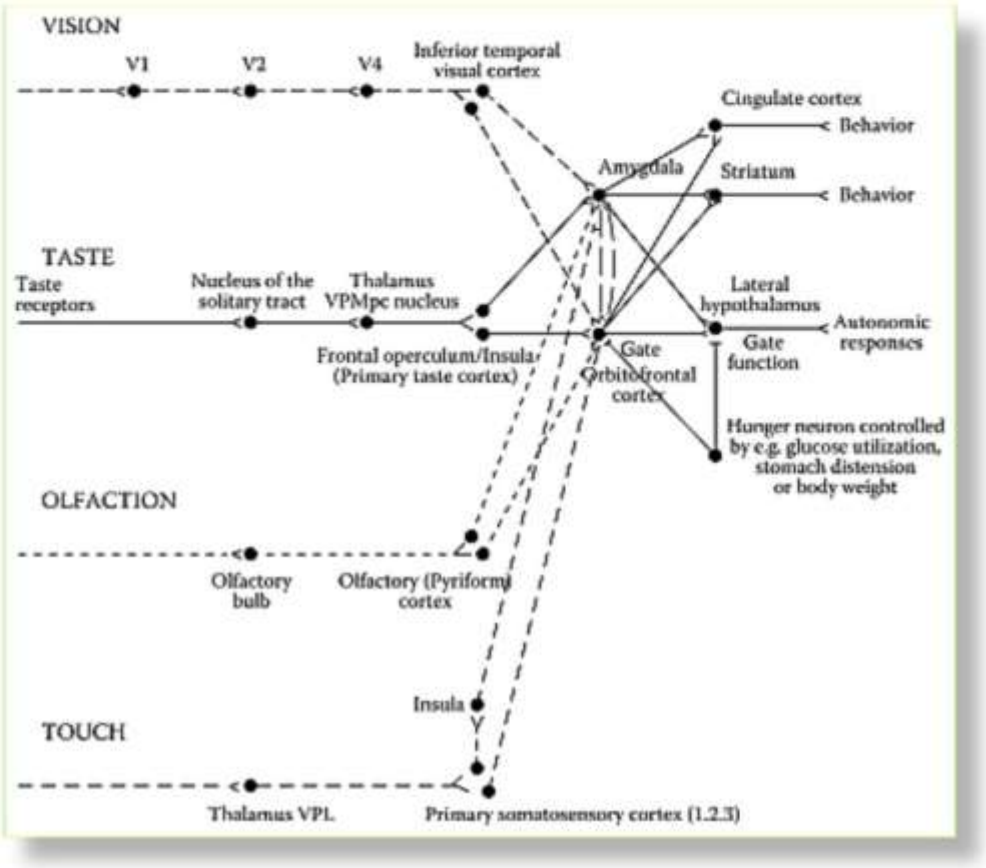

Figure 7. Schematic diagram of the brain pathways, responsible among other perceptions, of taste perception. Source: Rolls, 2010.

Roper (2007) reminds that humans can detect sucrose at a concentration of 6 to 7 mM and that they can recognize it as a sweet taste around 25 mM , but these values are subject to variations depending on the study. The detection threshold for NaCl ranges from 1 to 10 mM (NaCl solution in water); AFSSA, 2002; Roper, 2007. Receptors to salt start reacting at a concentration of 1.025 mM and many are stimulated at a concentration of 40 mM (AFSSA, 2002).

The perception of salty and sweet tastes can also be influenced by other orosensory features of food. For example, it was shown that taste perception in general, decreases with the increasing viscosity of the products; this was described for basic tastes such as sweet, salty or sour for instance (Pangborn, Trabue & Szczesniak, 1973). Another study demonstrated a positive correlation between salt release in saliva during chewing and salty perception Neyraud, Prinz & Dransfield, 2003. Further researches have shown this decrease to be due to interactions between the food tasted and the saliva (Ferry et al., 2006). Researchers identified the activity of the salivary enzyme α-amylase in the human mouth can reduce the perception of saltiness, especially in starch-thickened foods; thus, explaining the lower saltiness scores for subjects with higher amylase activity (Ferry et al., 2006).

2.2.2. The particular case of fatty taste

Two events are involved in fatty taste detection: early events and delayed events. Early events involve visual cues, smell/odor, texture and 'taste', whereas delayed events involve post-ingestive and post-absorptive signals (Gaillard, Passilly-Degrace & Besnard, 2008b). Here, we won't detail post-ingestive and post-absorptive signals involved in fatty perception (for a review see Gaillard et al., 2008b).

Concerning the early events, human psychophysical investigations used different processes in order to isolate the specific role of each taste component. The effects of visual cues can be minimized by using red lights during sensory testing. Concerning smell/odor, they can be minimized by using nose-clips as during food breakdown in the mouth, there is a flavor release (non-volatile to receptors in the mouth, and volatile to the receptors in the nose). Moreover, the textural cues of fats, considered as the major determinants of their detection, are minimized by using gums; for a review see Little & Feinle-Bisset, 2011. When all the taste components cited above are minimized, an oral detection of fatty acids in human is observed, underlining a pure taste detection of fats (Chale-Rush, Burgess & Mattes, 2007a; Mattes, 2009).

The structure and size of free fatty acids (FFA) makes them more likely taste signaling molecules than triglycerides, oils or solid dietary fats; thus, while investigating the existence of fat taste, researchers focused more on the study of FFA than on that of triglycerides, in oils or in solid dietary fats Mattes, 2010. Humans can detect a wide range of fatty acids in the oral cavity, with detection thresholds in the range of concentrations of the millimolar: PUFA (such as linoleic acid C18:2), MUFA (such as oleic acid C18:1), and SFA (especially the stearic C18:0, the lauric C12:0 and the caproic C6:0 fatty acids); for review see Chale-Rush et al., 2007a; Chale-Rush, Burgess & Mattes, 2007b; Mattes, 2009; Stewart et al., 2010. Detection threshold of fatty acids were investigated using a 3-AFC (3-alternative forced choice), ascending concentration presentation procedure and exhibited a wide inter-individual variability (see table 9).

Table 9. Average detection thresholds of fatty acids in humans.

Stimuli	Average detection threshold (mM)	Reference
Range for all FA tested	0.02 – 6.4	[1]
Lauric acid (C12:0)	2.7	[1]
Oleic acid (C18:1)	6.4 to 12 in overweight and obese 1 to 12 in lean subjects	[2]
	2.7	[3]
	2.2	[1]
	0.77	[4]
Linoleic acid (C18:2)	1.5	[1]
	1.2	[5]

[1] Stewart et al., 2010; [2] Stewart et al., 2011b; [3] Poette et al., 2011; [4] Chale-Rush et al., 2007a; [5] Chale-Rush et al., 2007b

To be definitively considered as a taste in humans, the transduction mechanisms of fat have to be identified (Chale-Rush et al., 2007a). The most recent review to date concluded to the inconclusiveness of transduction pathways for FA taste (Stewart et al., 2011a). So, these transduction mechanisms are still actively studied. Researches focus on some candidate receptors implicated in fat perception of rodents (Galindo et al., 2011):

- A fatty acid transporter CD36/FAT: present in the apical portion of circumvallate, foliate papillae and possibly in lower concentrations in fungiform papillae in mice (Laugerette et al., 2005). Furthermore, a CD36-mediated perception of LCFA in mice was seen to involve the gustatory pathway (Gaillard et al., 2008a). Thus, this transporter was investigated in humans, and was localized in the foliate and circumvallate papillae using CD36-specific antibodies (Simons, Kummer, Luiken & Boon, 2010). It binds LCFA, and besides being identified in taste receptor cells, it is located in other parts of the body such as muscle, intestine, adipose tissue, cardiac tissue (Stewart et al., 2011a).

- Two G-protein coupled receptors GPR40 and GPR120: the GPR120 binds medium- and long-chain FA and is localized in the circumvallate, fungiform and foliate papillae, whereas the GPR40 was reported in circumvallate papillae (Stewart et al., 2011a). A trained sensory panel tasted fatty acids with different chain lengths (C10:0 – C20:4), and was able to describe a “fatty” and a “scratchy-irritant” component (Galindo et al., 2011). Moreover, the LCFA (C18:1 and C18:2) were the only ones responsible to elicit a “fatty” perception. Besides, only the GPR120 was shown to be expressed in the human lingual epithelium. Thus, the authors concluded that the GPR120 may participate in the human gustatory FA perception, and do not exclude a possible interaction between the GPR120 and the CD36, with the possible existence of a signaling cascade involving more than a candidate receptor for FA detection in humans (Galindo et al., 2011).

Concerning fat perception some researchers were interested in the role of the enzymes composing the human saliva. Indeed, as TGs are not perceived *per se*, they have to be broken or cut in order to be perceived. The presence of salivary enzymes implicate was reported in rodents. Candidate human salivary enzymes thought to be implicated in fat perception are lipase and peroxidase. The role of salivary or lingual lipase in fat perception is still controversial (Salles et al., 2011), although some recent researches indicated that the lipolytic activity in saliva is sufficient to produce amounts of FA on the range of the demonstrated detection thresholds (Stewart et al., 2010). A recent work conducted in our laboratory, shows that the more important the lipolytic activity is, the higher the detection threshold (Poette et al., 2011). The hypothesis advanced is that when the lipolytic activity is important, the quantity of naturally present free fatty acids is important, thus to have a detection, the ‘background’ of free fatty acids must be risen (Poette et al., 2011). Moreover, Chalé-Rush and her colleagues (2007a) suggested that the detection thresholds of the oxidized linoleic acid could possibly involve the peroxidase activities.

In sum, the question of whether fat should or not be considered as a sixth basic taste for humans is still under debate (Mattes, 2009). Although, it is not clear to date how oral fat is sensed, or whether a gustatory component of fat exists in human, the majority of criteria necessary for defining fat as a sixth 'taste' are established in animal model studies (Stewart et al., 2011a).

2.3. Early development of the human gustatory system

2.3.1 Anatomical changes

In this section, anatomical changes of the gustatory system during the human development will be presented. The affective response to tastes in terms of intake and liking across subjects' ages will be detailed elsewhere (see part 3. of this manuscript), unless necessary for the comprehension of some parts of the present section and therefore specified here.

Taste cell synaptogenesis is observed between the 8th and 10th week of gestation, when the establishment of the first synaptic contacts between the gustatory cells and the gustatory nerves are observed. Then, the development of gustatory cells continues till the 10th week of gestation, when the circumvallate, foliate and fungiform papillae can be identified. The opening of the taste pores shown to develop since the 10th and till the 16th week of gestation, allows the access of gustatory stimuli to the taste buds. The taste buds have been identified in the fungiform papillae at the 16th week of gestation. Taste cells continue to mature, and it was seen that they are stimulated by the amniotic fluid that the fetus swallows as of the 12th week of gestation. At 15 weeks of gestation, anatomically complete taste buds can be identified in the human fetus. Some early indices on the functionality of the gustatory system in humans were given by studies on premature babies, as young as 6 months of gestation (Doty & Shah, 2008; Ganchrow & Mennella, 2003). Premature infants as young as 6-9 months of gestational age, who never experienced sucking (as they are fed by a stomach tube), have a more active sucking patter in response to sucrose solutions than they do for water (for a full review see Beauchamp & Mennella, 1998).

Term newborns have 2500 taste buds within the oral cavity (Doty & Shah, 2008). The number of taste buds rises during the first year of life, in circumvallate (250 taste buds) and in foliate (2500 taste buds) papillae and the maturation of the gustatory system continues at least till childhood. As they develop, children and teenagers have the same number of taste buds as do neonates, although their papillae differ in their size and distribution, and develop to look like what is observed in adults (for a full review on the anatomical development of the gustatory system in humans, see Doty & Shah, 2008).

Moreover, figure 8 illustrated recent research results showing that in general the reaction to tastes is more and more contrasted with age (Schwartz, Issanchou & Nicklaus, 2009).

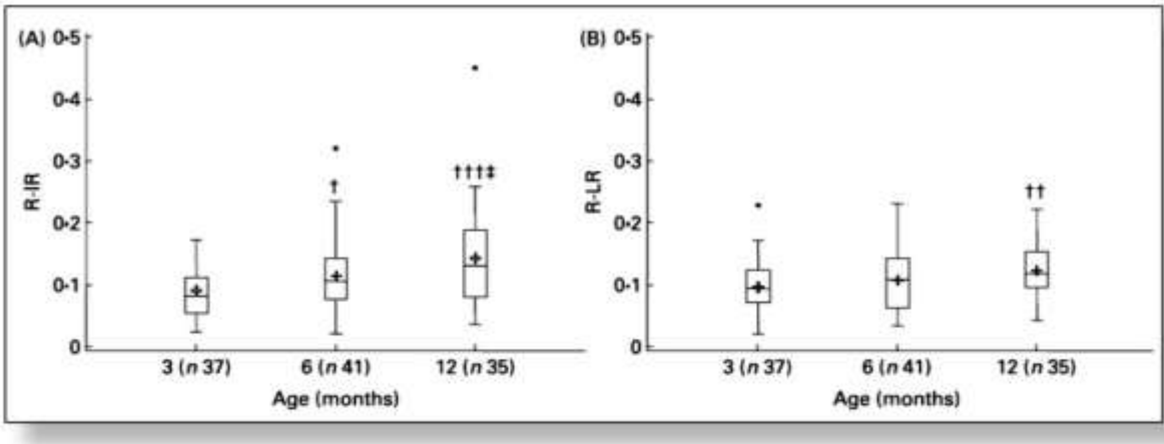

Figure 8. Representation of the global taste reactivity of infants of three age groups: 3, 6 and 12 month-old. Source: Schwartz et al., 2009.

Box plots of global taste reactivity based on ingestion ratios (R-IR) (A) and global taste reactivity based on liking ratios (R-LR) (B) for each studied age (3, 6 and 12 months old). For each box plot, the bottom and the top of the box are the 25th and 75th percentiles and the line within the box is the median; the **+** sign is the mean. The whiskers extend from the box as far as the data extend, to a distance of at most 1·5 x interquartile range. Any values more extreme than this are marked by a *. Mean value was significantly different from that at 3 months: † P=0·095 (marginal), †† P=0·015, ††† P=0·001 (LSMEANS and *t* tests). ‡ Mean value was marginally significantly different from that at 6 months (P=0·069) (LSMEANS and *t* tests).

2.3.2. Methodological investigations

In order to investigate taste perception in neonates and infants, researchers used numerous methodologies. Some of these methodologies consisted in the interpretation of facial expressions, heart rate, sucking patterns, tongue protrusion reflex or conditioned eye-blink reflex, or the evaluation of the intake of a tasting solution compared to water (Lawless, 1985; Schwartz et al., 2009). According to the few data available, it may be concluded that young children have detection thresholds in the same range than those of adults (Cowart, 1981). Researchers chose concentrations above the perception threshold of adults while conducting taste tests with infants and children, so that an absence of reaction will reflect indifference and not an absence of detection (Schwartz et al., 2009). Whether the infants' gustatory system is developed enough to perceive tastes as do adults, was investigated at different ages.

Whether young infants perceive and responses to salt oscillate between a neutral or indifferent response in 17 - 83 hour-old newborns (Maller & Desor, 1973), to rejection by 40-62 hour-old newborns (Crook, 1978). Some researchers attributed these responses to the incomplete development of the gustatory system involved in salty perception. The explanation according to Beauchamp and his colleagues would be that the receptors for salt are not mature yet at these ages, and that their maturation and thus an efficient perception for salty occurs later on, as it is the case in animals (Beauchamp, Cowart & Moran, 1986). Rather than been due to a maturation process, the emergence of a preference for salty taste in solution compared to water, observed between 3 and 6 months (Beauchamp et al., 1986; Schwartz et al., 2009), could be due to an exposure effect. Harris and booth argued that this effect could be due to the high exposure to salty foods during the weaning period occurring between 3 and 6 months for the majority of children, making it more likely to explain their response to salty at this age (Harris & Booth, 1987). Although this hypothesis would have been true in 1987, this might be questionable as the intensity of saltiness in foods presented to weaned infants, at least in France, is not that high compared to sweetness for example (Schwartz et al., 2010). Another hypothesis would be the higher physiological needs for salt during the first years of life, but this question was not investigated to my knowledge and would be worth exploring.

Concerning sweetness, it has been shown that human neonates are able to perceive sweetness, as they increase their intake of sucrose solutions over water (Desor, Maller & Turner, 1973). This effect was observed in infants as young as 1-3 days (Desor et al., 1973). These findings were recently confirmed by a longitudinal study of 3, 6, 12 and 20 month-old infants (Nicklaus, Chabanet, Schwartz, Szleper & Issanchou, 2011; Schwartz et al., 2009).

Steiner tested human infants few hours after birth, and interpreted their facial expressions in response to taste solutions displayed on their tongue (Steiner, 1979). The results indicate a clear detection by the infants; they displayed positive mimics towards sweet, which could be interpreted by an adult as an acceptance response. Besides being able to perceive sweetness, human newborns are able to distinguish different molecules responsible for the sweet taste in solutions, when presented at the same concentrations, and they prefer sucrose to glucose and lactose, and prefer fructose to glucose (Desor et al., 1973; Desor, Maller & Turner, 1977).

As far as fat is concerned and with regard to the discussed gustatory component, it is not surprising that data on its developmental gustatory system are scare. However, recent data collected by our team have shown that infants as young as 3, 6, 12 and 20 months respond to the presence of fat in a solution, as their intake decreases with age (figure 9; Nicklaus et al., 2011).

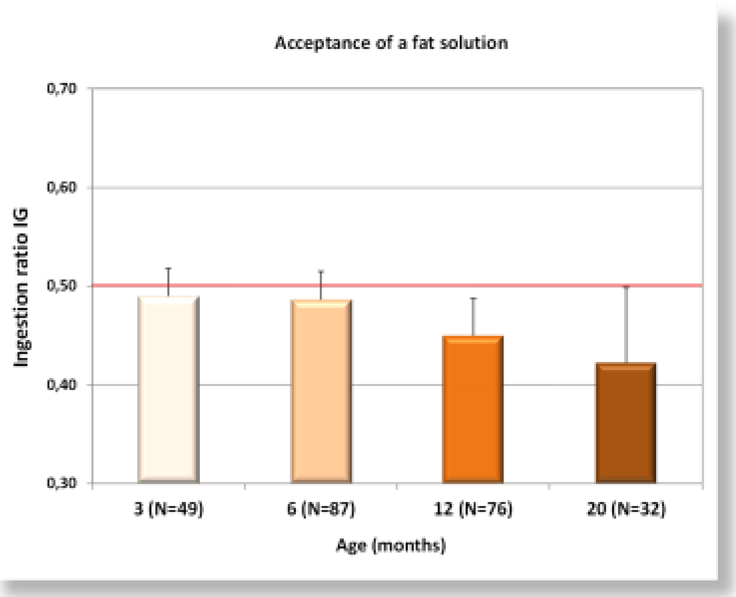

Figure 9. The evolution of ingestion ratio (IR) of a fat solution at the ages of 3, 6, 12 and 20 months. Adapted from: Nicklaus et al., 2011, personal communication.

2.4. Sensory interactions involving taste

"An increase in concentration of one of the tastants in a 'real food' might affect not only the perception of the taste quality of that manipulated tastant but also the other perceivable taste qualities"; Mojet, Heidema & Christ-Hazelhof, 2004.

Several kinds of sensory interactions can be reported: between taste interactions, within taste interaction as well as cross-modal interactions between taste, texture and aroma. Keast and Breslin (2002) describe three levels of interactions when taste-eliciting compounds are present in mixtures:

- Chemical interactions can occur in solution or in the food matrix. A direct effect on taste perception may be observed, as a result of the modification of taste intensity or the generation of new qualities due to an alteration of the structure of molecules. For example: 'gluten in bread may bind sodium, making it unavailable for taste reception'; Liem, Miremadi & Keast, 2011.
- Oral physiological interactions can occur in mouth. During such a physico-chemical peripheral effect (occurring at the epithelial/cellular level), one taste quality can interfere with the receptor cells or taste transduction mechanisms responding to another taste compound. For example: 'sodium interferes with the bitter taste transduction prior to the taste signal being sent to processing regions of the brain'; Liem et al., 2011.
- Cognitive interactions can occur when above threshold taste(s) mixtures induce suppression or an enhancement of individual qualities. This is described as a central cognitive effect and it can be observed even when physic-chemical mechanisms are avoided.

Across the literature several researchers have investigated the interrelationships within and between tastes in liquid or in solid media. The results of their work show that the intensity of the stimuli studied plays a major role on the amplitude and direction of the interaction and that different kinds of interactions within and between tastes in mixture do exist: enhancement, synergy, suppression and masking (Breslin & Huang, 2006; Keast & Breslin, 2002). Besides, multisensory-integration mechanisms were also described to explain perceptual interactions responsible for same flavor perceptions. In order for a cross-modal interaction to occur, a congruent and harmonious odor-taste or taste-odor pairs that are typically encountered together must be used. This can result in a 'taste enhancement', for example when a solution tastes sweeter when a strawberry tasteless aroma is present than when no aroma is added, or in a 'taste suppression', for example when a solution tastes less sweet when a ham aroma is added than when no aroma is added (Delwiche, 2004; Salles, 2006; Valentin, Chrea & Nguyen, 2006).

In the present section, given the extensive literature on sensory interactions and for more clarity and comprehension, only some cross-modal summations and the relationships between tastes studied in the present work, judged to be necessary for the comprehension of the work done during this thesis will be presented. The literature presented will be based on studies conducted with 'real life' foods as much as possible.

2.4.1. Interactions involving saltiness

Salty and bitter. Salt is known to be a taste modulator as perceived bitterness can be suppressed or lowered by salt addition (Breslin & Beauchamp, 1995; Breslin & Beauchamp, 1997; Doyle & Glass, 2010). Recent scientific work estimates 25% of the population to be insensitive to bitterness, while another 25% are very sensitive to ordinary levels of bitter compounds; although not précised by Doyle and Glass, two main bitter compounds are referenced across the literature: the 6-n-propylthiouracyl or PROP or the phenylthiocarbamide or PTC (Kilcast & den Ridder, 2007 cited by Doyle & Glass, 2010). Thus, the authors warn of the negative effect salt reduction might have on the last category of consumers Doyle & Glass, 2010. We may speculate that this issue will be more problematic if salt is reduced in foods served to children such as vegetable. In fact, vegetable intake in children is known to be below the recommended levels of 400 g a day as revealed by the French INCA2 study, where the mean (SD) intake of vegetables -excluding potatoes- hardly reaches 78.1 (56.6) g in children aged 3-14 years (AFSSA, 2009). Moreover, in a realistic setting such as nursery, vegetables are among the least chosen items by toddlers 2-3 year-olds (Nicklaus, Chabanet, Boggio & Issanchou, 2005b), and their lower intake was linked to their bitterness (Drewnowski & Gomez-Carneros, 2000). Concerning the impact of bitter taste perception on vegetable intake, a study including 65 pre-schoolers aged 3.5 to 4.5 years, confirmed that the majority of children identified as PROP non-tasters consumed significantly more bitter-tasting vegetables than did those children identified as PROP tasters (Bell & Tepper, 2006).

Salty and fatty. There is a wide range of foods available in the market with high salt and fat contents; e.g. snack foods, chips, pastries, fast foods, sausages… These foods appear to be the major sources of sodium in children and adults' diet (Meneton et al., 2009), and are among the most consumed by children and adults (AFSSA, 2009).
During cooking, the bitterness induced by the oxidation of fatty acids, was seen to be considerably reduces by adding salt, making it a great contributor to the palatability of high-fat foods (Nasser, 2001). Some studies have observed interactions between fat content, sodium release and salty perception in the mouth. During the chewing process, the authors observed a limited saltiness perception when fat was present in a model cheese. The observed impact of fat on saltiness perception could be explained by the slow diffusion of sodium into the saliva phase, as fat could have acted as a sodium barrier in the matrix, or salt was maintained inside the water due to the fat/water emulsions formation promoted by the presence of fat. Thus, the increase of fat content and the decrease of water content were seen to be related to a decrease in sodium release and thus a decrease of perceived saltiness (Phan, Yven, Lawrence, Chabanet, Reparet & Salles, 2008; Salles et al., 2011). In a recent work, fat addition in a model cheese (0 or 40 %) was seen to significantly increase salty perception, suggesting the existence of an interaction between salt and fat. This may be surprising for two reasons: first, salt being a hydrophilic compound it should not be retained in fat; second, the film supposed to be formed by fat on the tongue and palate should have limited the contact between taste buds and salt ions. However, other studies also reported such an increase in salty perception when fat was present in emulsions or in cheese (Panouillé, Saint-Eve, de Loubens, Déléris & Souchon, 2011).

In a work conducted by Warwick and Schiffman, a difference in saltiness rating in a salt-fat mixture was seen to depend on the subjects' weight status. Sixteen mixtures of liquid dairy products varying in fat content (% = dairy fat by weight): 0.5 % (skim milk), 3.5 % (whole milk), 11.5 % (half-and-half milk) and 36 % (heavy milk) and where salt was added in quantities ranging from 0, 0.146, 0.292 and 0.584 % (NaCl) were tested in a group of adult subjects. Their findings suggest that in normal weight subjects, saltiness intensity rating was affected by salt content and was unrelated to fat content, whereas within the overweight group, saltiness intensity rating was related to both salt and fat contents (Warwick & Schiffman, 1990). However, the mechanisms behind the observed interaction were not explicited.

Cross-modal interaction: example of odor-induced saltiness enhancement (OISE). A recent study investigated the possibility of an OISE in a solid food model system. The results obtained revealed that the perceived saltiness, of a solid food matrix with a low salt content, was enhanced by pairing with an odor associated to saltiness (Lawrence, Salles, Palicki, Septier, Busch & Thomas-Danguin, 2011). The authors support the cognitive origins of such an interaction.

2.4.2. Interactions involving sweetness

Sweet and sour. Sweet and sour taste are perceived by their own unique pathways (Chandrashekar et al., 2006). They interact with one another in foods and beverages, and it results in suppression or an enhancement of either taste. In fact, at medium and high intensities or concentrations, sweet suppresses the sour taste and other basic tastes (Keast & Breslin, 2002).

Sweet and fatty. Described cases of interaction between sweet taste and fatty correspond to a synergy. The majority if not all studies investigating this issue, used fat-sugar mixtures. In a yoghurt dairy food, fat was seen to enhance perceived sweetness and sucrose was seen to enhance perceived fattiness (Tuorila, Sommardahl, Hyvönen, Leporanta & Merimaa, 1993). At the same sucrose concentration, samples with varying fat content (skim milk, > 0.5 %; whole milk, 3.5 %; and heavy milk 36 %), were perceived as sweeter than water (Hayes & Duffy, 2007). In another study, fat was shown to contribute to sweetness scores of products tested, as the high-fat stimuli were judged sweeter (Drewnowski & Schwartz, 1990). When adults were allowed to add as much sugar as they wanted to a creamy white cheese of varying fat content, less sugar was added to high-fat versions compared to other variants (Daillant & Issanchou, 1993).

Part 3. Tastes: From detection to behavior

Eating behavior finds its origins during the first years of life (Birch, 1987), a key period when long standing food habits are established (Nicklaus et al., 2005a). In fact, when it comes to food preferences, known to be major determinants/predictors of food intake (Birch, 1999), they are formed early in childhood (Mennella, Jagnow & Beauchamp, 2001) and are likely to persist until adulthood (Nicklaus, Boggio, Chabanet & Issanchou, 2004). Thus, understanding its determinants early in life, appears of importance, especially regarding the influence of sensory properties of foods on intake, especially in young children (Drewnowski, 1997a).

The pleasantness of or the hedonic response to a food stimulus is linked to the intensity of its taste, and when the intensity deviates from an individual's optimum, the pleasantness decreases (Spetter et al., 2010). Differences in perceived intensity can have a strong impact on the palatability of the food tasted (Keast & Breslin, 2003), and thus its pleasantness.

3.1. Sensory testing with newborns, infants, toddlers and older children

Sensory evaluation is used as a mean to investigate the acceptability of foods and beverages and thus understand eating behavior. Newborns (just born) as well as infants (0-18 months) and toddlers (18 months – 3 years) '*present a challenge to sensory evaluation and consumer researchers because of their inability to communicate verbally*'; Guinard, 2001. Several indicators of hedonically motivated responses in this age group are semi-quantitative methods used by researchers: the interpretation cardiovascular and respiratory responses , the interpretation of sucking patterns (Mattes, 2005; Nysenbaum & Smart, 1982), of facial expressions (Schwartz, 2009; Steiner, 1979), and differential ingestion responses (Schwartz et al., 2009). To investigate 2-3 year old children's hedonic responses, some studies used discrimination tests i.e. paired comparison, duo-trio or intensity ranking. These tests as well as a 7-point hedonic scale appeared to be inappropriate for toddlers as young as 2-3 years (Kimmel, Sigman-Grant & Guinard, 1994). The only recommended evaluations techniques for toddlers less than 3 years of age are behavioral observations, diaries and consumption.

Nevertheless, some studies reporting the usage of methods other than those recommended for toddlers as young as 2-3 years old, generally compare different foods, and not different variants of the same food. Even if it would be interesting and useful to evaluate toddlers' ability to discriminate the sugar, fat and salt levels in food, to date no methodology adapted for such an investigation in 2-3 year-olds is available. In such cases, as children tend to focus on visual cues (Guinard, 2001). Thus, the same sample varying only in its salt, sugar or fat content will look the same for them and they would overlook taste.

Before going further, a distinction between preference and liking should be fostered. In 1986, Rozin and Vollmecke said that '*preference* assumes the availability of at least two different items, and refers to the choice of one rather than the other. *Liking* refers to a set of hedonic (affective) reactions to a food, usually indexed directly by verbal reports or rating scales, but sometimes indirectly by facial expressions'; Rozin & Vollmecke, 1986.

A study conducted by Wardle and co-workers' tested liking ratings (3-point face scale) and preference ranking (forced choice elimination ranking) for 6 vegetables, in a sample of 24-82 months of age children (Wardle, Cooke, Gibson, Sapochnik, Sheiham & Lawson, 2003a). However, only 34-82 months were able to comply with the study procedure. The author did not explain further the reasons for this withdrawal, but one could speculate that 2-3 year-olds are too young to be tested using such sensory evaluation method. Studies evaluating preference ranking and liking ratings in young children involved those 3 years or older (Birch, 1979b; Birch & Sullivan, 1991). In children younger than 7 years it is believed, with regard to the Piagetian theory, that infant displaying 'sensori-motor intelligence', and children displaying 'pre-operational thought' are not able to use causal reasoning. The logical reasoning develops later on, with the establishment of the 'concrete operational thought' and the 'formal thought' (Contento, 1981). Besides, sensory evaluation uses direct question, which are not suitable for use with young children. This implies that some evaluation methods have to be adapted to children, and indirect methods are sometimes used.

Methods used in adolescents (>12 years) are the same as those universally used with adults (ASTM, 2003; SSHA & Depledt, 1998). When it comes to younger children, methods do vary according to their cognitive developmental stage (language, motor skills), attention span and the number of products to be tested (ASTM, 2003). Table 10, adapted from the ASTM Standard Guide for Sensory Evaluation of Products by Children, summarizes the developmental stages and the adequate sensory measures for children aged 18 months to 10 years.

Table 10. Cognitive skills and advised sensory evaluation methods with children 0 to 12 years. Adapted from ASTM's Committee 18 on Sensory Evaluation: ASTM, 2003.

	Infants 0 - 18 m.	**Toddlers 18 m. to 3 y.**	**Pre-schoolers 3 to 5 y.**	**Beginning readers 5 to 8 y.**	**Pre-teens 8 to 12 y.**
Language	Pre-verbal	Begin to vocalize	Early language development	Moderate verbal and vocabulary	Improvement of self-expression
Cognitive skills	Rely on facial expressions	Adult interpretation required	Respond to questions and pictures	Reading and writing (variable)	Sufficient reading and writing
Attention span	Less than limited	Less than limited	Limited	Limited by the understanding of the task and interest level (tasks <15min)	Increasing attention span
Reasoning	Limited to pain and pleasure	Limited. Concept of 'no'	Limited. Beginning of the ability to verbalize what is liked and what is not	Cause/effect concept	Full ability. Capable of making a decision
Decision making	Do not make complex decisions	Do not make complex decisions.	Limited. But able to choose one thing over another	Increasing, but evident influence of adult approval	Capable of complex decisions
Understanding scales	Do not understand scales	Do not understand scales	Understanding simple scales. Sorting or identification tasks	Scale understanding	Capable of understanding scaling concept
Motor skills	Some gross motor skills	Great gross motor skills	Gross and fine motor skills	Gross and fine motor skills more refined	Hand to eye and other motor skills developed
Recommended evaluation techniques	Behavioral observations **Diaries** **Consumption** or duration **measurements**		Previous plus: paired comparison; sorting and matching; limited preference; ranking; one-on-one interview	Previous plus: simple attribute ratings; liking scales …	Previous plus: **hedonic scales**; simple attribute scaling and ratings

3.2. Impact of salt, sugar and fat content modifications in foods on hedonic responses

"The food itself is at once a source of nutrition […], a great source of pleasure and satisfaction"; Rozin & Vollmecke, 1986.

A wide range of individual variability in preference and hedonic responses to sensory stimuli was described since the early 70s. This individual variability is known to be 'emanating from differences in sensitivity, and in cognitive and experiential factors' (Pangborn, 1981). In 1970, Pangborn observed three hedonic functions for solutions of sucrose and sodium chloride: increasing, decreasing and parabolic with concentration (Pangborn, 1970). Ten years later, in a work conducted with Pangborn and Stern, Witherly distinguished four patterns of the relationship between concentration and hedonic preference or average hedonic rating (Witherly *et al*., 1980 cited by Drewnowski, 1987a). Thus, as shown in figure 10, the individual hedonic profiles were classified into:

- Type I response: an inverted U-shape for preference with a maximum hedonic response then a decline;
- Type II response: an increasing hedonic response with a plateau;
- Type III response: characterized by a monotonic decline in hedonic preference;
- Type IV response: a pattern where no change in hedonic response is observed with increasing concentration.

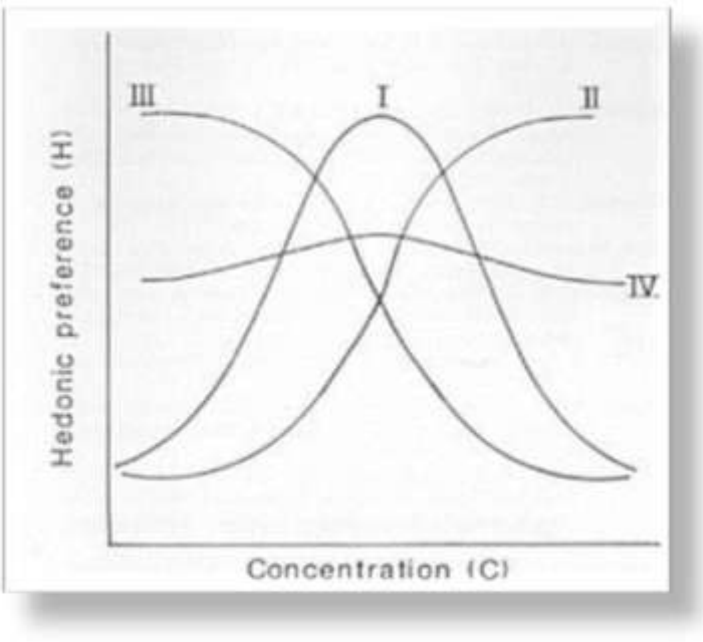

Figure 10. Idealized forms of some possible types of hedonic responses according to the concentration of a stimulus. Source: Drewnowski, 1987a.

Figure 11 illustrates the means of groups of several subjects, by opposition to Figure 10 where variations from the typical pattern are represented according to subjects (Cabanac, 1979). The main observation to be highlighted at this point concerns the mean reaction to sweet taste, which appears to be the most pleasant when compared to salty taste for instance. The individual and global patterns of response for salt, sugar and fat, will be detailed and discussed in the following parts.

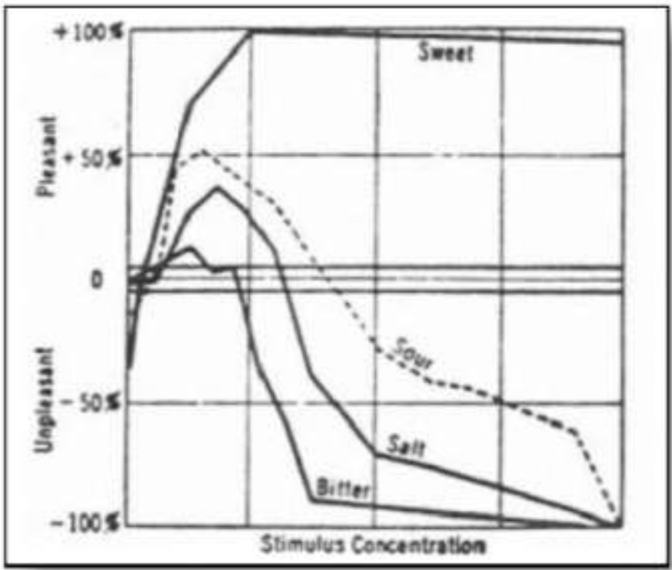

Figure 11. The preponderance of «pleasantness » or « unpleasant" judgments in relation to the stimulus concentrations, for means of groups of several subjects. Source: Cabanac, 1979.

3.2.1. Effect of salt on hedonics

Preferences for salt are shown to be influenced by prenatal factors. Infants as young as 16 weeks and adult offspring (18 – 43 years) of mothers who experienced moderate to severe vomiting during pregnancy, exhibit a high preference for salt in solutions (Crystal & Berstein, 1995; Crystal & Berstein, 1998).

An emerging preference for salty taste observed in aqueous solutions was observed at 4 months (Beauchamp, Cowart, Mennella & Marsh, 1994; Beauchamp et al., 1986). This observation was confirmed by recent work involving infants 3, 6, and 12 months of age (Schwartz et al., 2009), where the preference for salty taste was seen to emerge between 3 and 6 months, as it is shown in figure 12. In this study, results on preference were interpolated from an acceptance variable, evaluated using an ingestion ratio (IR) based on the volume consumed, and using a liking ratio (LR) based on the experimenter's judgment. The preference for salty taste observed at 6 months, continues to rise during the first year of life (Schwartz et al., 2009), and declines at the beginning of the 3rd year (Beauchamp et al., 1994; Beauchamp et al., 1986). Infants as young as 6 months start experiencing foods, several of which are salted and might not anymore associate this taste with water. Indeed, Harris and Booth have shown that infants' hedonic responses to salt were tightly linked to their recent dietary experiences with salted foods, which shapes their preference (Harris & Booth, 1987).

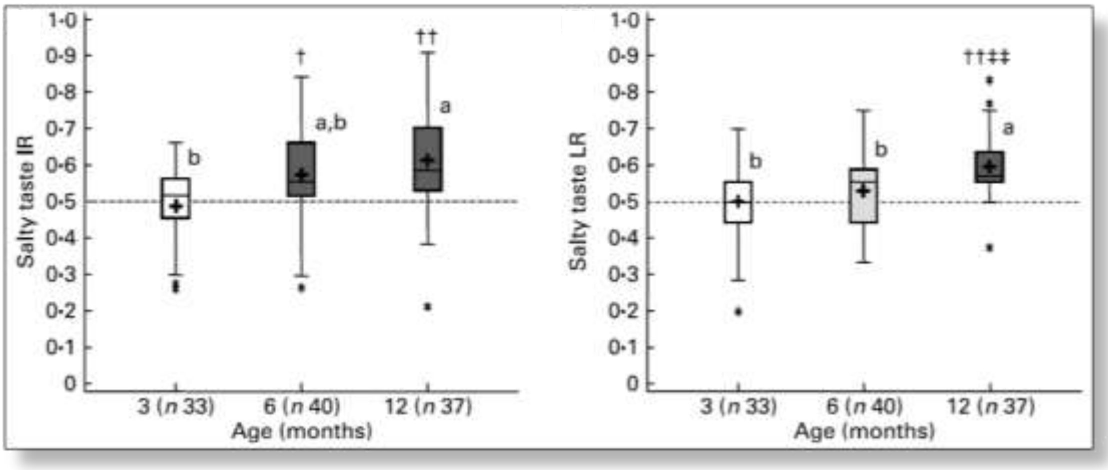

Figure 12. Evolution of 3, 6 and 12 month-old infants' acceptance to salty taste in solution.
Source: Schwartz et al., 2009.

When children start complementary feeding, around 5-7 months, the presence of salt or salty ingredients was seen to facilitate the acceptance of new vegetables compared to bitter vegetables –as evaluated by parents on a 4-point-scale- as shown in figure 13; Schwartz, Chabanet, Lange, Issanchou & Nicklaus, 2011.

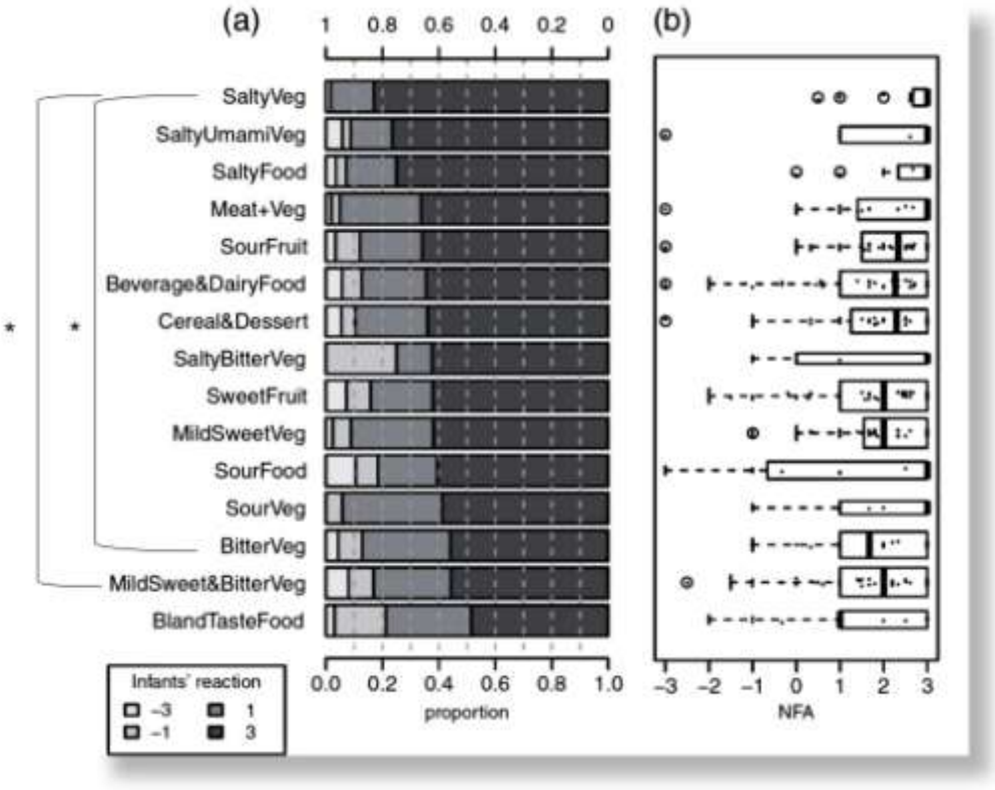

Figure 13. Five to seven month-old infants' reaction and acceptance of new foods at the beginning of complementary feeding.

a) Cumulative percentages of infants' reaction for each food group when tasting those foods for the first time between ages 5 to 7 months. b) Boxplots of individual new food acceptance (NFA) for each food group. Jittered observed values are superimposed. The height of each box is proportional to the number of infants. Infants' reaction should be read as follows: '3', very positive, '1', positive, '−1', negative, '−3', very negative. Adapted from: Schwartz et al., 2011.

In children, the exposure to salt in foods after the age of 2 years was seen to result in liking for salt in foods (Beauchamp & Moran, 1984; Cowart & Beauchamp, 1986; Sullivan & Birch, 1990). In other respects, Cowart and Beauchamp investigated 20 3-6 year-olds preference for a soup broth with varying salt contents[9] from 0, 0.99 and 1.98 % NaCl (0; 0.17 and 0.34 M) using paired comparisons, and found the majority to best preference the salted soup (n=11) compared to the moderately salter (n=6) and to the unsalted soup (n=3) (Cowart & Beauchamp, 1986). These results were further confirmed in 3-7 year-olds, also

[9] 1 mol/l (M) = 5.844 g/100g (%) of NaCl

using paired comparisons, between soups with varying salt contents, and results have shown that 7 out of 14 toddler preferred the 3.27 % (0.56 M) NaCl soup to either the 0.06, 1.05 or 1.98 % (0.01, 0.18, 0.34 M) soups. In a second series of experiments where an additional higher salt concentration was added, half the toddlers still chose high concentrations (≥ 3.27 % or 0.56 M) as their preferred (Beauchamp & Cowart, 1990). A comment should be highlighted at this point, as this comparison method was criticized by some authors. Kôster, 1981 argued that a subject will always choose the sweeter beer (+1) compared to an unsweetened one (0), but is presented with another pair of beers (+1 and +2) he will chose the sweeter one (+2) and so on. However, if presented (in a natural condition such as a bar) with the sweet beer (+2) he chooses during the comparison test he will reject it and judge it to be 'appallingly' or awfully sweet. Thus, the results obtained from this kind of tests should be interpreted cautiously if the aim is to define a concentration to use for real life foods. Anyways, even when preference for popcorns with varying salt contents (0, 5.8, 11.68, 17.53 or + 17.53 % equivalent to 0, 1, 2, 3 or + 3 M), was evaluated on a 7-point Likert scale from 1=least preferred to 7=most preferred, children 7-12 years, preferred the more concentrated salt in popcorn (17.53 % and + 17.53 % or 3 and +3M); Verma, Mittal, Ghildiyal, Chaudhary & Mahajan, 2007; salt contents used during this experiment might seem high but it's worth reminding that 14 ml of each salty solution was sprayed into a plain/bland popcorn preparation using a spray bottle.

Do children and adults differ on their preference for salty foods? A group of adolescents (9-15 years) and a group of adults were asked to taste four samples of salty solutions and to rank them from the most to the least preferred. Using this ranked preference test, it was shown that the younger subjects preferred greater saltiness in solutions than did adults (Desor, Greene & Maller, 1975). When the comparison was made using real foods, children (7-12 years) preferred more salty popcorn (17.53 % or +3M) than did adults (11.68 % or 2 M), and compared to their parents, 3-7 years preferred higher levels of salt in a soup than did their parents (Beauchamp & Cowart, 1990; Verma et al., 2007).

The acceptability of saltiness is shown to be product-specific (Adams, Maller & Cardello, 1995). Besides, the preference for salty taste cannot be generalized to all foods (Bertino, Beauchamp & Engelman, 1986; Shepherd, Farleigh & Land, 1984).

Concerning salt, three of the four types of hedonic responses were observed: Type I, II and III. The proportion of subjects for whom an optimal level of salt is reached before a decline in pleasantness in observed, thus displaying a type I response profile, were found to be equivalent to 26 % in adults; the optimum was observed between 0.15 and 0.45 % salt, (0.025 and 0.077 M). The proportion of subjects judging increasing concentrations of salt in solution, up to 0.87 % salt (0.15 M), as being decreasingly pleasant, thus displaying a type III response profile, was equivalent to 65 %. And finally, the proportion of subjects judging increasing concentrations of salt in solution as being increasingly pleasant, thus displaying a type II response profile, were found to be equivalent to 9 % in adults for solutions 0.87 % (0.15 M) of NaCl (Pangborn, 1970). The same proportion of 10 % was found in adults when salt solution was concentrated up to 2.33 % (0.40 M), and it was found to be equivalent to 20 % in children 9-15 years of age (Desor et al., 1975).

3.1.2. Effect of sugar on hedonics

Sugar provides a sweet taste that endows a pleasure response, and provides a motivation to continue feeding (Blass, 1987). Indeed, sweetness appears among the most important parameters guiding preference for foods in children, alongside high energy density and familiarity (Birch, 1979b; Drewnowski, 1989b; Drewnowski & Almiron-Roig, 2010).

Unlike what is observed for salty taste, humans exhibit an innate preference for sweet solutions, already observed few hours after birth (Steiner, 1979). Besides, infants 3, 6 and 12 month-olds clearly prefer sweet solution (lactose) over water and ingest more of it (Schwartz et al., 2009), as depicted in figure 14, thus confirming previous findings when sucrose and glucose were presented in solutions (Beauchamp & Moran, 1982; Desor et al., 1977). Another important point highlighted by experimental studies with infants, is the infants' ability to distinguish sweet compounds one from another, and to prefer those associated to higher degrees of sweetness: prefer sucrose and fructose to lactose and glucose (Desor et al., 1977). Moreover, neonates were seen to prefer sweet solutions with the higher concentrations (Ganchrow, Steiner & Daher, 1983), and Infants aged 1-4 days exhibited a high preference for intensely sweet solutions, and were even able to discriminate them according to their sweetness intensity and to prefer the sweetest ones (Cowart & Beauchamp, 1990; Rosenstein & Oster, 1988). In children under the age of 2 years, sweet solutions generally elicit acceptance (Beauchamp & Moran, 1984). Besides, the sweeter the solution the more it is consumed by toddlers 2-3 year-olds (Vasquez, Pearson & Beauchamp, 1982).

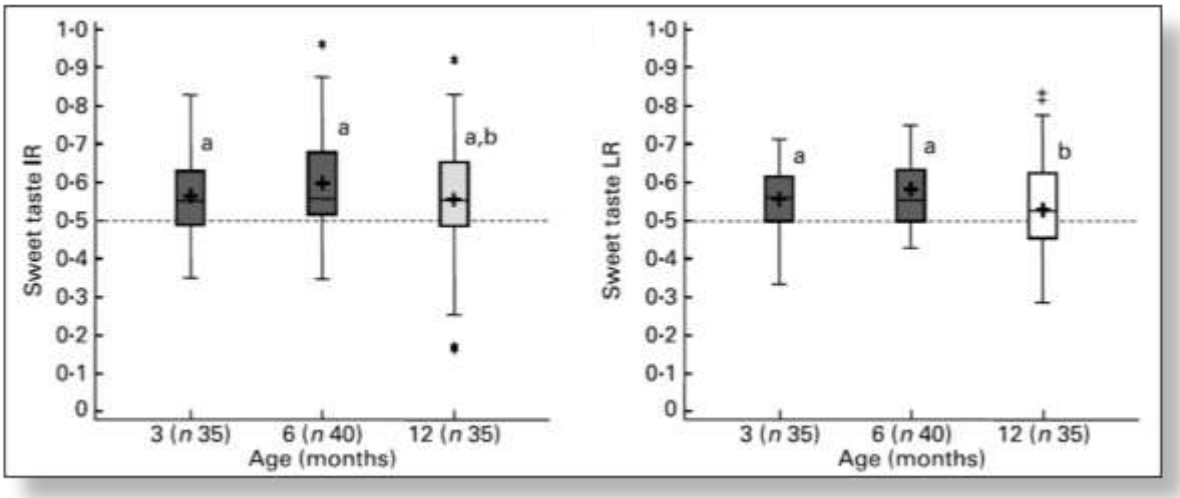

Figure 14. Evolution of 3, 6 and 12 month-old infants' acceptance to sweet taste in solution. Source: Schwartz et al., 2009.

Is there an age-related effect in sweet preference? Pleasantness in response to variations in sucrose concentrations in an orange beverage (8.24, 10.71, 13.93, 18.10 and 23.53 % of sucrose) was investigated in children (6-12 years), adolescents (13-18 years), adults (19-34 years), older adults (50-65 years) and elderly subjects (65 years and over). Pleasantness was accessed using a 5-point hedonic scale with faces indicating the degrees of like/dislike (sip-and-swallow procedure). Results show an age effect on pleasantness ranting; as mean pleasantness ratings were higher for children than for the other groups (Zandstra & de Graaf, 1998). The age-related decline in sweet preference was documented elsewhere: sweet preference is high in childhood (Pepino & Mennella, 2005) and early adolescence and decreases in early adulthood (Desor & Beauchamp, 1987). Long-term repeated exposure to sweet tastes during infancy was suggested to enhance young children's preferences for this taste (Beauchamp & Moran, 1984). As children grow to adulthood, their liking for sweet taste decreases (Desor & Beauchamp, 1987; Liem & Mennella, 2003). Thus, preference for sweet taste drops between childhood and adolescence (Drewnowski, 1989a). The decline of preference for sweet observed at 12-14 y.o. and although still observable, sugar consumption then declines in adult life (Drewnowski, 2000).

As depicted in figure 11, the global picture of pleasantness response reveals that contrarily to salty taste, for which unpleasantness is observed at high concentrations, sweet taste ranks first for pleasantness compared to other tastes (Cabanac, 1979). Indeed, data presented by Pangborn revealed that the majority of subjects (65 %) display a type III hedonic response to salt, whereas 55 % of subjects display a type II hedonic response for sugar (Pangborn, 1970). Concerning individual differences in liking for highly sweetened foods, a splitting of the population into several groups with regard to their preference for increasing sugar concentrations, as illustrated in figure 10, was previously reported (Sørensen et al., 2003). As far as sucrose [10] is concerned three of the four types of hedonic responses were observed by Pangborn in 1970: Type I, II and III. The proportion of adult subjects for whom an optimal level of sucrose is reached before a decline in pleasantness in observed, thus displaying a type I response profile, were found to be equivalent to 20 %; the optimum was observed between 2.0 and 4.4 % sucrose, (0.058 and 0.128 M). The proportion of subjects judging increasing concentrations of sucrose in solution, up to 8.0 % (0.233 M), as being decreasingly pleasant, thus displaying a type III response profile, was equivalent to 25 %. And finally, the proportion of subjects judging increasing concentrations of sucrose in solution as being increasingly pleasant, thus displaying a type II response profile, were found to be equivalent to 55 % in adults for solutions 8.0 % (0.233 M) of sucrose (Pangborn, 1970). This proportion was equivalent to 25 % in adults when sucrose solution was concentrated up to 20.53 % (0.60 M), and it was found to be equivalent to 50 % in children 9-15 years of age (Desor et al., 1975). This observation was confirmed by further works. A study comparing children and adults revealed that responses corresponded to the inverted U-shape and preferred concentrations were higher for children than for adults (de Graaf & Zandstra, 1999).

[10] 1 mol/l (M) = 34.23 g/ 100 g of sucrose

3.1.3. Effect of fat on hedonics

Unlike sweet for example, whether liking for fat is an innate feature or not is still unclear. Work starts investigating this question when the fetus is still in his mother's womb. A study conducted in the early 70s by Liley, has shown that an intra-amniotic injection of 3 – 8 ml of fat (Lipiodol, an iodinated poppy seed oil), reduces fetal drinking (Liley, 1972 cited by Mattes, 2005). Even if the product injected has a strong flavor explaining the negative response observed, interpretation of this reaction as a rejection of the fetus towards fat cannot be rejected (Mattes, 2005).

In newborns (half a day to 4 days of age), no specific reaction was found in response to oral stimulation by oil deposed on the tongue (Graillon, Barr, Young, Wright & Hendricks, 1997). Preference for fat was investigated by monitoring sucking behavior and by using an 'ecological' media: milk (breast or formula); the rationale for breast milk is that its fat content increases during sucking and thus an active sucking pattern, would reflect a preference for fat (Mattes, 2005). Babies (n=24; 4-9 days) were given breast milk varying in fat content (low-fat (LF) & high-fat (HF)) by the bottle (40 ml) for a maximum of 5 minutes. Three groups were set: a control group1 (LF/LF), a control group2 (HF/LF), and the last was the experimental group (LF/HF) was a simulation of breastfeeding (Woolridge, Baum & Drewett, 1980). The authors found no effect of fat content on sucking patterns. In a study where formula milk was used, Nysenbaum & Smart gave 48 and 120 hour-old infants on day 1 a high-fat then a low-fat milks (each for 2 minutes) and on day 2, the opposite was tested. Infants had a more active sucking behavior for high-fat milk, maybe reflecting a higher preference (Nysenbaum & Smart, 1982). This conclusion was especially true, with regard to the greater sucking behavior observed when the high-fat milk was followed by the low fat one.

Mattes reported a study conducted with Rankin in 1996, where the children's preference for a novel high-fat food was higher than that of novel foods flavored with sweet, sour, bitter or salty tastes (Rankin & Mattes, 1996 cited by Mattes, 2005); no indication of the children's age was provided. Due to the discrepancy of the results surrounding the question of whether fat is liked or disliked early in life, further investigations are needed in that field of research. In the same infants seen at aged 3, 6, 12 and 20 months, acceptance of a fat solution was seen to decrease with age, as it was shown in figure 9. In studies with adults, liking increases with increasing fat content in foods (Drewnowski & Greenwood, 1983). The preference for fat is believed to further decrease with age. In fact, in elderly (82.3 +/- 6.4 years), the pleasantness for fat mixtures with either salt or sucrose was shown to be independent of the mixture's fat content (Warwick & Schiffman, 1990).

In young children it may be more difficult to separate and study the impact of taste modality alone on their preference, due to the complexity of its perception. Few studies in children have addressed the hedonic responses to fats in foods (Fisher & Birch, 1995; Gazzaniga & Burns, 1993). In their study, Fisher and Birch accessed fat preference in 3- 5 year old children using a rank ordering preference procedure (after tasting them). A set of foods served during lunch and dinner were categorized as lower in fat (3-25%) or high fat content (55-98 %). Children exhibited a higher preference for high fat foods than for low fat ones (Fisher & Birch, 1995).

As it was discussed elsewhere in this manuscript, the presence of fat in food matrices is responsible for aroma release during eating and this plays a role on palatability and attractiveness (see parts 1 and 2). Compared to a low-fat yogurt (0.2%), flavor release was more persistent in a high-fat one (10%) Brauss, Linforth, Cayeux, Harvey & Taylor, 1999; thus, the higher the fat content, the 'slower' its flavor release is, and the more pleasure it provides during eating. The hedonic valence of fat might be overruled by dietary experiences (Mattes, 2005), as the frequency of sensory exposures to fats in the modern diet could have exerted a strong influence on liking (Mattes, 1993). The preference for fat in food might not be innate, but seems to develop throughout the exposure to an environment where rich-fat foods with a high energy density are widely available, shaping children's preference through a high-fat diet. This was investigated using approaches based on learning mechanisms, and focused on the positive post-ingestive consequences of fat. It was shown that fat post-ingestive consequences can condition the preference for new flavors in preschool children. Children acquire preferences for flavors associated with high-fat contents (see Birch, 1992; Birch, McPhee, Bryant & Johnson, 1993; Johnson, McPhee & Birch, 1991; Kern, McPhee, Fisher, Johnson & Birch, 1993, these approaches will be further detailed in part 4).

Unlike what is observed for saltiness and sweetness neither a global nor an individual picture of hedonic response profile to fat was studied. A set of studies conducted by Mela attempted to clarify this issue, but using foods not solutions as it was the case for the two other sensory qualities (Mela, 1990). Naïve assessors were asked to rate different foods, each with 5 five levels of vegetable oil, for pleasantness and perceived 'fat content'. The pattern of 4 individual foods (mashed potato, tuna fish, Felafel mix and vanilla pudding), revealed an 'unusual and apparently food-specific preference patterns'. Thus, authors aggregated data from these and other battery of foods, which generated a 'much more familiar picture of sensory response', is it was reproduced in figure 15. This would suggest a type I response in the case of fat.

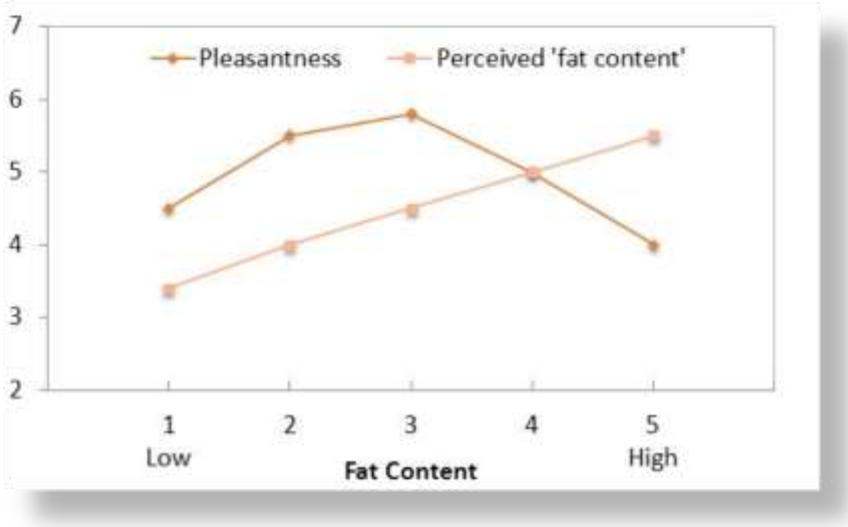

Figure 15. Combined ratings of pleasantness and perceived fat content responses to 8 foods.

Ratings were obtained on 9-point category scales, from 'extremely unpleasant' to extremely pleasant' and from 'none' to 'extreme' respectively for pleasantness and for perceived 'fat content'. Adapted from Mela, 1990.

3.1.4. Is there a link between weight status and preference for salt, sugar or fat in foods?

"The wisdom of the body leads the organism to seek pleasure and avoid displeasure, and thus achieve behaviors which are beneficial to the subject's physiology"

Cabanac, 1992.

The citation above could have been true in another time period -in a perfect world. However, in recent years, it is assumed that the taste preference for palatable foods contributes to the obesity epidemic (Salbe, DelParigi, Pratley, Drewnowski & Tataranni, 2004). Hedonic responses to salt, sugar and fat are supposed to reflect the pleasure linked to the intake of foods carrying them. Unfortunately, in our modern societies and with the wide range of available high-salt, high-sugar and high-fat foods, their intake is sometimes linked to one of the major health concerns: overweight and obesity.

Concerning sugar, some studies concluded to an inverse relation between the hedonic response to sweet taste and weight (Drewnowski, Brunzell, Sande, Iverius & Greenwood, 1985; Salbe et al., 2004). In adults the majority of obese subjects exhibited a sweet-dislike pattern of response (Grinker and Hirsh, 1972 and Underwood et al., 1973 cited by Cabanac, 1979).

Concerning fat, researchers have found obese subjects to have a higher preference for fat (presented in fat-sucrose mixture) compared to normal weight ones (Drewnowski et al., 1985). Besides, it was suggested that preference for higher fat levels in normal weight subjects, might depend on their weight status (see figure 16): subjects with a currently 'appropriate' weight-for-height but with a former history of obesity, prefer a higher level of fat than never-obese subjects (Drewnowski et al., 1985; Warwick & Schiffman, 1990). In other respect, Warwick and Schiffman cited a talk given by Drewnowski and his colleagues during the 11th annual meeting of the ACS in 1990, during which the researcher argued that the preference for fat-sucrose mixtures is higher in subjects with an obesity installed since childhood, than in subjects with adult-onset obesity (Warwick & Schiffman, 1990). In fact, Drewnowski et al., 1985, based on the Response Surface Method (RSM) indicated that in obese subjects, a 34 % fat / 4 % sucrose was considered as an optimal dairy product, while for normal-weight subjects the optimal dairy product was a 21 % fat / 8 % sucrose, as illustrated in figure 16. This study confirms the data obtained by other authors using the RSM in normal weight subjects as the optimal fat/sucrose mixture was 20% fat / 9 % sucrose (Drewnowski & Greenwood, 1983; Moskowitz, 1974).

Some laboratory studies have supported the link between obesity and liking for higher fat foods (Drewnowski & Greenwood, 1983), while other studies were less supportive (Salbe et al., 2004). Moreover, heightened fat liking is associated with increased intake (Duffy, 2007 and adiposity, in normal weight adults (Mela & Sacchetti, 1991) and overweight/obese men (Duffy, Lanier, Hutchins, Pescatello, Johnson & Bartoshuk, 2007). Other works confirm these findings, as they found obese subjects to have a greater preference for high-fat foods, compared to non-obese ones (Bartoshuk, Duffy, Hayes, Moskowitz & Snyder, 2006; Drewnowski et al., 1985; Drewnowski, Kurth & Rahaim, 1991).

A link between the preference for high-fat foods and body mass index was described in 9-12-year-old children (Ricketts, 1997). Moreover, children's 'fat appetite' –i.e. their preference for high-fat snack foods - was linked to their parents' and to their own body mass index Fisher & Birch, 1995.

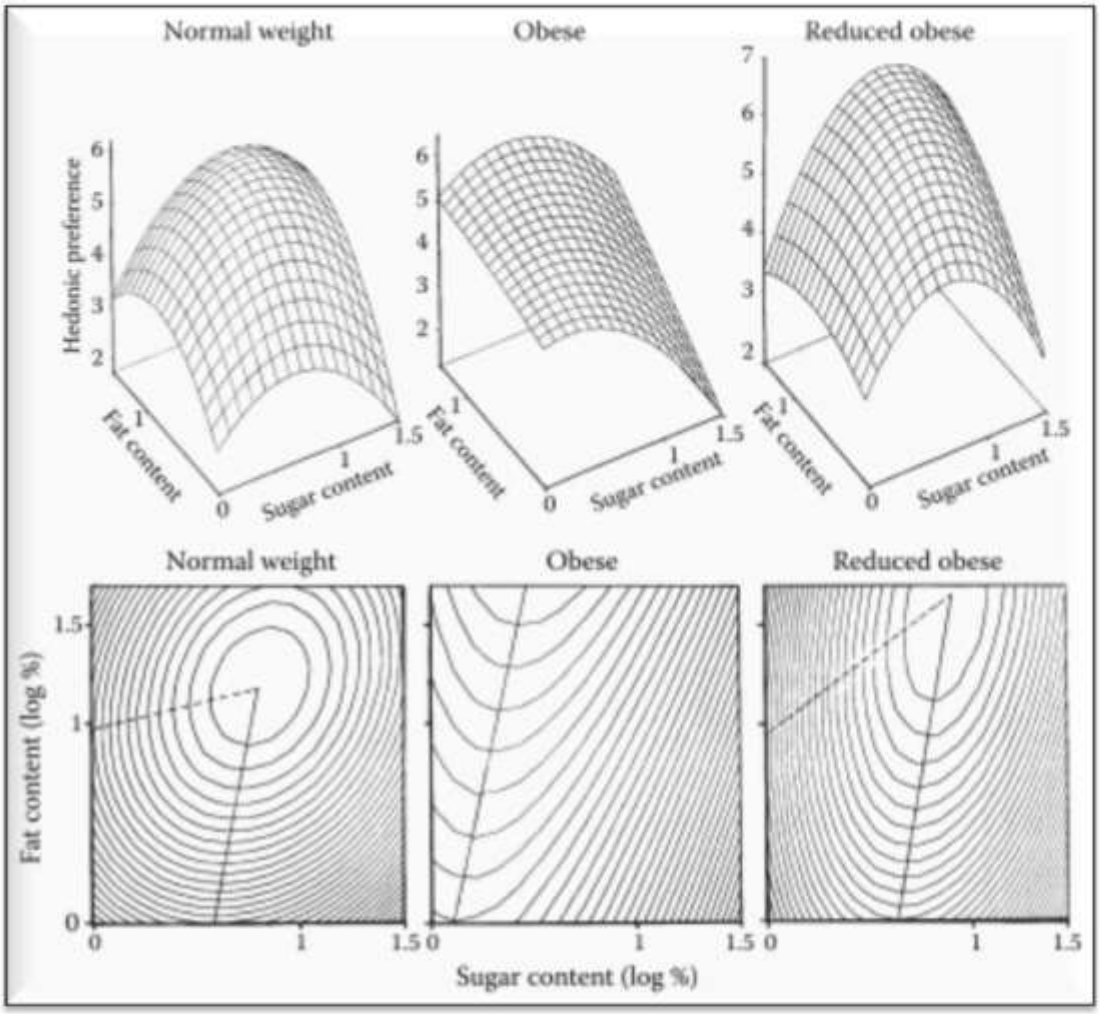

Figure 16. Three dimensional hedonic response surface (top panel) and isopreference contours (bottom panel) of hedonic preference ratings for dairy products with differing sucrose (0, 5, 10 or 20%) and fat (0.1, 3.5, 11.7, 37.6 and 52.6%) contents.

The data represented concern normal weight (n=15; 21.6 ±0.5 kg/m²), obese (n=12; 34.4 ±1.7 kg/m²) and 'reduced' obese (n=8; 23.6 ±0.9 kg/m²) subjects with mean age of 33.5 ±1.7 years). *Source:* Drewnowski et al., 1985.

3.2. Impact of salt, sugar and fat on food intake

3.2.1. Food intake: definitions and background

In order to estimate salt, sugar or fat intakes in an individual's diet, researchers use measures such as frequency surveys, food diaries or dietary records. However, these measures rely on memory and are subject to an individual's subjectivity, hence, they are difficult to interpret. Moreover, some individuals have a tendency to under or over report information on their intake.

As far as the food ingestion is concerned, many terms are used across the literature, and sometimes the definitions are not consistent with one another. Thus, before going further in this section, some definitions are worth clarifying. A recent review commissioned by the Appetite Regulation Task Force of the European branch of the International Life Science Institute (ILSI Europe), gives updated definitions of the 'appetite system' operations (for review see (Blundell et al., 2010). The term *'appetite'* usually used in connection with short-term fluctuations in motivation to eat (Parkinson, Drewett, Le Couteur & Adamson, 2010). The updated definition refers to appetite as covering the whole field of food intake, selection, motivation and preference; more specifically, it refers to the qualitative aspects of eating, sensory aspects or responsiveness to other stimuli such as energy deficit etc. (Blundell et al., 2010). The *palatability* of a food, as defined by Yeomans, refers to the hedonic evaluation of oro-sensory food cues under standardized conditions (Yeomans & Symes, 1999). Food consumption is mostly driven by food palatability, and a highly palatable food stimulates appetite leading to an excessive food intake (Yeomans, Blundell & Leshem, 2004). Another term involved in the appetite system is '*hunger*' the construct connoting the drive to eat (Blundell et al., 2010). In other respects, the use of '*satiety'* and '*satiation*' terms is still confused in the scientific literature. For example, Schwartz and his colleagues, in an article published in Nature in 2000, said that "The major determinant of meal size is the <u>onset of satiety</u> […] generated during food ingestion that leads to <u>meal termination</u>" (Schwartz, Woods, Porte, Seeley & Baskin, 2000). Others define the control of meal size, due to a cessation of the desire to eat and conducting to the termination of the meal, as 'satiation' or intra-meal satiety (Blundell et al., 2010). Whereas 'satiety' also named post-ingestive or inter-meal satiety, refers to the inhibition of further eating and the decline of hunger (Blundell et al., 2010); this later construct will not be described or discussed further in this manuscript as it is outside the scope of this thesis work. These two aspects form the '*satiety cascade'* proposed by Blundell in 1987 and revised by Mela in 2006, as illustrated in figure 17. Another term to be defined would be *sensory specific satiety,* reflecting the decrease of pleasantness for a food that has been just eaten, without affecting the pleasantness for other foods. It integrates current pleasure and past experiences (Rolls, Rolls, Rowe & Sweeney, 1981). This concept was further defined as *sensory specific satiation* not satiety (Blundell et al., 2010).

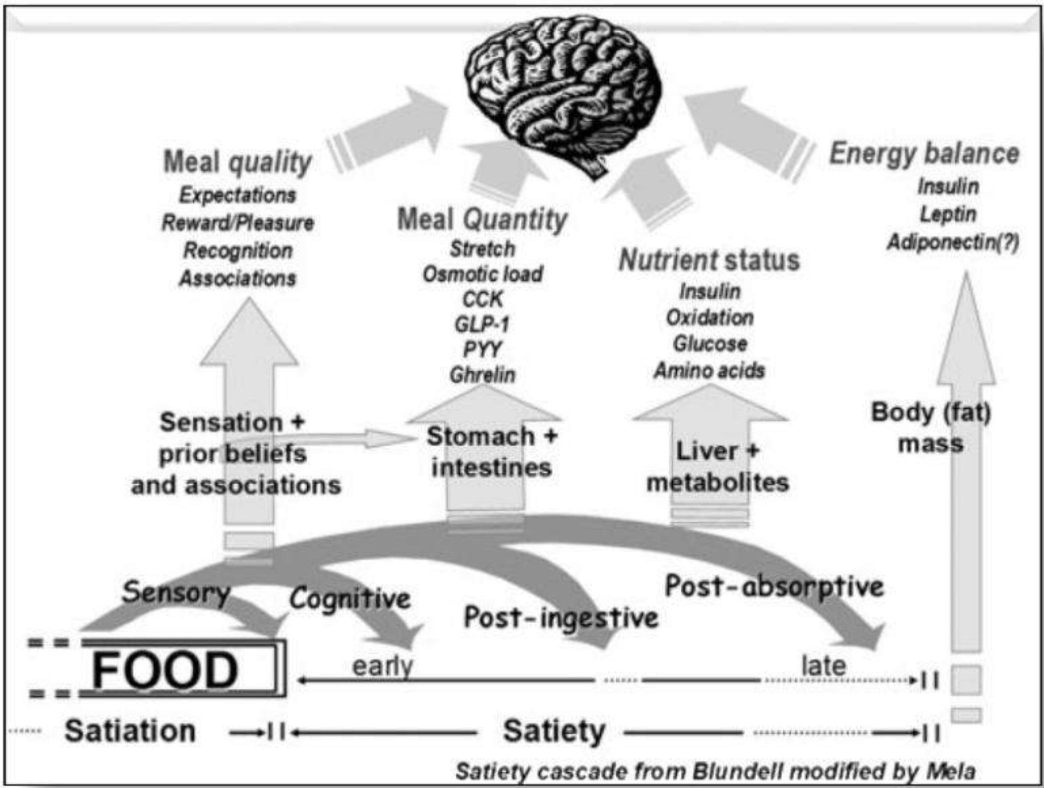

Figure 17. The Satiety cascade, revised. Source: Blundell et al., 2010.

Food intake can be evaluated in terms of weight (or grams) consumed, or in terms of corresponding calories ingested i.e. energy intake. How much we eat can be influenced by how foods taste and their effects on physiological responses, with the food palatability guiding this behavior (French & Robinson, 2003; Sørensen et al., 2003). In fact, sensory factors play an important role in how much we eat, as illustrated by the satiety cascade (see figure 17). The process of eating is influenced by other factors such as cognition, as humans while growing, learn to 'know' how much of a food they have or could eat in order to get satiated (for a review see Blundell et al., 2010). This learned response might indeed lead to a high intake of a given food, even when its content is manipulated in order to reduce its energy density for example, resulting in an excess energy intake, which might be deleterious for long-time maintenance of body weight (for a review on the subject see Rolls, 2009).

3.2.2. Effect of salt on intake

Salt is known as an appetite stimulator (Leshem, 2009; Shepherd, 1988). When infants 4 to 7 months were offered salted (9 to 440 mg of sodium i.e. 0.0225 to 1.1 g of salt per 100 g of food) or unsalted foods (5 to 130 mg of sodium i.e. 0.0125 to 0.325 g of salt per 100 g of food) on alternate weeks, no impact of salt was observed on intake (Fomon, Thomas & Filer, 1970). The author explains the absence of an effect by the experimental conditions. In fact, foods were fed by the infant's mother, and no instructions were given on when she had or not to stop feeding, suggesting that they might have given both versions with regard to their own interpretation on the infant's 'limit of ingestion'.

In another study where instructions were given to the mothers, newly weaned 6 month old infants were offered low-sodium baby cereals (10 mg of sodium i.e. 0.025 g of salt per 100 g of food) or high-sodium baby cereals (100 mg of sodium i.e. 0.25 g of salt per 100 g of food), they consumed the salted cereals in a significantly greater amount (83.6 g vs. 57.3 g) compared to the slightly salted ones (Harris & Booth, 1987). The same group was tested at 12 months of age, using unsalted mashed potatoes (3 mg of sodium i.e. 0.0075 g of salt per 100 g of food) or salted mashed potatoes (100 mg of sodium i.e. 0.25 g of salt per 100 g of food). The results were the same as when they were 6 months: they consumed significantly more of the salted compared to the unsalted mashed potatoes (70.7 g vs. 54.9 g); the intake of other items served during the meal, was not affected by salt content neither at 6 nor at 12 months (Harris & Booth, 1987). Children aged 3-6 years consumed significantly more of a saltier soup than of a less salty version (Cowart & Beauchamp, 1986). Moreover, a context-specific reaction for salty taste was observed at this age, as children consumed unsalted water, but rejected it when it was supplemented with salt, which was explained by the fact that at this age, salt is more associated with other media than water due to the exposure to foods (Cowart & Beauchamp, 1986). This context-specific reaction was confirmed by recent results with French infants who rejected salty compared to unsalted water at 20 months (Schwartz, 2009).

To our knowledge no other studies have investigated the intake response of older children to foods varying in their salt content.

3.2.3. Effect of sugar on intake

While some researchers have found over-sweetening to have less impact on decreasing intake than does under-sweetening (Vickers, Holton & Wang, 1998), others reached the opposite conclusions (Lucas & Bellisle, 1987; Vickers, Holton & Wang, 2001). A study conducted in 1999 by De Graaf and Zandstra favors the hypothesis of a more detrimental impact on intake of over sweetening. Subjects 8-10, 14-16 and 20-25 year-old, were asked to sip-and-swallow a range of increasingly sweetened waters and orangeades, and their subsequent intake was recorded (de Graaf & Zandstra, 1999). The results illustrated in figure 18, show that the intake of highly sweetened variants of orangeade: tended to decrease in young adults (20-25 years); whereas intake was plateauing among younger subjects (8-10 and 14-16 years) at higher concentrations.

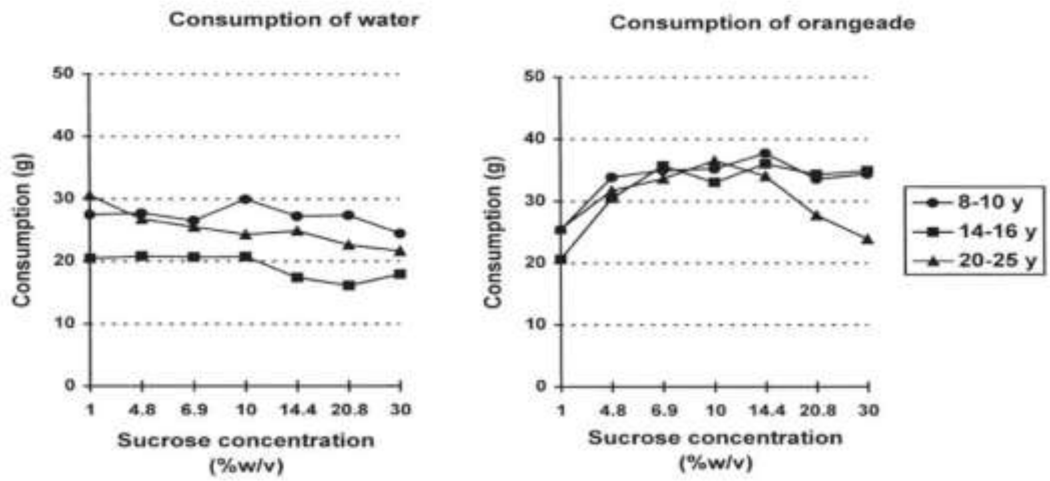

Figure 18. Mean consumption of increasingly sweetened water and orangeades, by subjects 8-10, 14-16 and 20-25 years of age. Source: de Graaf & Zandstra, 1999.

The authors also found children to be less sensitive to sucrose, which is in line with previous findings, indicating that sucrose thresholds are higher in 8-9 year-old children compared to adults (James, Laing & Oram, 1997). Besides, children were less able to discriminate different sucrose concentrations compared to adolescents and adults (de Graaf & Zandstra, 1999).

Another study conducted by Zandstra and De Graaf in 1999, evaluated the amount consumed by 36 subjects aged 18-27 years during an *ad libitum* test where five yogurts varying on their sucrose content (1, 5.9, 10, 17.6 and 30%) were served (Zandstra, de Graaf, van Trijp & van Staveren, 1999). The 30 % yogurt was significantly less consumed than the four other ones; and the 5.9 % was more consumed than the 17.6 % yogurt.

3.2.4. Effect of fat on intake

Fat is desirable and people would adopt a high-fat diet if given the possibility (Reed, 2010). In fact, fat is attractive due to its sensory properties (Drewnowski, 1987b; Reed et al., 1992). Moreover, fat affects satiety by slowing gastric emptying and intestinal motility (Uauy & Dangour, 2009). Fats are known to be less satiating than proteins and carbohydrates (Drewnowski & Almiron-Roig, 2010). The high palatability of high-fat foods, results in a behavior defined as "passive overconsumption" (Blundell, Lawton, Cotton & Macdiarmid, 1996), palatability-induced hyperphagia (French & Robinson, 2003); also named 'non-homeostatic eating' (Mela, 2006).

In 1975, based on behavioral observations, Barbara Hall hypothesized that breastfed infants have a more efficient appetite-control mechanism than do bottle-fed ones (Hall, 1975). Her rationale was that high-fat content of breast-milk provides a signal to the baby to stop feeding. However, this hypothesis was not verified by experimental studies. Some researchers investigated the effect of fat content variation on newborns' intake. No significant effect of fat content was found either in 4-9 day-old newborns' breast milk intake (Woolridge et al., 1980) or in 2-5 day-old newborns' or 5-week-old infants formula milk intake (Chan, Pollitt & Leibel, 1979; Nysenbaum & Smart, 1982). This absence of fat effect on intake could be due to other signals linked to breastfeeding such as milk flow changes during the course of a feed for example (Woolridge et al., 1980), and not to the absence of fat effect *per se*. Nevertheless, Chan and colleagues proposed an alternative to that hypothesis, and another explanation would be that the effect on intake, highlighted by Hall, is not linked to a specific nutrient content such as fat, but to the corresponding energy density of the milk, regardless of its nutrient type. In that line, effects of a higher palatability influencing intake were evidenced by a later research (Chan et al., 1979).

In a recent study, decreasing the fat content of a food (to decrease its energy density) was associated with a decrease in energy intake but to a slight increase in weight intake (Leahy, Birch & Rolls, 2008). However, whether this behavior is due to palatability or to satiety, is less known (Drewnowski & Almiron-Roig, 2010).

3.2.5. Is there a link between weight status and intake of salt, sugar or fat in foods?

In terms of intake, heavier infants tend to consume greater absolute volume of sweetened water than lighter ones (Doty & Shah, 2008).

A study identified heavier subjects to eat high-fat foods more frequently than did normal weight ones (Pangborn, Bos & Stern, 1985). Can sensitivity to fat be related to obesity? Recently, researchers identified subjects as hypo- or hypersensitive to fatty acids using a screening procedure, and found the proportion of hypersensitive subjects to the C18:1 (mainly present in foods for example) to be equivalent to 22 %, and this percentage can even reach 46-80 % in other studies (Nasser, Kissileff, Boozer, Chou & Pi-Sunyer, 2001). Researchers found differences in total energy and fat consumption among hypo- and hypersensitive subjects to oral fatty acids: hypersensitive subjects consumed less dietary fat, and were able to differentiate between custards based on their fat content (Stewart et al., 2010). Thus, attenuated taste sensitivity could be implicated in foods' overconsumption, and especially fat-rich ones. Indeed, in studies were the link between taste sensitivity, body weight and fat consumption was investigated, researchers found energy and fat intakes in particular, to be consumed in excess among obese subjects who also had a significant attenuation in taste sensitivity to C18:1 (for review see Little & Feinle-Bisset, 2011; Stewart et al., 2011a).

3.3. Link between preference and intake

In the case of salt, the preference for salt in 2 year-old toddlers was not related to measures of salt consumption and salt usage as reported by their mothers (Beauchamp & Moran, 1984). Another work showed however that salt use during the second year of life is related to salt intake at 4 years (Yeung, Leung & Pennell, 1984). Studies investigating the link between hedonic measures and salt intake in adults revealed that a higher preference was sometimes related to a higher salt intake, whereas other sensory measures such as threshold or suprathreshold sensitivity were not related to salt intake (Shepherd, 1988). In fact, due to the use of different methodologies to estimate an individual's salt intake (as discussed in part 1), the relationship between sensory reactions to salt and salt intake is not clear. Nevertheless, a recent study in adults found a positive link between liking and sodium intake (Hayes, Sullivan & Duffy, 2010). A study with 10-12 year-old children revealed that those who liked salty foods (reported by their mother), ingested more salt (estimated by urinary sodium excretion); Matsuzuki, Muto & Haruyama, 2008. A study in adults compared hedonic ratings after a sip-and-spit tasting of a food and *ad libitum* intake of the same food varying in salt content (Bellisle, Giachetti & Tournier, 1988). It showed that the optimal salinity revealed by hedonic rating was higher than the optimal revealed by intake data.

Elevated sweet preference is associated with a greater intake of added sugars and consumption of sweet foods (Pangborn & Giovanni, 1984). The preference for sweet taste may be linked to a high consumption of sweet foods: 4-year-old children who prefer the sweetest solution choose the sweetest apple juice at snack time (Olson & Gemmill, 1981). To access the link between preference and intake, Birch asked 17 toddlers (3 years 7 months and 4 years 7 months) to rank order open-faced sandwiches, with 8 kinds of spreads, some of which are sweet (margarine, margarine and mint jelly, peanut butter, peanut butter and grape jelly, cream cheese, cream cheese and honey, cream cheese and caviar and cheddar cheese spread), according to their preferences (Birch, 1979b). The participants were allowed to select and consume as much as they wanted of the sandwiches. The results, presented in figure 19, show that the more the sandwich is preferred, the more it was consumed by the child. The conclusion of that work was that 3-4-years children, preference for sweet foods is a great indicator of their intake.

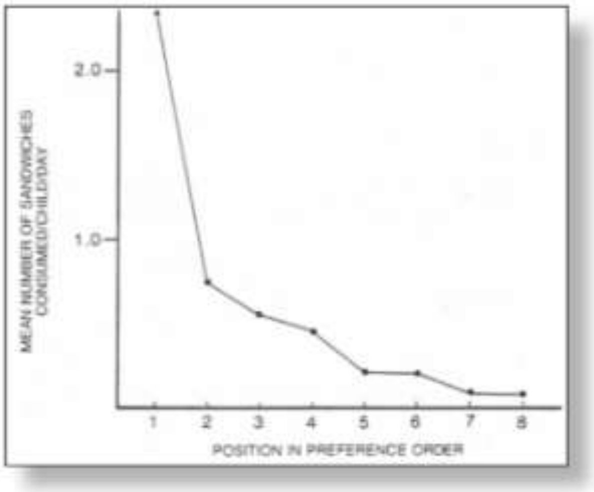

Figure 19. The relationship between the position in preference order of sandwiches with different spreads, and their intake by 3-4 year toddlers. Source Birch, 1979b.

In the case of sweet taste, studies in adults have shown that liking tests do not necessarily predict consumption; thus the intake of overly sweet foods is lower than would be predicted by taste test liking scores (Lucas & Bellisle, 1987; Vickers & Holton, 1998). Other studies have found hedonic ratings to predict consumption, as similar patterns exist between liking ratings of sweetened foods and their consumption (Vickers et al., 1998; Zandstra et al., 1999).

As far as fat is concerned, a study involving 3-5-year children assessed their preference for fatty foods and their intake using a 30-h weighed food intake data from a standard menu. This study revealed a positive relationship between the preference for fat in foods and the consumption of fat foods (Fisher & Birch, 1995); revealing that 'children with high-fat intake were more likely to have strong preferences for high-fat foods, than were children with low fat intake'.

Part 4. Exposure and learning mechanisms involved in the acquisition of food preferences

Humans are born with an innate preference for sweet taste and an aversion for bitter taste. In other respects, the majority of food likes and dislikes are learned and can be acquired thanks to experience and learning mechanisms (Birch, 1999). This could explain why familiarity with a food appears to be a key driver for children to consume and choose this food, as it accounts for 50-60 % of variance in their preference (Birch, 1979a).

Several mechanisms were identified in order to explain the acquisition of food preferences and may be used as a mean to overcome prior dislikes: Mere exposure (ME), Flavor-Flavor Learning (FFL) and Flavor-Nutrient Learning (FNL); for review see Havermans, Ronald Ross & Victor, 2010. The definition of these mechanisms and the experimental studies investigating them in children and adults are summarized below.

4.1. Mere exposure mechanism

As depicted in figure 20, human newborns are exposed since their early (uterine) life to various flavors (taste, aromas), starting when the fetus is in the womb, and continuing when the infant is exposed to adult foods. These experiences shape later food acceptance and preferences. It was shown that taste preferences acquired for aversive tastes such as bitter taste acquired through by repeated exposure during infancy are reliably stable over time. That is to say, children exposed when infants to hydrolyzed formula or to soy formula both described as sour and bitter tasting, exhibit a great preference for a sour or a bitter tasting juice -respectively- at 4-5 years, and their mothers reported them as more likely to prefer broccoli, compared to children fed with milk formula when infants (Liem & Mennella, 2002; Mennella & Beauchamp, 2002).

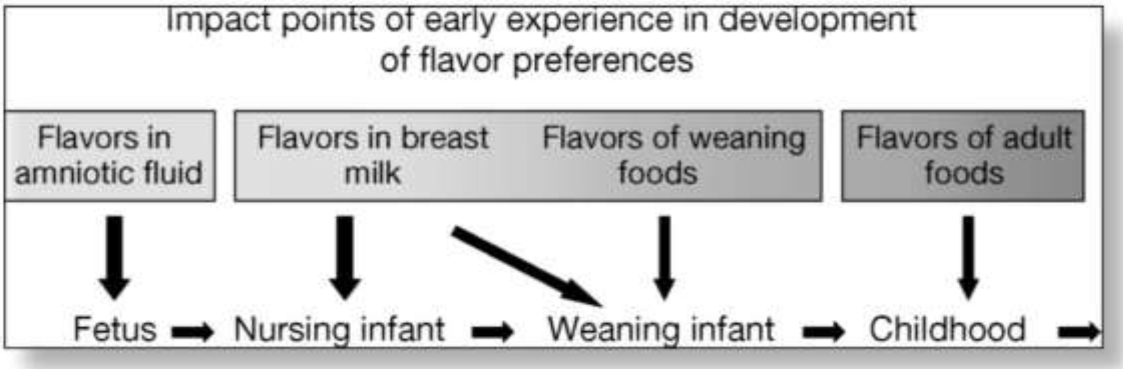

Figure 20. Flavor experiences throughout childhood. Source: Beauchamp & Mennella, 2009.

Evidences of experience with taste influencing preferences were supported by several studies in children. Infants exposed at birth and during the first 6 months of life to a sweetened water on a regular basis, exhibited a greater preference (measured through volume consumed) for a sweetened water compared to water alone at the age of 2 years old, compared to those children who were not exposed (Beauchamp & Moran, 1984). In the same line, 4-7 year-old children whose parents are used to add sucrose to their diet have higher preference for sweeter apple juice and cereals, than children of parents who do not do so (Liem & Mennella, 2002). Elevated preference for a given concentration of sucrose in orangeades can be was observed even after a short repeated exposure (8 days) to this concentration (Liem & de Graaf, 2004). Hence, repeated exposure seems to be an effective strategy to enhance liking, willingness to eat and intake of initially novel or unfamiliar foods, and was then applied in other studies with both adults and children.

In 1968 Zajonc suggested that "mere exposure of the individual to a stimulus enhances his attitude toward it" Zajonc, 1968, or as specified by Pliner, 1982, the 'affective response to a stimulus increases with increasing exposure". Mere exposure (ME) was first described for images, visual stimuli and sounds, and it was further applied to foods. The principle was that repeated experiences with food through exposure would result in an increased preference for this food.

The first experimental demonstration of this mechanism in the food domain was brought by Pliner in 1982. She exposed students to 4 tropical fruit juices, which they pre-tested, and were selected on the basis of a low familiarity score and similar scores of liking.

During a first session of 'exposure', each student was exposed to 35 samples of three of the four selected juices, either 20 times to one, 10 times to another, 5 times to a third and never to the fourth. The experimenter stressed their attention on the bitterness of the samples by asking them to rate bitterness intensity (distractive task), and warned them not to be surprised if some samples had the same taste. Then the four juices were presented once again to be scored for liking on a 7-point category scale. One week later, during a second session, the four juices were presented and the students had to rate their liking again. Liking ratings increased with increasing exposure; but this effect was smaller in the 2nd session than in the 1st session (Pliner, 1982). The same year, another study was conducted with children by Birch and Marlin. A first group (n=6) of 2 year-old toddlers was exposed either 20, 15, 10, 5 or 2 times to five initially novel cheeses (they received one pair of cheeses each day during 26 consecutive days). A second group (n=8) tasted a pair of initially novel fruits once a day during 25 consecutive days and thus were exposed either 20, 15, 10 or 5 times or never to a given fruit. Following the exposure period, children received the 10 possible pairs of cheeses (or fruits) and for each pair they were asked to choose one food to "eat more of it". The results confirm the ME hypothesis: preference was an increasing function of exposure frequency (Birch & Marlin, 1982).

Studies attempted to investigate the role of exposure on children's willingness to taste vegetables, on liking and on intake, since the beginning of complementary feeding. Some works focused on whether ME could be efficient in order to increase vegetable acceptance especially among children. Sullivan and Birch exposed 36 infants (4-6 months), to peas or green beans (a new food for them), once a day during 10 consecutive days)Sullivan & Birch, 1994). The foods were available in two versions, unsalted or salted (0.3 %), and each child was fed one food either salted or not. A first observation was that the intake of the vegetable they were exposed to, increased on average by 100 % at the post-exposure, from ~30 to ~60 g. A second observation was that no effect of salt was observed, as all groups increased their vegetable intake, whether salt was added to the recipe or not. In this study, the intake was not recorded during the exposure period. However, in another study with weaning-age infants, an effect of exposure on intake was observed after just a single exposure (Birch, Gunder, Grimm-Thomas & Laing, 1998). In fact, the authors noted that much of the post-exposure increase in intake occurred between the pre-exposure and the 1st day of exposure (35 to 61 g then from 61 to69 g during the 10 exposure days.)

In a study conducted with 4-5 year-old children, three groups were exposed to a plain, an unsalted or a sweet version of a novel food (tofu). The exposure took place twice a week over 9 weeks (15 exposures), and children were encouraged to 'take a taste'. Preference was measured using first a categorization on a 3-point scale with faces, then a ranking within each category. The results showed that preference increases after exposures only for the version children were exposed to (Sullivan & Birch, 1990).

Other studies demonstrated the efficiency of repeated exposure on increasing the acceptance of an initially disliked vegetable. After 14 consecutive days of in-home exposure, toddlers 2-6 year-olds' intake increased from 47 % at pre-intervention to 77 % at post-intervention, and the exposure also resulted in a significant improve of preference ranking for the target vegetable (Wardle et al., 2003a). In children aged 5-7 years old, 8 daily exposures to sweet red pepper (identified as being novel and relatively disliked in same-age children, and unfamiliar to the target study population), induced an increase in the number of pieces tasted from 2 to almost ten, as well as a significant linear increase in liking over the 8 exposure sessions (Wardle, Herrera, Cooke & Gibson, 2003b). Here again, a single exposure to the vegetable induced an significant increase in intake but not in liking in the control group; That was ”sufficient to reduce typical neophobic response of reluctance to taste, but that further exposures are required to effect changes in liking” (Wardle et al., 2003b; p 346).

It is noteworthy that the majority of parents stop trying to feed vegetable to their children after few attempts, if they consider that their children dislike it (Maier, Chabanet, Schaal, Issanchou & Leathwood, 2007a). However, the studies on ME demonstrate that it is worth keeping trying. In fact, as depicted in figure 21, a comparison of the effect of exposure on the intake of an initially liked and an initially disliked vegetable shows a great increase on the intake of the disliked vegetable by 4-7 month olds, after only 8 exposures.

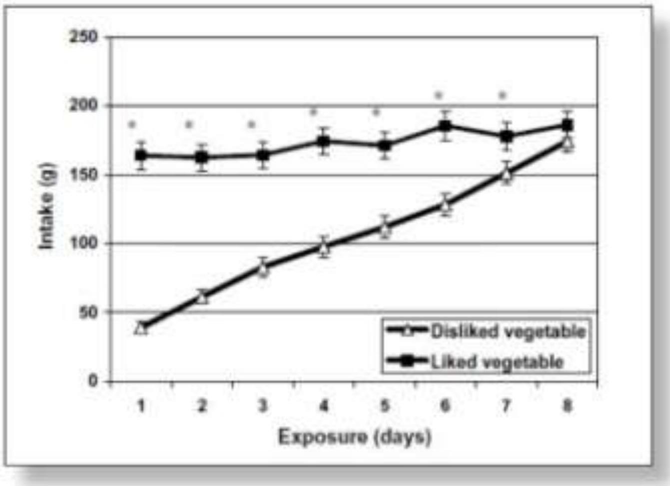

Figure 21. Effect of mere exposure on the intake of an initially disliked and an initially liked vegetable.

*Mean intake of the initially disliked vegetable is significantly different from mean intake of the initially liked vegetable (P<0.05). Mean intake ± SEM. Source: Maier et al., 2007a.

In sum, if an individual is repeatedly exposed to a food, his liking for the food increases as measured in adults and in children, as does his willingness to eat more of it as measured in children, as does his intake as measured in infants. ”The exposure effect thus seems so consistent, powerful and universal that in later studies, it was considered as the ‘‘gold standard’’ against which any studied mechanism should be tested” Nicklaus, in press; p 2. The exposure has to be performed through tasting the food and not only looking at it (Birch, McPhee, Shoba, Pirok & Steinberg, 1987).

4.2. Flavor-flavor learning mechanism

Flavor-Flavor Learning (FFL) is a learning mechanism corresponding to a form of ‘Pavlovian conditioning’. The Pavlovian conditioning was first described for its importance in acquiring emotional response in animals, and was further applied to studies in humans (Rozin & Zellner, 1985). This associative conditioning learning is based on pairing a conditioned stimulus (CS) to an unconditioned one (US). In the food domain, the CS is generally an initially novel or neutral flavor/food, and the US is a stimulus that triggers a positive response with biological robustness. The principle is that when the US is removed, the affective value of the CS remains. In the case of FFL studies in human, the US often chosen is the sweet taste (without the accompanying calories), due to its high affective value (Havermans et al., 2010).

In a study involving infants at weaning age, researchers investigated this mechanism, hypothesizing that 'infants would be more accepting of green beans if their previous dietary experience with this food was associated with a fruit' Forestell & Mennella, 2007. Thus, two groups of 4 to 8 month-olds followed an 8 consecutive day home-exposure to green beans (GB group) or to green beans followed one hour later by peach purée (GB-P group). Their preference and intake data were recorded. After the exposure period, infants in the GB group increased their intake of this vegetable, but their liking did not increase. However, when considering the GB-P group, not only their intake of green beans increased compared to the group GB, but also did their liking (fewer facial expressions of distaste during feeding). Thus, infants might have associated the sweet taste of peaches to the taste of green beans, which led to an increase liking of green beans compared to the other group.

Associating the taste of vegetables with the sweet taste was proved to enhance older children's liking. A study was conducted with 4-6 year-old children who, on the first day, ranked the taste of 6 vegetable drinks from the most liked (1) to the least liked (6) (Havermans & Jansen, 2007). The target vegetables (CS) were those ranked 3 and 4. In a randomized design conducted the second day, each child was served the pair of vegetables: one with added dextrose, eliciting a sweet taste (CS+); and the other one without it (CS-). Children had to take a sip and swallow from both cups for approximately one hour at a rate of one sip every 5 minutes. The day after the same process was applied, followed by a post-test where the 6 initial vegetables were served unsweetened and liking ranking was measured. As shown in figure 22, the vegetable ranked as just ok during pre-test, and served sweetened during exposure, was better liked at post-test. So, a positive shift in preference was observed on a short-time period (3 days).

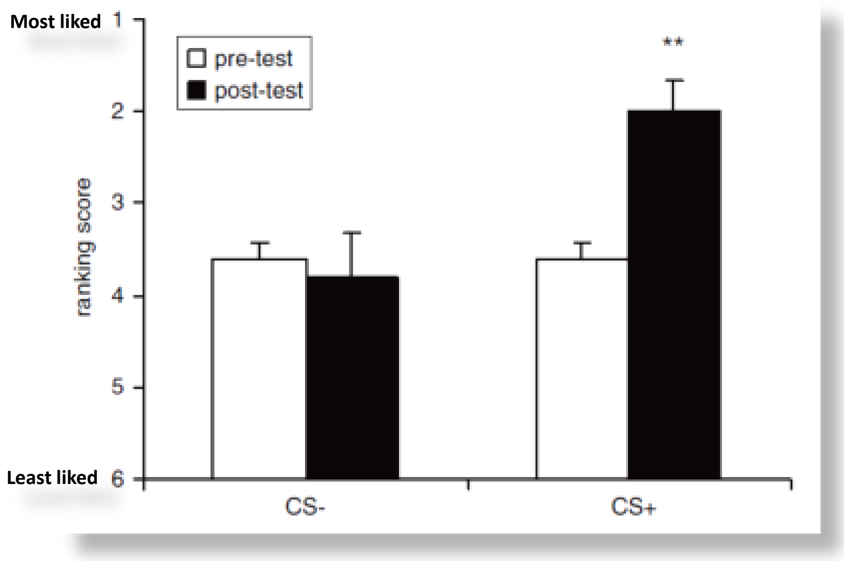

Figure 22. Mean ranking scores of 4-6 year-olds for vegetables, before and after a flavor-flavor learning, with sweet taste as the US.

Means ± SEM. ** refers to $p < 0.01$ for the difference from pre-test rank. Adapted from Havermans & Jansen, 2007.

Other experiments investigated FFL in children as young as 2-5 years old, for a period of 20 consecutive days, in order to increase their preference for a sour tasting grapefruit juice (Capaldi & Privitera, 2008). The juice was chosen to be initially neutral or disliked, and was associated to the sweet taste of sucrose. As shown in figure 23, compared to the measure at baseline, an increase in liking was observed after the 20 day exposure period when the sweet taste was removed. The following day, a post test (test 1) was conducted and the effect was still observed. The increase in liking was persistent even after a 2 week following the exposure period (test 2); both tests 1 and 2 were conducted during 5 days. A point to be highlighted concerns children who initially liked the taste of the plain juice: for them sucrose addition did not increase liking.). The same authors tested a similar approach in adults, and found that after only 3 days of exposure with a sweetened version of an initially disliked bitter vegetable (broccoli or cauliflower), an increase in liking for this vegetable was observed when sucrose was removed. The authors proposed to explanations for the adults' increase in liking ratings: either this would reveal a long lasting shift in liking due to a 'transfer of affect', which would have consequences on a longer term; or this result reflects a 'conditioned responding', that would depend on further associations with sweetness and thus predicting a quick extinction (Capaldi & Privitera, 2008).

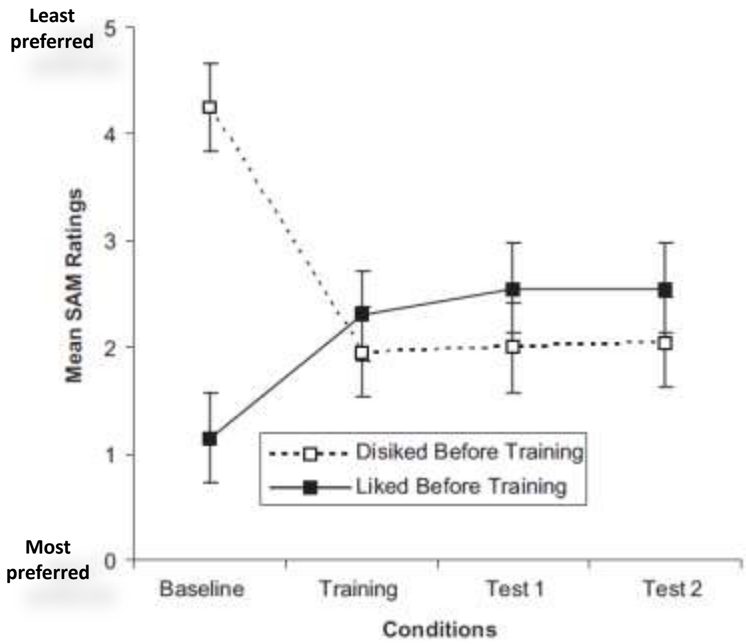

Figure 23. Effectiveness of flavor-flavor learning on liking of 2-5 year-old initial likers or dislikers of a grapefruit-juice.

Exposure (training) lasted 20 days; a 2 week break separated test 1 and test 2, both performed during 5 days. Liking and disliking were assessed using a rating scale with 5 pictures named SAM for Self-Assessment Manikin. Means ± SEM. Adapted from Capaldi & Privitera, 2008.

In this study, adults (18-23 years) were also exposed during the 3 days of FFL to another vegetable (broccoli or cauliflower), which constitutes a ME condition. Liking for this food was not significantly increased. The authors concluded that FFL is more effective than ME, which is not sufficient to produce a significant shift in liking and questioned the efficiency of ME (Capaldi & Privitera, 2008). In another study where adults were exposed to plain spinach during a period of 5 weeks, a significant effect of exposure on liking was observed (Bingham, Hurling & Stocks, 2005). Thus, it is possible that 3 days of exposure, as tested in the study of Capaldi and Privitera, was not a long enough period to elicit a shift in liking.

The FFL mechanism was applied to vegetables in order to enhance their liking and intake in children and in adults as depicted in table 11. Other flavors were used with adults as US, monosodium glutamate (MSG) for example.

Table 11. Example of studies investigating flavor-flavor learning in children and in adults.

Target food(s) or CS	N / Age group (years)	Sensory manipulation or US	Number of exposures	Measured outcome	Observed effect	Reference
	Children					
Zucchini - pumpkin - peas -cauliflower - broccoli – carrots	13 / 5	Dextrose None	6	Preference	Increase of preference No increase	Havermans & Jansen, 2007
Grapefruit juice	49 / 2- 5	Sucrose	20	Liking	Increase in liking (initial dislikers)	Capaldi & Privitera, 2008
	Adults					
Cabbage - broccoli - Spinach -cauliflower	40 / 18-64	Cream + cheese + spice	5	Liking	Increase in liking (initial dislikers)	Bingham et al., 2005
Broccoli/cauliflower	29 / 18-23	Sucrose	3	Liking	Increase in liking	Capaldi & Privitera, 2008
Spinach	32 / 22-24	MSG	4	Intake Liking	Increase in intake & liking	Yeomans, Gould, Mobini & Prescott, 2008

4.3. Flavor-nutrient learning

Flavor-Nutrient Learning (FNL) is another type of Pavlovian conditioning, and it is the last of the three mechanisms exposed herein. The principle is to pair a conditioned stimulus (CS), generally a new flavor or food (CS) to an unconditioned stimulus (US) which absorption or ingestion brings positive consequences in order to produce a post-ingestive reinforcement and induce a shift in liking for the CS even when the US is removed. As it will be developed hereafter, the reinforcement is generally achieved by increasing the energy density of a food by adding fat or caloric sweeteners.

FNL was studied with children (Birch, McPhee, Steinberg & Sullivan, 1990): two groups of 11 toddlers aged 3-5 years old, were served two drinks either low in energy density (LE, sweetened with aspartame; <5 kcal/150 ml) or high in energy density (HE, sweetened with maltodextrin; 155 kcal/150 ml), which were paired to two different and unfamiliar flavors (A-orange-chocolate or B-bubble gum). The first group was exposed to A/HE and B/LE and the second group to A/LE and B/HE. Before and after a 5-week conditioning period during which subjects consumed the drinks twice a week, a preference test (like, just ok or dislike) and a two-flavor choice tests (intake was recorded) were performed. The intake of both novel drinks increased at post-test, while only the preference for the flavor paired with the HE increased compared to that of the LE paired one, thus revealing a conditioning effect of the energy density.

In the previously described experiment, carbohydrates were used in order to manipulate energy density, and in another study variations in fat content were applied in order to achieve changes in energy density (Johnson et al., 1991). In this study, 12 toddlers aged 3- 4 years old, were served a LE yogurt drink (110 kcal/100 g) or a HE one (220 kcal/100 g) either paired with a pumpkin or with an orange-chocolate flavor, on 8 occasions. Results show that the preference for the flavor paired with the HE yogurt increased compared to the one paired with the LE yogurt. However, the increase in intake was similar for both drinks, confirming the previous results obtained when energy density was manipulated with carbohydrates. In another study (Kern et al., 1993), the authors suggested that the absence of any effect of FNL on intake in children could be associated to their state of hunger, which was not controlled during pre- and post-test and during the exposure phase; indeed, if learning is based on post-ingestive consequences, the absence of hunger could explain the absence of effect; thus, the effect of hunger state on FNL was investigated. In this FNL trial, 27 toddlers aged 3-4 years old, were exposed to a HE and a LE yogurt (fat manipulation), paired with one of 5 flavors (banana, cherry, grape, peach or raspberry). During the 6 weeks of conditioning, they received the LE (or HE) drink twice a week and the week after they received the HE (or LE) drink. A conditioning (150 ml) and a mere exposure (just taste; ≤ 16g) groups were set, and received the drink in a hungry or in a full state i.e. before or after a meal. The experiment's conclusions, illustrated by figure 24, confirm the previous findings of a preference conditioning mediated by fat in 3-4 year-olds. The effect was further strengthened by the post-ingestive consequences of fat, and the effect was stronger in the group who had the opportunity to consume a great amount than in the group who just tasted the stimulus: confirming that a hungry state favors FNL. The hypothesis of the impact of hunger on FNL effect is also supported by later work, as an FNL is more noticeable when subjects are in a state of high-energy requirement i.e. when they are hungry (Appleton, Gentry & Shepherd, 2006).

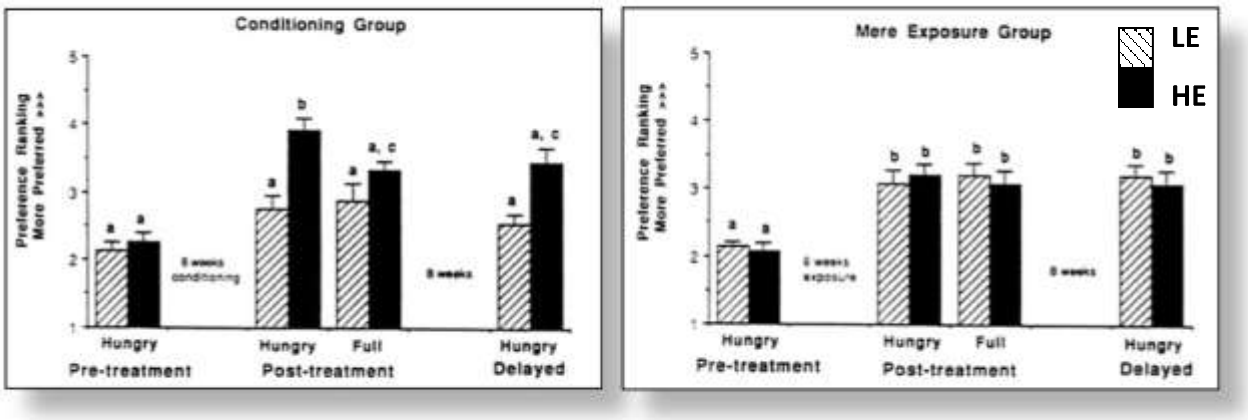

Figure 24. Effect of hunger state on flavor-nutrient learning or mere exposure on 3-4 year-olds' preference for a high-energy (HE) or a low-energy (LE) paired flavors. Source: Kern et al., 1993.

As it was the case for FFL, researchers were interested in testing the effectiveness of FNL in enhancing vegetable intake as the low-energy density provided by vegetables, is proposed to be one of the reasons making them among the least chosen and consumed foods by children (Gibson & Wardle, 2003; Nicklaus et al., 2004). In a study published recently by Zeinstra and co-workers Zeinstra, Koelen, Kok & de Graaf, 2009), a 14-day conditioning trial was scheduled with children aged 7-8 years old, in order to investigate FNL on vegetable liking/intake. From a list of six vegetables (cucumber, iceberg lettuce, carrot, red pepper, tomato and beetroot), the two middle ranked ones were selected according to a preference ranking test. Vegetable drinks were prepared and the HE one was obtained by adding maltodextrin. Both LE and HE drinks were sweetened with aspartame, 150 ml were served during children's morning snack, and preference and intake were measured. During consumption, children completed a questionnaire on the drinks' sensory characteristics (liking, sweetness, sourness, saltiness, bitterness and thickness). This study did not run as the authors wished, as at the mid-trial period, the procedure was interrupted because children did not consume enough drinks. In fact, in the FNL studies described above, completion with the conditioning trial occurred when the child consumed at least 80% of the volume offered (Johnson et al., 1991; Kern et al., 1993). In Zeinstra's study, as shown in figure 25, a reason explaining the 'failure' of the experiment might be their taste, judged as very strong and intense (≈ 3 out of a scale of 1 to 5). Thus no effect of pairing with energy on liking was observed and ratings were very low for both drinks. Unfortunately, the 'hard-wired aversion to the vegetable tastes', limited ingestion and prevented from observing any effect of FNL on vegetable liking and intake (Zeinstra et al., 2009). Several points should be highlighted in order to explain the results of this study: the vegetables were served during the morning snack (10:15 am), which might be an inappropriate time to consume vegetables; besides, the vegetables were mixed and served as drinks, which might not have been an appropriate form of presentation for children aged 7-8 years old; moreover, children this age may have already developed expectations concerning congruence between tastes and foods, and pairing vegetable drinks with a sweet taste might have been incongruous.

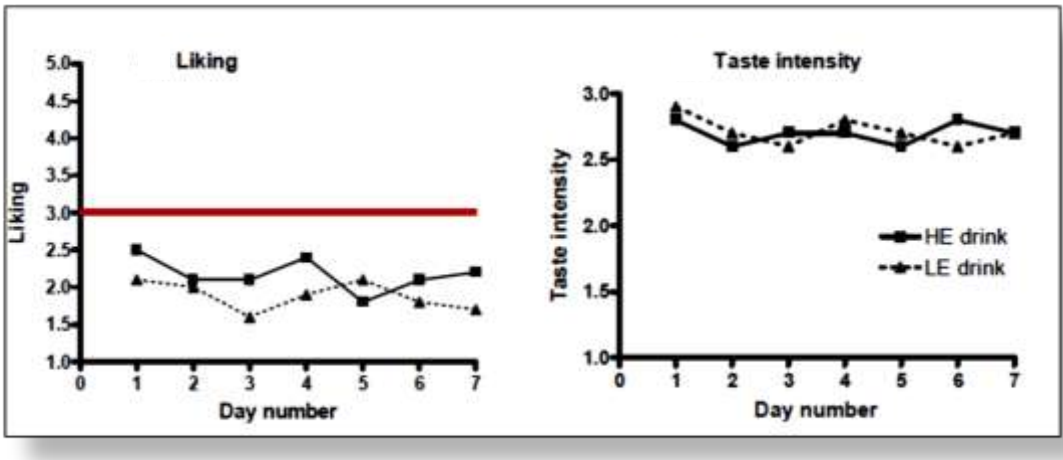

Figure 25. A 'failed' flavor-nutrient learning experiment inducing vegetable liking in 7-8 year-olds. Adapted from: Zeinstra, 2010.

4.4. Comparison between mechanisms

4.4.1. What influences the efficiency of mechanisms

The efficiency of each mechanism might depend on the goal to reach; e.g. if the aim is to increase liking or to increase intake. For example, 8 exposures were sufficient to increase infants' intake and liking of green beans in a FFL procedure, whereas this number of exposure was not enough to increase liking in a ME procedure, as only intake increased (Forestell & Mennella, 2007).

Besides, the number of exposures might be of importance, as for example, FFL was seen to be more efficient than ME after 3 consecutive days of exposure (Capaldi & Privitera, 2008); moreover one exposure a week for five weeks induced a greater efficiency of FFL compared to ME (Bingham et al., 2005). Whether this was due to the different affective value of both tested sensory qualities i.e. sweetness in the first experiment and saltiness in the second experiment, or to the number of exposure, cannot be concluded.

It is argued that "it may be easier to modify the preferences of children by repeated exposures than those of adults, because adults' rich experience of foods may have limited their idea of what foods should be like, thus resulting in rigid expectations" Tuorila, 1996. Thus, changing an adult's preference might appear to be harder than that of children. However, there is no indication on the number of exposures necessary for a learning effect on preference, liking or intake to occur. This might depend on the target CS, on the nature of the US, on the age of the subjects. Besides, no systematic view is given on how often exposures should take place e.g. many times a week, on separate or on consequent days,

4.4.2. Can learning effects be separated as ME, FFL and FNL?

One question that could be raised is: in the different studies presented above, are the different mechanisms really distinct or are there some overlaps? In some of the cited above studies, the frontier between the definitions of the used terms for mechanisms is sometimes thin.

It was argued that if ME is expected to dissipate neophobia, as suggested by Zajonc in 1968, then mere exposure effect would not occur with already familiar foods (see Pliner, Pelchat & Grabski, 1993 and discussion in Pliner, 1982). Familiarity implies that the food was already tasted by the subject (at least once!) before the treatment or exposure period. However, in general it is unclear whether familiarity should be related to the food as a whole, with the usual way it is prepared, with a specific recipe thus having a specific flavor and taste? with the texture (pureed or chunks; cooked or raw); e.g. if repeated exposure was conducted with a mashed version of a food, would it imply that the food presented in chunks will also be further accepted? In some studies cited above investigating repeated exposure, test foods were chosen on the basis of novelty or unfamiliarity or on the basis of prior dislike. It was argued that ME would be more efficient to induce a shift in liking if the food is willing to be consumed and if it is initially neutral or disliked (Capaldi & Privitera, 2008).

In the study of Sullivan and Birch (1990), even if the authors described the three conditions (plain, salted, and sweetened tofu) as mere exposure, one could consider that the exposure to the salted or to the sweetened version as a FFL with salt or either an FNL with sweet taste.

In this experiment, children liked the taste of tofu they were exposed to, and not the others (Sullivan & Birch, 1990). At this age (4-6 years) children exposed to one version (salted or sweetened) might have learned that it was *the* appropriate seasoning. In another study with younger children (4-6 months), a generalization of the exposure effect occurred when the experience with salted peas enhanced acceptance for unsalted peas; the same pattern was observed for green beans (Sullivan & Birch, 1994); indicating an 'intra-product generalization'. This generalization effect was also observed in another study with 4-7 month-old infants (Birch et al., 1998). In this study, after 10 days of exposure to a target food (fruit), infants who further received the same food prepared by another manufactured or a similar food from the same category, increased their intake, compared to infants who received a different food from another food category. To sum up, several points can be described in order to define each mechanism (table 12).

Besides, in real life, can we consider that mere exposure is really 'mere'? Indeed, in normal eating-occasions, food intake is always followed by a post-ingestive effect or by an associated pleasure. This would be more described as a FNL or a FFL.

Table 12. Comparison between ME, FFL and FNL.

	ME	FFL	FNL
Number of exposures	8-15; 10 optimal	Less than ME	Less than ME
Initial familiarity/liking	Neutral / disliked	Neutral	Neutral
Quantity	Just a taste is enough	Just a taste is enough	Critical
Hunger state	----	---	Critical

Part 5. Thesis outline and research questions

As it was discussed throughout the previous parts, early childhood is a key period in the development of eating behavior. Understanding the determinants of food preferences and food intake in toddlers as young as the age of 2-3 years seems of a great importance from the perspective of establishing long-lasting healthy eating habits. Some food ingredients such as salt, sugar and fat are targeted by nutritional policy makers and public health services. Their reduction is recommended especially in foods for children. However, precise data about amounts of reduction and their consequences in term of preference and intake in actual foods (in opposition to solutions or model foods) are not available.

Our main aim during this thesis work was to investigate toddlers' (2-3 years) response to salt, sugar and fat variations in actual foods. In order to have 'ecological' data, experiments took place at the toddlers' usual food intake environment i.e. nursery canteen, during their normally scheduled meal time (lunch or snack). A secondary objective was to conduct comparisons with older children or with adult population whenever possible. During this PhD thesis four papers, aiming at answering the following research questions, will be presented and discussed.

Research questions and objectives concerning salt:

As was reviewed previously, in general toddlers prefer and consume salty foods and this preference maintains into later childhood.

Hypothesis 1: Compared to the usual version of a food, saltier versions would be consumed in a greater amount, and unsalted versions would be less consumed.

*» This question is investigated in **chapters 2 & 3***

It was established that a context-specific effect for salt preference exists. Likewise, we could speculate that a food-specific effect of salt level on intake does exist.

Hypothesis 2: The effect of salt level variations on intake would be different in a highly consumed food by toddlers and children (e.g. a starchy food), compared to a less consumed food (e.g. vegetables).

*» This question is investigated in **chapters 2 & 3***

With regard to the greater experience of 8-11 year-olds with salty foods, compared to toddlers, their intake of saltier versions would be more important compared to the usual version of a food.

Hypothesis 3: Children aged 8-11 years would display a different pattern of response in terms of intake than would 2-3 year-olds.

» This question is investigated in ***chapters 2 &3***

Food intake is influenced by food preferences. But which role does salt level play in this relationship? Because of some methodological constraints (making it difficult to directly explore preferences of toddlers toward salt level variations in foods) only older children aged 8 to 11 were involved in this part of the work.

Hypothesis 4: In children aged 8-11 year-olds, salt content would impact preference, and consequently intake.

» This question is investigated in ***chapter 3***

As salt plays an important role in liking foods, especially vegetables, one could speculate that it could play a role on learning to like a disliked or neutrally liked vegetable. However, due to the long-lasting adverse health effects of salt intake, public health authorities aim at reducing salt levels in foods especially for children. Different strategies are explored among which the use of aroma or salty ingredients such as spices evoking salty taste was proposed.

Hypothesis 5: Salt may be used as an unconditioned stimulus (or a conditioning agent) in order to increase the acceptance of a vegetable in a flavor-flavor learning experiment.

Hypothesis 6: A part of salt used while preparing foods, could be replaced by a congruent salt-associated spice in a flavor-flavor learning experiment.

» These questions are investigated in ***chapter 4***

Research questions and objectives concerning sugar:

Preference for sweet taste is innate, and children exhibit an elevated preference for sweet taste, as they consume more of the sweeter liquids (solutions and orangeades). However, toddlers' intake of a food varying in its sugar content was not investigated yet.

Hypothesis 7: Toddlers would consume more of the sweeter versions of a food than of the less sweet versions.

Hypothesis 8: This effect would be food-specific, and different for an initially sweetened and an initially unsweetened food.

» This question is investigated in ***chapter 2 and in a study presented in appendix #1***

Research questions and objectives concerning fat:

Children learn to prefer energy-dense foods, the majority of which are high in fat content. This could explain the high palatability of high-fat foods.

Hypothesis 9: As palatability appears to be an important driver of intake, one might hypothesize that a decrease in fat content of a food would induce a decrease in its weight intake.

» This question is investigated in ***chapters 2 & 5***

Besides, in preload studies, children were more efficient than adults at regulating their energy intake when fat content varied. Would this still be the case when observing intake within a meal?

Hypothesis 10: As far as energy density (related to fat content) is concerned, a decrease in a food's energy density would induce an increase in its intake in order to 'compensate' for the missing energy.

Hypothesis 11: Toddlers would better regulate their energy intake when fat content varied than adults would.

» These questions are investigated in ***chapter 5***

Chapter 2. The impact of salt, sugar and fat on toddlers' food intake

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***British Journal of Nutrition*, 2011, 105 (4) : 645 - 653**

British Journal of Nutrition (2011), **105**, 645–653 doi:10.1017/S0007114510003752

The impact of salt, fat and sugar levels on toddler food intake

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(Received 30 April 2010 – Revised 17 August 2010 – Accepted 25 August 2010 – First published online 7 December 2010)

Abstract

Understanding the early determinants of food intake, in particular the role of food sensory quality, is a necessary step to improve the prevention of unhealthy food habits. However, the extent to which food intake varies according to salt, fat and sugar content is imperfectly known. The present study aimed at evaluating whether toddler food intake varied during lunches or snacks in which salt, fat or sugar contents had been modified in common foods. Seventy-four children (30 (SE 0·5) months old) participated in the study in their usual day-care centres. Every other week, they were served lunches composed, among other items, of green beans and pasta with varying salt (0, 0·6 and 1·2% added salt) or fat (0, 2·5 and 5% added butter) levels and afternoon snacks composed of fruit purée varying in sugar level (0, 5 and 10% added sugar). During each meal, children could eat as much as they wanted from the target foods. Each food was weighed before and after the meal. Salt level had a positive impact on the intake of the target foods. On the contrary, no impact of added fat or added sugar levels was observed. This implies that fat and sugar addition could be avoided in foods for children without having an impact on palatability, allowing the energy density of children's diet to be limited. Salt addition should be limited, but its suppression in vegetables, whose intake is to be promoted, should be considered cautiously.

Key words: Children: Food intake: Salt: Fat: Sugar: BMI

Over the past few years, many countries have developed governmental policies aimed at decreasing the intake of salt-rich, fat-rich and sugar-rich foods[1]. In France in particular, the National Nutrition and Health Programme aims at limiting the quantity of salt, fat and sugar in foods[2]. However, data measuring the impact of these reductions on food consumption, in particular in young children, are not available. Regarding the influence of sensory properties of foods on intake, especially in young children[3], and the importance of early childhood in the development of long-standing food behaviour[4], studying the impact of the recommendations on child food intake seems necessary. Few studies to date have evaluated the link between ingredient levels and intake especially among young children. In the present context of the universal prevalence of paediatric obesity and of the wide availability of palatable processed foods, it is essential to take into account the sensory drivers of eating.

In infants, previous studies using aqueous solutions showed that a preference for salty taste in a solution emerges at about the age of 4 months and persists till the age of 2 years[5–7]. Unlike what is observed for salty taste, humans exhibit an innate preference for sweet solutions, already observed a few hours after birth[8], with newborn babies preferring highly sweet solutions[9]. Furthermore, during the first year of life, sweet and salty solutions are consumed on average more than water[7]. In children under the age of 2 years, sweet solutions generally elicit acceptance[10], and the sweeter the solution the more they consume[11]. This preference may be linked to a high consumption of sweet foods: 4-year-old children who prefer the sweetest solution choose the sweetest apple juice at snack time[12].

Beyond studies using solutions, research on foods showed that the preference for salt in foods, which appears at the age of 6 months, is reinforced by repeated exposure to salty foods, at least till 12 months of age[13]. Moreover, a context-specific preference for salt appears in early childhood. On the one hand, 2-year-old children tend to ingest more salty soup and carrots than the plain versions. On the other hand, children between 3 and 6 years old reject salty solutions but not salty soup[10,14]. This suggests the emergence of a preference for salty solutions between 3 and 6 months, followed by a preference for salty over non-salty versions of foods.

In other respects, fat-rich and sugar-rich foods, frequently associated with the high prevalence of overweight and obesity, are both highly palatable and appealing[15],

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while providing dietary energy at low cost[16]. Sugar and fat are liked food features by children and the sweet and fat taste preference in childhood can be explained by the pleasure or by the energy they provide, considering the energy needs while growing[17]. Furthermore, an age-related decline in sweet preference was documented: it is high in childhood[18] and early adolescence and decreases in early adulthood[19]. As far as fat is concerned, it has positive post-ingestive consequences, since it conditions the preference for new flavours in preschool children[20,21]. However, the impact of fat level on child preference was not studied directly as was that of salt or sugar level. In a recent study, decreasing the fat content of a food (to decrease its energy density) was associated with a decrease in energy intake and also with a slight increase in weight intake in relation to energy compensation[22]. Moreover, child preference and consumption of fats appear to be linked to those of their parents and to their own BMI[23].

The present study aims at evaluating the impact of salt, fat or sugar levels in common foods on children's intake, during a normal meal in a familiar environment. The general hypothesis is that salt, fat and sugar increase food palatability, which can potentially lead to an increase of food intake[24]. Three ingredient levels were studied: no addition, usual level used while preparing foods for children and twice the usual level. Our hypotheses were that children would (i) consume more of the saltier version and less of the salt-free one than of the usual one and (ii) consume more of the high-fat version and less of the fat-free one compared with the usual one considering that a positive effect of fat on palatability might be observed. Concerning added sugar variations, they were tested in a fruit purée. It could be supposed that children would (iii) consume more of the sweeter versions of this food than of the unsweetened version. Moreover, the existence of a food-specific effect of ingredient level on intake can be hypothesised, as for instance context-specific effects were shown for salt preference. In particular, the ingredient level effect may be different according to the hedonic value of the target food, since sensory variations in a liked food might affect the intake differently compared with in one that is rather disliked. Hence, variations of salt and fat were tested in two foods: one vegetable and one starchy food.

Participants and methods

Participants

All children attending one of three nurseries in Dijon (France) were proposed to take part in the present experiment. The children's body weight (kg) and height (m) were measured by the nursery doctor and *z*-scores for BMI were calculated using the French and International Obesity Task Force standards for child overweight and obesity[25]. Their parents received an information sheet and signed a consent form. One of the children's parents did not allow their child participate due to food allergies. The study procedure was conducted according to the guidelines laid down in the Declaration of Helsinki, and approved by a local ethical committee (Comité de Protection des Personnes Est I Bourgogne).

A total of seventy-four children (forty-two girls and thirty-two boys) participated in the study. Their average age was 30 (SE 4) months old (range 18–37 months), with average BMI of 15·9 (SE 0·1) kg/m^2 (range 13·5–18·9 kg/m^2) and average BMI *z*-score −0·2 (SE 0·1) (range −2·3–2·0).

General experimental design

The present study aimed at evaluating toddler food intake according to variations of salt, fat and sugar levels in usual foods in a natural setting. It took place in the children's usual nursery in order to minimise the effects due to context changes. Before the beginning of the study, the experimenters visited the nurseries several times in order for the children and the nursery staff to become familiar to them. The nurses were asked to behave as normal (keep an eye on the children during the meal and help maintain calm conditions). Moreover, they were instructed neither to exhort children to eat nor to urge them to eat. Children were seated by groups of four to six around a table and one nurse was in charge of one table as usual. All the children were able to eat alone with a spoon or a fork, without any help. All the menu items were prepared in each nursery kitchen by the nursery cook and were pre-weighed by experimenters before lunch. The children could eat as much as they wanted from the target foods by asking for additional servings if they wanted to, which was a common practice in each nursery. All the servings were registered during the meal or snack for each child on a record sheet. Intake (g) of each food item of the lunch or snack was measured by weighing each food before and after consumption to the nearest 1 g (Soehnle, Leifheit Group, Germany). The nurses were blind to the modifications brought to the target foods.

Added salt and fat level variations during lunches: procedure. For salt or fat level variations, the target foods were green beans and pasta served at lunch. The experimental fixed menu was served every other week at 11.00 hours. It was composed of vegetable salad (a unique serving of 50 g), chicken (a unique serving of 50 g), green beans (an initial serving of 50 g and up to a complement of 125 g on the basis of 25 g for each additional serving), pasta (an initial serving of 50 g and up to a complement of 125 g on the basis of 25 g for each additional serving), yogurt (a unique serving of 125 g), bread (*ad libitum*) and water (*ad libitum*). Portion sizes were adapted from the French guidelines for school canteen usage[26]. Vegetable salad and chicken were prepared as usual. Frozen green beans and pasta (small macaroni) were boiled with tap water (11 and 8 min, respectively) without adding salt, oil or any flavouring. The experimenters further added salt or butter according to the experimental design and stirred the foods homogeneously.

Added salt level variations were studied in a first series of five observations and added fat level variations were studied in a second series of five observations. According to the nursery cooks, the average levels used for preparing foods for children were 0·6 g of salt/100 g of food and 2·5 g of butter/100 g of food. These usual levels were then chosen to be the 'control' values (0·6 % salt and 2·5 % butter). We varied these levels by adding no salt or no fat (0 %) or by doubling them (1·2 % salt and 5 % butter). Thus, three levels were tested for each target food and each ingredient. For both the series, the first observation was a control for salt and fat. Then the order of the four other observations was different across the nurseries (Table 1). For each observation, salt or fat level varied only in green beans or in pasta. For instance, if salt level varied in green beans, their fat content was the control level and pasta was served with control levels of salt and of fat.

After the serving of the fixed amount of vegetable salad, children were served chicken and green beans; afterwards, they were served pasta and then the yogurt. This order of presentation was chosen in order to allow the children to eat the vegetable first, which was generally less consumed by children compared with pasta[27] – this also being the usual practice in the nurseries.

Added sugar level variation during snack time: procedure. For sugar level variations, the target food was a fruit purée served during the afternoon snack every other week at 15.30 hours. In order to evaluate the children's intake depending on added sugar level, an unsweetened apple–peach purée (Hero, 26400 ALLEX, France) was chosen and three levels of added sugar were tested. One level corresponded to the plain fruit purée (unsweetened; variant named 0 %); for the second one, 5 g of sugar was added to 100 g of purée (variant named 5 %); and for the third one, 10 g of sugar was added to 100 g of purée (variant named 10 %). The order of presentation for the three variants was different across the nurseries. A first portion of 100 g was served and additional servings of 50 g were available, up to a complement of 200 g. Afterwards, the children were served a maximum of 20 g of biscuits then *ad libitum* milk and/or water.

Sensory description of food variants

A sensory description of the food variants was performed with an adult panel. In fact, regarding the young age of the children participating in the main study, sensory description was not possible[28,29]. A trained sensory panel of twelve adults (six women and six men), aged between 27 and 66 years, evaluated all variants of the three target foods. The panel members were screened for their olfactory abilities, their abilities to perceive and identify the basic tastes and for their capability to use a linear scale to score intensities. The intensity scales from the Spectrum™ method for descriptive analysis were used to describe sweet, salty, sour and bitter tastes[30]. A Spectrum™-like scale was developed for fattiness perception. After eleven training sessions on the way to use the scale for each taste or perception, the panellists were asked to score the perceived intensities of each variant of each target food presented monadically, from 'not perceived' (0) to 'very intense' (10). Two replications were performed.

Statistical analyses

Statistical analyses were carried out using SAS System for Windows version 9.1 (SAS Institute, Inc., Cary, NC, USA). Significance was set at $P<0·05$. For sensory description, an ANOVA was performed using a linear model (SAS GLM

Table 1. Experimental design for varying added (g/100 g) salt and fat (butter) in green beans and pasta for each nursery

			Salt level variations					Fat level variations				
			Observation number									
Nursery	Food	Ingredient	1*	2	3	4	5	6*	7	8	9	10
A	Green beans	Salt	0·6	0·6	0	0·6	1·2	0·6	0·6	0·6	0·6	0·6
		Butter	2·5	2·5	2·5	2·5	2·5	2·5	5	2·5	0	2·5
	Pasta	Salt	0·6	1·2	0·6	0	0·6	0·6	0·6	0·6	0·6	0·6
		Butter	2·5	2·5	2·5	2·5	2·5	2·5	2·5	0	2·5	5
B	Green beans	Salt	0·6	0	0·6	1·2	0·6	0·6	0·6	0·6	0·6	0·6
		Butter	2·5	2·5	2·5	2·5	2·5	2·5	2·5	5	2·5	0
	Pasta	Salt	0·6	0·6	1·2	0·6	0	0·6	0·6	0·6	0·6	0·6
		Butter	2·5	2·5	2·5	2·5	2·5	2·5	0	2·5	5	2·5
C	Green beans	Salt	0·6	0·6	1·2	0·6	0	0·6	0·6	0·6	0·6	0·6
		Butter	2·5	2·5	2·5	2·5	2·5	2·5	0	2·5	5	2·5
	Pasta	Salt	0·6	0	0·6	1·2	0·6	0·6	0·6	0·6	0·6	0·6
		Butter	2·5	2·5	2·5	2·5	2·5	2·5	2·5	5	2·5	0

* Control observations.

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procedure) for each taste quality and for the three variants of each target food. The model was:

$$\text{Taste quality} = \text{panellist} + \text{ingredient level} + \text{panellist} \times \text{ingredient level} + \text{error},$$

where 'panellist' was considered as a random effect. Means for the three variants were compared with one another using t tests.

To analyse the children's consumption, the outcome was intake (g) of green beans, pasta or fruit purée. For each series, paired t tests (t_{df}) were used to determine, over the five lunches, whether the average intake of green beans was different from the average intake of pasta. For each ingredient (fat, salt or sugar) and each target food intake (green beans, pasta or fruit purée), data were analysed using a mixed linear model (SAS Mixed procedure), with 'child effect' considered as random. The primary factor tested in the model was ingredient level. The children's BMI z-score was introduced as a covariate to evaluate to what extent it might affect the target food intake. Interactions between ingredient level and BMI z-score were investigated: if no significant effect was found, the interaction was removed from the model. The 'Empirical' option was specified so as to use a sandwich estimator for the variance–covariance matrix of the fixed-effect parameters. For the comparison of means, the Dunnett test was applied: the control variant (0·6% added salt, 2·5% added butter or 5% added sugar) was compared with the other variants. Data are reported as mean values with their standard errors.

Results

Sensory description of food variants

Salt level variations in green beans and pasta. Saltiness intensity differed according to added salt levels in green beans and in pasta ($F(2,36) = 71·47$; $P < 0·0001$ and $F(2,36) = 95·52$; $P < 0·0001$, respectively): the three variants of each food were significantly different (Table 2). Moreover, the 0% added salt green beans were perceived as more sour ($F(2,36) = 4·35$; $P = 0·02$) and less fatty ($F(2,36) = 17·89$; $P < 0·0001$) than the two other variants.

Fat level variations in green beans and pasta. Fattiness perception differed according to added fat levels in green beans and in pasta ($F(2,36) = 4·10$; $P = 0·025$ and $F(2,36) = 24·30$; $P < 0·0001$, respectively), as shown in Table 2. However, panellists were not able to distinguish the 2·5% and the 5% added fat green beans, whereas the three variants of pasta were distinguished from each other.

Sugar level variations in fruit purée. Sweetness intensity differed according to added sugar level in fruit purée ($F(2,36) = 74·89$; $P < 0·0001$) as reported in Table 2: the three variants were significantly different, as the more the added sugar the higher the perceived sweetness. They also differed with regard to sourness and bitterness intensities, ($F(2,36) = 8·34$; $P = 0·0011$ and $F(2,36) = 9·57$; $P = 0·0005$, respectively): the 0% added sugar variant was perceived more sour and more bitter than the two other variants.

Children's intake during experimental lunches

Intake when added salt level varied. From the initial sample of seventy-four children, only those who were present for at least two meals out of five were considered for the present analyses, resulting in a final group of sixty-six children. No child reached the maximum serving of 175 g for green beans and only 7% reached this maximum for pasta. A paired t test comparing the average intake of green beans and pasta over the five lunches showed, as expected, that pasta intake, 87 (SE 4) g, was greater than that of green beans, 32 (SE 3) g, where $t_{65} = 12·5$ ($P < 0·0001$).

Table 2. Sensory description of food variants

Food	Ingredient	Level (%)	Salty*	Sweet	Sour	Bitter	Fatty
Green beans	Salt	0	$0·25^a$	$0·38^a$	$0·62^b$	$0·21^a$	$0·50^a$
		0·6	$2·10^b$	$0·59^a$	$0·40^a$	$0·15^a$	$1·15^b$
		1·2	$3·81^c$	$0·55^a$	$0·49^a$	$0·17^a$	$1·11^b$
	Butter	0	$2·22^a$	$0·76^a$	$0·63^a$	$0·18^a$	$0·77^a$
		2·5	$2·24^a$	$0·73^a$	$0·44^a$	$0·17^a$	$1·08^b$
		5	$2·20^a$	$0·79^a$	$0·53^a$	$0·18^a$	$1·16^b$
Pasta	Salt	0	$0·26^a$	$0·69^a$	$0·38^a$	$0·21^a$	$1·58^a$
		0·6	$2·54^b$	$0·79^a$	$0·26^a$	$0·14^a$	$1·90^a$
		1·2	$3·94^c$	$0·86^a$	$0·31^a$	$0·44^a$	$2·29^a$
	Butter	0	$2·14^a$	$0·54^a$	$0·24^a$	$0·07^a$	$0·82^a$
		2·5	$2·45^a$	$0·65^a$	$0·25^a$	$0·05^a$	$1·28^b$
		5	$2·47^a$	$0·59^a$	$0·32^a$	$0·08^a$	$1·99^c$
Fruit purée	Sugar	0	$0·19^a$	$1·45^a$	$3·96^b$	$0·65^b$	$0·21^a$
		5	$0·14^a$	$3·10^b$	$2·84^a$	$0·37^a$	$0·27^a$
		10	$0·12^a$	$4·64^c$	$2·33^a$	$0·25^a$	$0·34^a$

a,b,c Mean values within columns with unlike superscript letters were significantly different for each food, each ingredient and each sensory descriptor ($P < 0·05$).

* For each sensory descriptor, the used scale ranged from 0 to 10.

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The impact of salt level variations on intake was evaluated separately for each target food. The results are shown in Fig. 1(a). Concerning green beans, the salt level had an impact on the intake ($F(2,106) = 10·89$; $P < 0·0001$; n 63): 0 % salt green beans were less consumed than 0·6 % salt green beans. Concerning pasta, the salt level also had an impact on the intake ($F(2,92) = 3·19$; $P = 0·045$; n 60): 1·2 % salt pasta was more consumed than 0·6 % salt pasta. Hence, the suppression of salt decreased green bean intake and the addition of salt increased pasta intake, compared with the usual salt level. Despite this difference in salt level effect observed through the analyses conducted separately for each target food, a global analysis performed on data of both target foods did not reveal a significant food × salt level interaction.

Intake when added fat level varied. From the initial sample of seventy-four children, only those who attended at least two meals out of five were considered for the present analyses, resulting in a final group of sixty-nine children. No child reached the maximum serving of 175 g for green beans and only 11 % reached this maximum for pasta. A paired t test comparing the average intake of green beans and pasta over the five lunches showed, as expected, that pasta intake, 99 (SE 4) g was greater than green bean intake, 43 (SE 3) g, where $t_{68} = 12·23$ ($P < 0·0001$).

The impact of fat level variations on intake was evaluated separately for each target food. Fat level had an impact on neither the intake of green beans ($F(2,103) = 0·45$; $P = 0·63$; n 62) nor on that of pasta ($F(2,99) = 0·48$; $P = 0·62$; n 60). The results are shown in Fig. 1(b).

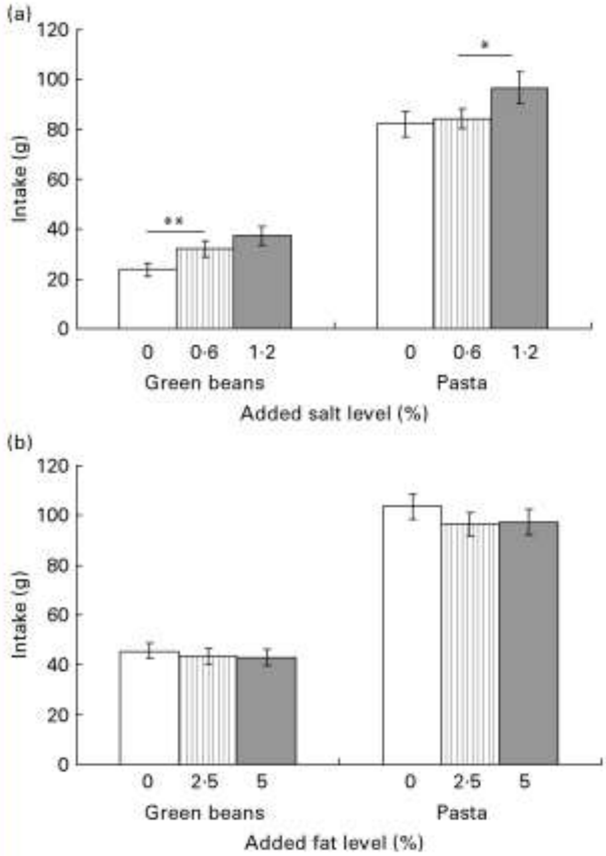

Fig. 1. Impact of added salt ((a) 0, 0·6 and 1·2 % added salt) and added fat ((b) 0, 2·5 and 5 % added butter) level on green bean and pasta intake in 2–3-year-old children (least squares mean values with their standard errors). For mean comparison, a Dunnett test was applied: the 0·6 % added salt or the 2·5 % added butter sample was compared with the other samples respectively for added salt and added fat level analysis. Mean values were significantly different: *$P < 0·05$; **$P < 0·01$.

Children's intake during experimental snacks when added sugar level varied

From the initial sample of seventy-four children, only those who attended the three snack sessions in which sugar level varied in fruit purée were considered for the present analysis (n 61). Only 4 % of children reached the maximum serving of 300 g of fruit purée.

Sugar level variations did not have an impact on fruit purée intake ($F(2,120) = 1·43$; $P = 0·24$), as shown in Fig. 2.

Effect of BMI z-score on intake

The children's BMI z-score was not related to intake when added salt level varied, neither in green beans ($F(1,106) = 0·78$; $P = 0·38$) nor in pasta ($F(1,92) = 0·61$; $P = 0·44$). When added fat level varied, BMI z-score did not affect the green bean intake ($F(1,103) = 1·77$; $P = 0·18$), but it had an impact on pasta intake ($F(1,99) = 6·01$; $P = 0·01$): the higher the BMI z-score the higher the pasta intake. Further examination of data revealed that BMI z-score was especially related to intake of the 5 % added butter pasta. When added sugar level varied in fruit purée, no impact of BMI z-score on intake was observed ($F(1,120) = 0·32$; $P = 0·57$).

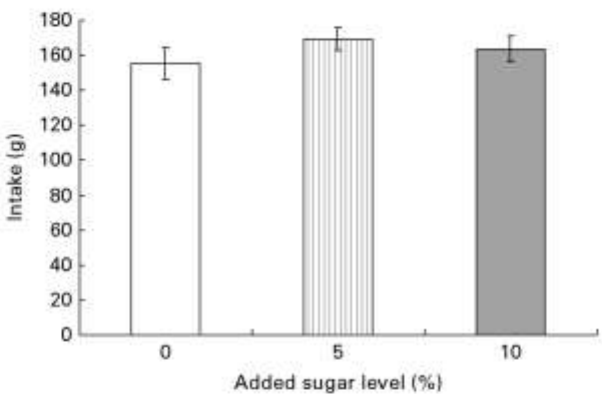

Fig. 2. Impact of added sugar level (0, 5 and 10 %) on fruit purée intake in 2–3-year-old children (least squares mean values with their standard errors). For mean comparison, a Dunnett test was applied: the 5 % added sugar sample was compared with the other samples. Mean values were not significantly different.

Discussion

Added salt and fat level variations

The present study aimed at evaluating the impact of salt and fat variations on the intake of two different foods by 2- to 3-year-old children. Two main findings can be highlighted: first, salt had an impact on intake but fat did not; secondly, intake increased with salt level (compared with the usual salt level, a suppression of salt induced a 25% decrease in green bean intake, whereas an addition of salt induced a 15% increase in pasta intake). Moreover, pasta was more consumed than green beans.

Salt (NaCl) is known as an appetite stimulator, a taste modulator and a flavour enhancer[31]. An increased preference for salty over non-salty foods appears at 2–3 years of age[5], leading to a preference for saltier foods in children than in adults, whether in a liquid[32] or in a solid medium[33]. In addition to the fact that salt preference is context specific in infants and children[10], it may also be food specific. In fact, other researchers showed that the perception and acceptability of saltiness are product specific[34]. In the present experiment, the hypothesis of a food-specific effect of salt is not confirmed (no significant food × salt level interaction). However, when salt was suppressed in green beans, intake was reduced by 25% and was very low (24 g), whereas intake was still high in pasta (82 g). Here, suppression of salt appeared critical in a not well-liked food, whereas it was not the case in a liked food. Salt is also known to be a taste modulator, as perceived bitterness can be suppressed by salt addition[35]. Here, variations in bitterness could have explained the decreasing intake of green beans when no salt was added, but the trained panel scored bitterness in green beans as very low (mean bitterness intensity over the three variants of 0·18 on a scale from 0 to 10) and failed to find any difference in bitterness between green bean samples. However, the unsalted green beans were scored sourer and less fatty. We hence cannot exclude that variations in tastes, other than salty taste, have an impact on children, which accounts for the decreased intake of the unsalted version, whereas pasta samples differed clearly only in salty taste.

In other respects, it seems that human avidity for salty taste is not due to a 'sodium appetite' (a desire for sodium) as is the case for animals, but is most probably related to a 'salt appetite' (seeking the taste of NaCl), an attraction to the taste of salt independently from the subject's Na status (for a review, see Leshem[31]). Nevertheless, due to its potential long-term detrimental effect on health[36], strategies to reduce Na intake have been developed[37]. One of them is related to the flavour-enhancing properties of salt: adding an odorant associated with saltiness through previous experience (i.e. bacon flavour) to a slightly salted solution enhanced its perceived saltiness[38]. This strategy would be interesting to promote vegetable intake in children by maintaining a salty perception without adding large amounts of salt.

Concerning the absence of fat impact on intake, two hypotheses can be formulated: either the changes of fat content over different meals were not perceived by children or fat is not an immediate hedonic determinant of food intake in children. Here, the trained panel could differentiate the 'fattiness perception' in the three samples of pasta varying in fat content, and concerning green beans, the panel could only differentiate the 0% added butter green beans from the other two samples. Despite these sensory differences, children's intake was not affected by fat content. Hence, fat sensory properties may not be well detected by human subjects[3,39]. In fact, studies in adults using dairy products showed that participants could not distinguish between the samples according to their fat content, but liked the fattiest samples[39,40]. Yet, it is admitted that the preference for high-fat foods is not based on a 'conscious' perception of fat content[41]. However, as for salt, fat in foods is also known to enhance their flavour and their acceptability and may lead to an increased preference, and by extension, to an increased intake[15,20,21]. However, we did not observe such an effect. That the fat level in food is poorly perceived is also revealed in a study examining the link between energy density associated with varying fat content and intake of macaroni and cheese in 2- to 5-year-old children[22]. Intake for two fat levels (39·8 and 19·7% total fat) was measured in separate meals: when the fat level decreased, a small but significant increase in intake was observed (+7%). In the present study, the addition of 5% butter corresponded to 4·1% fat: thus when total fat varied from 4·1 to 0%, a 6·5% increase in intake was noticed, which did not reach significance (P=0·27). Nevertheless, these fat levels correspond to those generally used in nursery canteens for preparing food for children. With such a range of fat content, the presentation of an *ad libitum* meal was not associated with children's adjustment for varying palatability and/or energy density in the short term. Similarly, concerning the green beans, the decrease in fat content in green beans (from 4·1 to 0% total fat) resulted in a 4·5% increase in intake, which again was NS. Hence, contrarily to our initial hypothesis, no direct effect of fat on intake was observed.

The present data also showed that preschool children with a higher BMI z-score consumed more pasta when fat level was higher, thus confirming the previous result highlighting that fatter children prefer high-fat foods[23]. Moreover, child 'fat appetite' was previously linked to their parents' body mass, and a link between child preference for high-fat snack foods and their consumption of a high percentage of total energy from fat was also shown[23]. A similar link between the preference for high-fat foods and BMI was described in 9- to 12-year-old children[42]. Longitudinal approaches would help us to understand the contribution of fatty food intake in the development of obesity. Alternatively, this effect could be related to sensitivity to fatty acids which is lower in heavier adult subjects, as reported recently[43]. To date, such an effect has not been studied in children.

In other respects, two main reasons are generally put forward to explain the low intake of vegetables among young children: the first one is the absence of reinforcement resulting from their low energy density[44,45] and the second one is their disliked taste that might be due to their bitterness[46] (as was discussed earlier). The lower intake of green beans compared with that of pasta confirms the previous data showing that, in a free choice situation, vegetables are among the least selected foods by young children compared with starchy foods and animal products[27], despite their recognised beneficial effects on health[47]. Moreover, the low variety of consumed vegetables observed in early childhood persists through to adulthood[4,45]. Hence, increasing vegetable intake by children is a major issue and more knowledge is needed about factors influencing their acceptance. Several studies have investigated the methods for improving preschool children's vegetable consumption by evaluating the effect of learning practices (for a review, see Havermans[48]) as well as the effect of varying ways of preparation[49]. The last study showed that children prefer boiled vegetables, which was the preparation method chosen for the present study. Clearly, sensory properties of vegetables are largely modified by preparation and much is to be learned to fully understand their impact on vegetable intake by children.

Added sugar level variations

The present findings indicate that 2- to 3-year-old children's intake of a fruit purée was not affected by its added sugar content. Contrarily to our initial hypothesis, no increase in intake with increasing added sugar level was observed. With regard to the recognised effect of sweetening on enhancing the palatability of foods, three hypotheses may explain this result. First, when tasting the fruit purée, children may not have perceived the difference in sweetness intensity between the variants over separate snack days. However, the results obtained by the trained panel showed a clear distinction between the samples in terms of sweetness and to a lesser extent of sourness and bitterness. Secondly, the unsweetened variant may have been sweet enough for children to like and consume it, due to the natural sweet taste of the fruits. Thirdly, the intake of all versions of the fruit purée, 163 g on an average, was far above the median portion of commercialised products (100 g), therefore suggesting a ceiling effect.

Individual differences in liking for sweet taste were previously reported[24,50]. In a study comparing children's and adults' liking related to sugar concentration, responses fitted an inverted U-shape for preference with a maximum hedonic response for both groups, but preferred concentrations were higher for children than for adults[17]. In the present study, taken as a group, the intake by children levelled up with increasing added sugar level, with neither increase nor decrease depending on the sugar level, suggesting an indifference to sugar level. Hence, the hypothesis of a tolerance zone to sweet taste, in fact a 'large tolerance plateau', could be considered.

The high consumption of fruit purée is consistent with the fact that liking sweet-tasting foods is strongly linked to the child's basic biology (for a review, see Pepino & Mennella[51]), and the absence of sugar level effect on intake is in line with the observation that most studies in adults failed to find an effect of sugar level on food intake[24]. That is to say, one may not need to add sugar to fruit purée to enhance its intake. Furthermore, an interventional study in children aged 7–11 years showed that the more children consume sweetened drinks, the higher their risk of being overweight or obese[52]. Altogether, these findings suggest that in order to promote a healthy energy balance in children and to favour the consumption of healthy foods such as fruits, limiting or suppressing added sugar may help to prevent excess energy input without altering their intake. Similar data concerning different foods with different textures and different levels of sugar, such as drinks, cakes or dairy products would also be useful and should be investigated.

Methodological considerations

Here, an initial portion of 50 g was served of green beans and pasta and of 100 g of fruit purée. This did not lead to a systematic consumption of this portion: at the group level, green bean intake was always below the served portion, revealing that children did not clean up their plates but pasta and fruit purée intake was always above the served portions; so, children did not hesitate to ask for additional servings. The fact that 'additional serving' was a usual practice in nurseries could account for this observation, which is consistent with the results showing that children below 3 years old are not systematically influenced by portion size[53]. Thus, this procedure seems appropriate to observe the effects of subtle sensory manipulations.

Conducting such a study where children did not have the opportunity to compare variants directly, no difference in intake was observed when fat and sugar level varied. Another protocol using a paired preference test might have yielded different results. However, studying intake appears more relevant in order to understand the actual children's behaviour in response to a given level of ingredient, because it is closer to everyday eating experiences where children are not able to compare the products.

General conclusion

Salt level had a positive impact on the intake of target foods, whereas fat (butter) content did not, either for green beans or for pasta. Besides, sugar level did not elicit any difference in the intake of fruit purée. That is to say, lowering the addition of fat or sugar while preparing children's foods, especially when the food is liked without any extra ingredients being added (such as starchy foods or fruit purée), may appear to be a useful way to reduce energy intake and to avoid the development of a habit of consuming foods rich in fat and sugar. Furthermore, these findings suggest that there is no need to add salt to pasta which is consumed anyway. On the contrary, salt suppression in vegetables, whose intake is to be promoted, should be considered cautiously. Alternatively, seasoning vegetables using salt-associated spices may be an efficient and necessary strategy to promote their consumption in children. Hence, nutritional policies regarding the use of salt, fat and sugar aimed at children could take these results into account and could emphasise that salt addition should be limited and that fat and sugar addition can be avoided, helping to prevent obesity from an early age on and to establish healthier food habits.

Acknowledgements

The present work was funded by the Nutrition, Chemical Food Safety and Consumer Behaviour Division of INRA (French National Institute for Agronomical Research) and the Regional Council of Burgundy. The authors thank the Early Childhood Department of the city of Dijon as well as the nursery directors and caregivers. Cooks from the nurseries and from the Dijon University Hospital central kitchen are especially thanked for their support. S. B. thanks the children and their parents who took part in the present study; Dr Vincent Boggio for his general support and his advice; Claire Chabanet for her assistance on conducting statistical analyses; Christophe Martin for conducting sensory profiling; Valérie Feyen, Emilie Szleper, Céline Magnan, Camille Schwartz, Audrey Perot and Françoise Durey are thanked for their assistance with food preparation and data collection. S. B. and S. N. designed the study with the help of S. I. The collection of data, the data analysis and the writing of the paper were performed by S. B. and S. N. with the help of S. I. The authors have no conflict of interest to disclose.

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Chapter 3. Salt content: effect on preferences and consequences on intake in children

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Submitted

ABSTRACT

Background & aims: Although salt reduction is recommended and despite the importance of sensory properties of foods in child eating behavior, the impact of salt on child liking and intake has received little attention. The impact of salt content of two common foods on children's intake and liking was investigated.

Methods: Children (n=75; 8-11 years) participated to 5 test lunches in their school canteen in a cross-over design. The salt content of two foods (green beans and pasta) varied (0, 0.6 or 1.2 %). Intake was recorded. Children scored foods for liking and ranked them for preference and saltiness. Data were modeled using Structural Equation Modeling (SEM).

Results: Suppressing salt decreased green bean intake, whereas increasing it increased pasta intake. For both foods, children could rank the variants according to salt content and preferred the two saltier ones. SEM showed as expected, that salt influenced intake through preference, and allowed prioritizing factors affecting food intake: hunger, liking of the food regardless of its salt content, and liking for a particular salt content in the food.

Conclusions: Salt has a positive and food-specific effect on intake. Taking into account children's liking may lead to salt addition beyond the content which is sufficient for a food to be consumed.

Keywords: Children; salt; food intake; food preferences; BMI

1. Introduction

The relationship between dietary sodium intake and the prevalence of some pathologies later in life has long been recognized (1). Reducing dietary sodium intake has become among public health priorities in several countries, resulting in governmental policies aiming at reducing salt content in foods (2). Childhood plays an important role in the establishment of eating behavior (3, 4), thus analyzing the role of salt in children's intake appears of importance. In children, the role of food sensory qualities on eating behavior is important (5), so salt reduction might impact food liking and consequently food intake (6, 7). A recent study on the role of salt in 2-3 year-old children's food intake showed an overall positive effect of salt content on food intake (8). Whether salt has the same impact on food intake in older children and the role played by sensory preference on intake in this population are unknown.

The relationship between sensory reactions to salt and salt intake is not clear and in studies with adults, inconsistent findings might be explained by the use of different methodologies to estimate an individual's salt intake (see 7 for a review). Studies investigating the link between sensory measures and salt intake in adults revealed that a higher preference was sometimes related to a higher salt intake, whereas other sensory measures such as threshold or suprathreshold sensitivity were not related to salt intake. For this reason, it seems relevant to investigate preference as a primary sensory measurement to establish an individual's reaction to salt, especially when working with children. A recent study in adults combined two strategies to access sodium intake (quantitative food frequency over one year and a five-non-consecutive-day food record) in order to evaluate the link between liking for salty foods and consumption of high-sodium foods (9). A positive association between perceived saltiness of snack foods and liking was found; as well as a positive link between liking and sodium intake. Moreover, saltiness impacted differently liking depending on the type of food tested, supporting the hypothesis of a food-specific preference for high salt contents suggested elsewhere (10, 11). A study with 10-12 year-old children revealed that those who liked salty foods (reported by their mother), ingested more salt (estimated by urinary sodium excretion) (12). Another alternative approach to evaluate the impact of salt consists in a direct measurement of food intake in response to varying salt content in a given food. A study in adults compared hedonic ratings after a sip-and-spit tasting of the food and *ad libitum* intake of the same food varying in salt content (13). It showed that the optimal salinity revealed by hedonic rating was higher than the optimal revealed by intake data. Another study examined the role of salt content on food intake in 2-3 year-old children (6), but could not address the role of hedonic rating in children this young for methodological reasons (8, 14).

The objectives of the present study were the following: (1) evaluate the effect of salt content on school-age children's intake of two foods (green beans representing vegetables and pasta representing starchy foods), and (2) prioritizing the relative importance of salt content, preference, hunger and weight status on children's food intake. Here, BMI was considered in the analyses because of the recognized relationship between food intake and BMI (15), and because a positive significant association between the intake of an energy-dense food and toddler's BMI *z*-score has already been observed (8).

2. Materials and methods

2.1. Participants

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by a local ethical committee (Comité de Protection des Personnes Est I Bourgogne). Parents received written information about the study and allowed for the participation of their child with a signed consent form. They provided information about their child's body weight (kg) and height (m); z-scores for BMI (further noted z-BMI) were then calculated (16). All children aged between 8 and 11 years old and attending one school in

Dijon (France) were proposed to participate in the present study, representing a group of N=106. Two couples of parents refused the participation of their child.

2.2. Study design

The study took place from October to December 2009 in Dijon (France). On the basis of a previous study conducted with toddlers in which salt content modification had a significant effect on green bean intake and on pasta intake (8), a power analysis (17) showed that 33 children were necessary to detect a difference in green bean intake of 8 g, with a significance level of 0.05 for type I error and a power of 0.80.

Children's intake was monitored five times in a school canteen every other week, at lunch time (between 11:45 am and 12:45 pm). Target foods, in which salt content was manipulated, were green beans and pasta. The experimental fixed menu was composed of vegetable salad (a unique serving of 50 g; 0.87 kcal/g or 3.64 kJ/g), chicken (a unique serving of 50 g; 2.26 kcal/g or 9.46 kJ/g), green beans (an initial serving of 100 g and a second serving of 100 g; 0.37 kcal/g or 1.55 kJ/g), pasta (an initial serving of 100 g and a second serving of 100 g; 1.4 kcal/g or 5.86 kJ/g), cheese (a unique serving of 18 g; 2.39 kcal/g or 10 kJ/g), fruit puree (a unique serving of 100 g; 0.57 kcal/g or 2.38 kJ/g), bread (*ad libitum*; 2.65 kcal/g or 11.1 kJ/g) and water (*ad libitum*). Portion sizes were adapted from the French guidelines for school canteen usage (18). Vegetable salad and chicken were prepared by the canteen cook as usual. Green beans and pasta were boiled with plain water, without adding salt, oil or any flavoring. Butter (2.5 %) and salt were further added by the experimenters according to the experimental design, and were stirred homogeneously. Salt contents were the same as those used in a previous study with 2-3 year-old children (8): no added salt (0 %), intermediate salt content (0.6 %) or twice this amount (1.2 %). The intermediate salt content is the usual one for such foods in school canteens and can thus be considered as a reference salt content. The first observation was a control, where green beans and pasta were prepared with the intermediate salt content. For each following observation, salt content varied only in green beans or in pasta. For instance, if salt content was different from 0.6% in green beans, pasta was served with the 0.6% salt content. For each food of the menu, intake data (serving minus leftovers, in g) was recorded for each child to the nearest 1 g (Soehnle, Leifheit Group, Germany). Children were blind to the aim of the study. The school team was aware that salt content was modified but was blind to the experimental design.

After the five lunches at school, children participated in a sensory evaluation session organized at 10:00 am at the Centre des Sciences du Goût et de l'Alimentation laboratory, as part of a school outing. During this session, they were first presented with the three salt content variants for each target food in a sequential monadic way and rated their liking on a five point facial scale (from 'very bad', -2, to 'very good', +2) (14). Second, they were served the three variants simultaneously and were asked to rank them according to their preference (from 'most preferred', +1, to 'least preferred', -1), and then according to their saltiness (from 'most salty', +1, to 'least salty', -1). Ties were not allowed. Here both liking and preference ranking were measured since these two variables provided us with different information about the child's perception of the food. The liking score made it possible for a child to score a food negatively whatever its salt content, if s/he did not like it in general. The preference ranking 'forced' the child to decide which variant was preferred whatever her/his liking of the given food. Foods were maintained warm after salt addition (45 °C) and 30 g were served in a 6 oz (175 ml) isotherm covered cup.

2.3. Statistical analysis

First, univariate analyses were carried out to investigate the salt content and the z-BMI effects on target food intake, liking and preference ranking. Second, a multivariate analysis was performed using Structural Equation Modeling (SEM) in order to evaluate the respective weights of different variables on target food intake. Significance was set at $P<0.05$.

Univariate analyses

Analyses were carried out using SAS System for Windows version 9.1 (SAS Institute, Inc., Cary, NC, USA). Intake and liking data (g) for both target foods were analyzed using a linear mixed model (SAS Mixed procedure), with 'child' considered as a random effect. The primary factor tested in the model was salt content. Children's z-BMI was introduced as a covariate. The model was the following: Intake (or Liking) = food + salt content + z-BMI + food x salt content + food x z-BMI + error. The 'Empirical' option was specified so as to use a sandwich estimator for the variance-covariance matrix of the fixed-effect parameters. If a significant food x salt content interaction was found, data were analyzed for each target food separately using the linear model: Intake (or Liking) = salt content + z-BMI + error.

Preference and saltiness rankings for each food were analyzed according to the model: Ranking = salt content + error. If a significant effect was revealed, multiple mean comparisons were applied using the Dunnett test: the reference salt content (0.6 % added salt) was compared to the other variants. Data are reported as Mean ± SEM.

Structural Equation Modeling (SEM) analysis

Structural Equation Modeling and bootstrap were conducted with R2.10.1, using SEM package (19) in order to assess a causal scheme derived from theoretical grounds (20).

Variable definition and dataset structure. The SEM method can deal with multilevel data (21). Here, the variables taken into account were: intake (of salad, chicken, green beans and pasta), liking, preference, salt content and z-BMI. The data were two-level data, as some variables were recorded at the meal level (level-1; intake of each food item), whereas others were recorded at the child level (level-2; liking of green bean or pasta at a given salt content, z-BMI). For green bean and pasta, liking for the 0.6 % added salt content was used as a measure of the 'overall liking' of each food; and the difference in liking relative to the 0.6 % added salt variant was used as a measure of the 'relative liking'. These measures will be compared with the preference ranking measures, as they actually measured the same construct. A latent construct was defined to estimate ‘hunger’ at the meal level, through the child’s intake of both target foods and all foods consumed before them or at the same time (salad and chicken).

The modeling approach was based on a correlation matrix, in order to evaluate and compare the respective variables influencing food intake (20): salt content, liking and preference ranking, the child’s ‘hunger’ and z-BMI. Pearson (when both variables were continuous), polyserial (when one variable was discrete and the other was continuous) or polychoric correlations (when two discrete variables) were calculated (22).

Model construction and estimation. The maximum likelihood estimator was used to estimate the model parameters (loadings). A first model based on theoretical considerations included an effect of the overall liking for green bean and pasta (0.6 % added salt liking) on their respective intake; this effect occurred at the child level. It also included one latent variable per target food named preference, which was measured by two observed variables, preference ranking and relative liking. An effect of preference on intake was taken into account for both target foods. A quadratic salt content effect on preference was added, for green beans and pasta. Thus, the salt content variable and its square after orthogonalization were included (23). Thereby, we considered that a higher salt content could involve a higher preference, which in turn could involve a higher intake.

The same scheme was set up for green beans and for pasta. A correlation between overall and relative liking was included. Moreover, a correlation between green bean overall liking and pasta overall liking was added to the model, to take into account inter-individual differences in liking scale use. In addition, the effect of salad intake on green bean, pasta and chicken intakes was added and set to the same value to take into account the fact that a child who had eaten a large amount of salad might not have been as hungry for the remaining of the meal.

The nested model obtained by applying equality constraints between green bean and pasta parameters (salt content effect on preference, preference effect on intake, overall liking effect on intake, preference loadings on preference ranking and relative liking) was compared to the unconstraint model.

Lastly, a z-BMI effect on 'hunger' was investigated, supposing that the higher the z-BMI the higher the overall intake. Then, modification indices were considered for model improvement. They led to consider a specific z-BMI effect on pasta liking and intake. When both effects were introduced in the model, the z-BMI effect on 'hunger' was no longer significant, suggesting a specific effect of z-BMI on pasta intake rather than a general effect on hunger. Modification indices also suggested a link between overall green bean liking and salad intake; this link was added to the model. Such an effect makes sense, since vegetable salad included pieces of green beans; as expected, it appeared significant and positive.

Comparisons between the theoretical model and the modified models were tested by likelihood ratio tests ($P<0.05$).

Since the observations were clustered by child, the independence hypothesis between observations was not met. Therefore, the parameter standard errors and parameter 95% confidence intervals were obtained by bootstrap resampling (B=100), with resampling performed at the child level (21, 24).

Model validation and indicators of model fit.

The fit is considered as good when the model is parsimonious and when fitted covariance is close enough to observed covariance. As recommended, several fit indexes that address different aspects of model fit were reported here: chi-square test, Tucker-Lewis Non-Normed Fit Index (NNFI), Bentler Comparative Fit Index (CFI) and Root Mean Square Error of Approximation (RMSEA). RMSEA is expected to be as close as possible to 0, with a value around 0.05 considered as good, while NNFI and CFI are expected to be as close as possible to 1 with values around 0.90 considered as acceptable. The chi-square statistic is also reported as a relevant index but not used to perform a formal test since models are usually rejected (25).

3. Results

Data for 75 children (thirty-five girls), for whom all data was complete were taken into account in the present analysis. Children's mean age was 9.4 ± 0.1 years (range 8 to 11 years), with a mean BMI of 17.1 ± 0.3 kg/m² and a mean z-BMI of 0.49 ± 0.15 SD (range -2.28 to 4.23 SD). Of these children, 11 % were considered overweight or obese (z-BMI > +2 SDs) (26). Three hundred and forty nine meals were recorded, involving 75 children who attended a maximum of 5 meals. One, 3, 17 and 54 children took part in 2, 3, 4, and 5 meals respectively.

Over the five lunches, total intake was 367 ± 9 g (range 137 to 520 g) and total energy intake was 412 ± 9 kcal (range 172 to 570 kcal) or 1723 ± 38 kJ (range 720 to 2384 kJ). A paired *t* test showed that over the five lunches green bean intake (75 ± 4 g ; range 4 to 160 g) was lower than pasta intake (136 ± 5 g ; range 61 to 200 g) (t_{74} = 10.48 ; P<0.0001).

Univariate analysis: Impact of salt content on intake. A significant food x salt content interaction effect on food intake was found (F (2, 271) =15.24; P<0.0001). Further analyses were run separately for each target food. Salt content impacted green bean and pasta intake significantly (respectively F (2, 137) =5.65; P<0.0045 and F (2, 134) =12.26; P<0.0001) and differently, as unsalted green bean was less consumed than the 0.6 % added salt variant, whereas the 1.2 % added salt pasta was more consumed than the 0.6 % added salt variant (**Figure 1**). Green bean intake was not related to z-BMI, whereas pasta intake was higher when z-BMI was higher (respectively F (2, 137) =0.08; P=0.77 and

F (2, 134) =18.21; *P*<0.0001): for an increase of 1 SD of z-BMI, pasta intake increased by 15g.

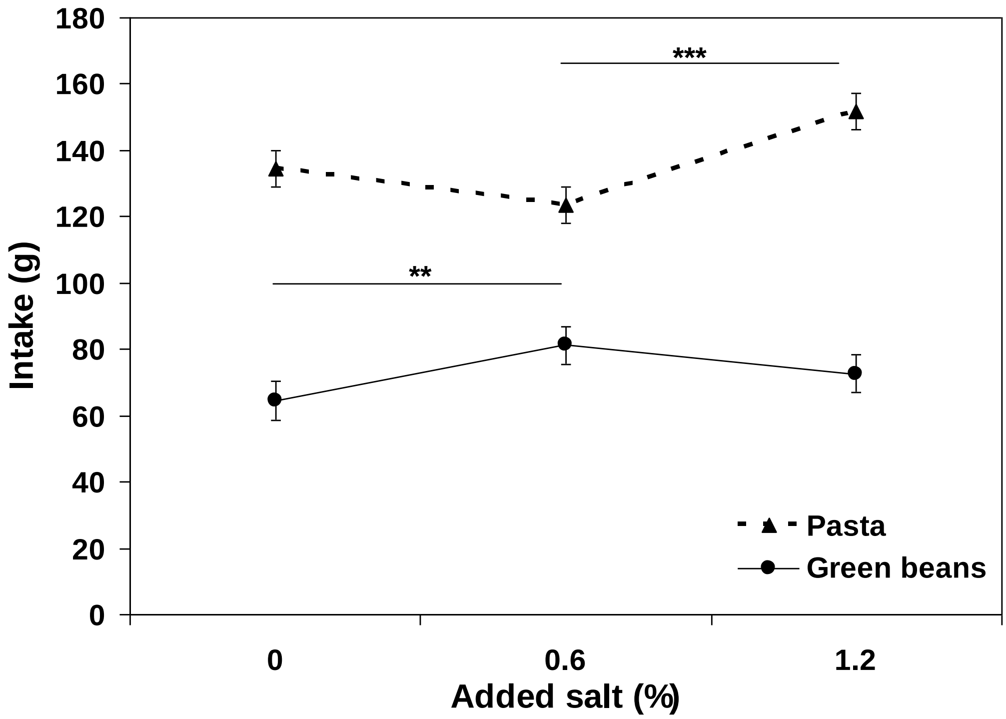

Figure 1. Impact of added salt content (0, 0.6 or 1.2%) on green bean and pasta intake in 8-11 year-old children (least squares means with SEM). The 0 and 1.2 % salt contents were compared to the 0.6 % level with a Dunnett test: ** *P*<0.01 and *** *P* <0.001.

Univariate analysis: Impact of salt content on liking and saltiness. For liking data, the food effect (*F* (1, 74)=2.79; *P*=0.10) and the interaction between food and salt content (*F* (2, 296)=1.17; *P*=0.31) were not significant. The food factor was removed from the model and only the salt content factor was taken into account: the 0.6 and 1.2% added salt variants were more liked than the unsalted one (*F* (2, 298) =32.32; *P*<0.0001; **Figure 2A**). In the initial model, the effect of the interaction between food and z-BMI on liking was significant (*F* (1, 292) =4.52; *P*<0.035); but in separate models for each food, the effect of z-BMI on liking was not significant (*F* (1, 136) =1.22; *P*=0.27 for green beans and *F* (1, 156) =0.27; *P*=0.60 for pasta).

For ranking data, the salt content factor was significant: for both target foods the 0.6 and 1.2% added salt variants were preferred to the unsalted one (F (2, 298) =53.48; P<0.0001). Mean rankings for the 0, 0.6 and 1.2 % added salt green beans were -0.61, 0.39 and 0.23 respectively (with scores varying from -1 to 1); whereas for pasta, mean rankings were -0.60, 0.24 and 0.36 for the 0, 0.6 and 1.2 % added salt variants respectively. When asked to rank their perceived saltiness, children were able to distinguish the three variants for both foods (F (2, 298) =328.86; P<0.0001; **Figure 2B**).

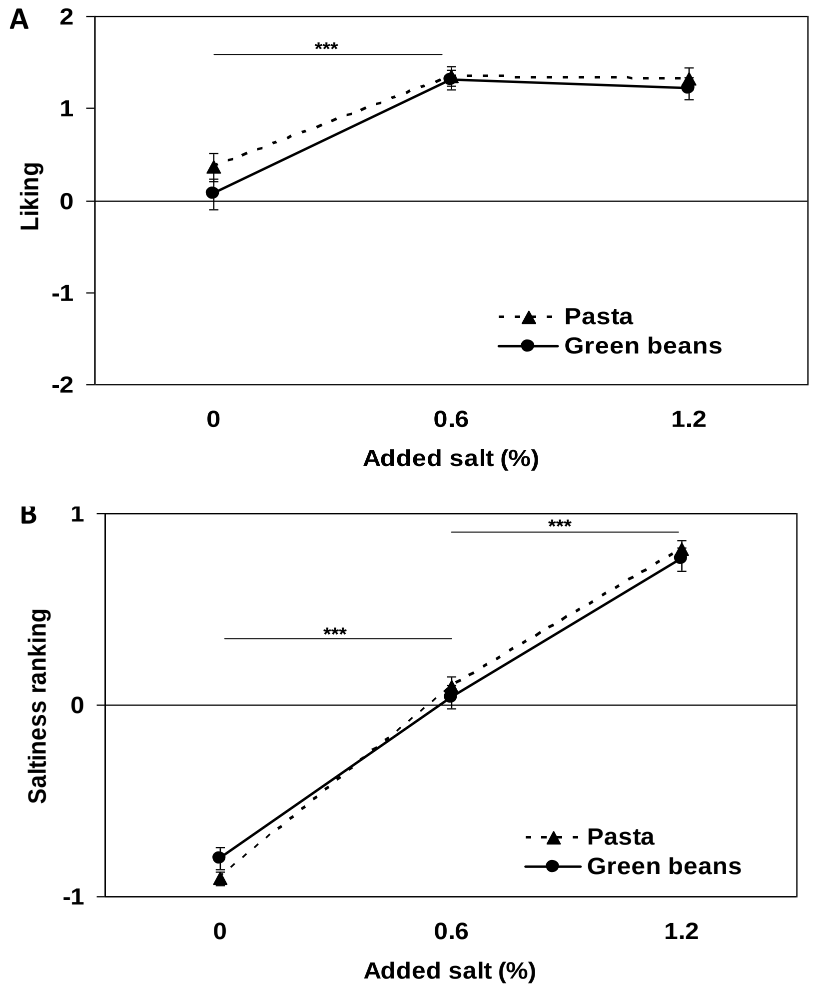

Figure 2. Impact of added salt content (0, 0.6 or 1.2%) on green bean and pasta sensory scores, in 8-11 year-old children. Least squares means with SEM for (a) liking (from ‘very bad’, -2, to ‘very good’, 2) and (b) saltiness ranking (from ‘least salted’, -1, to ‘more salted’, 1). The 0 and 1.2 % salt contents were compared to the 0.6 % level with a Dunnett test: ** P<0.01 and *** P <0.001.

Structural Equation Modeling: relationship between salt content, liking and intake. SEM was used to test the theoretical model that salt content influences intake through its impact on preferences. The final model is represented in **Figure 3**. All fit indices suggested a good fit. The model explained 21 % and 26 % of the variance for green beans and pasta intake respectively. The results were the same without the equality constraints between green bean and pasta parameters.

The primary determinant of intake was the latent variable 'hunger' (r = 0.46), followed by the overall liking (r = 0.18), and by the relative liking (r = 0.10) which was related to salt content, for both green beans and pasta. The z-BMI impacted pasta intake: the higher the z-BMI, the higher the pasta intake.

Bootstrap resampling showed that all parameters were significant (P = 0.05), but the z-BMI effect on overall pasta liking (P = 0.10) suggested by modification indices.

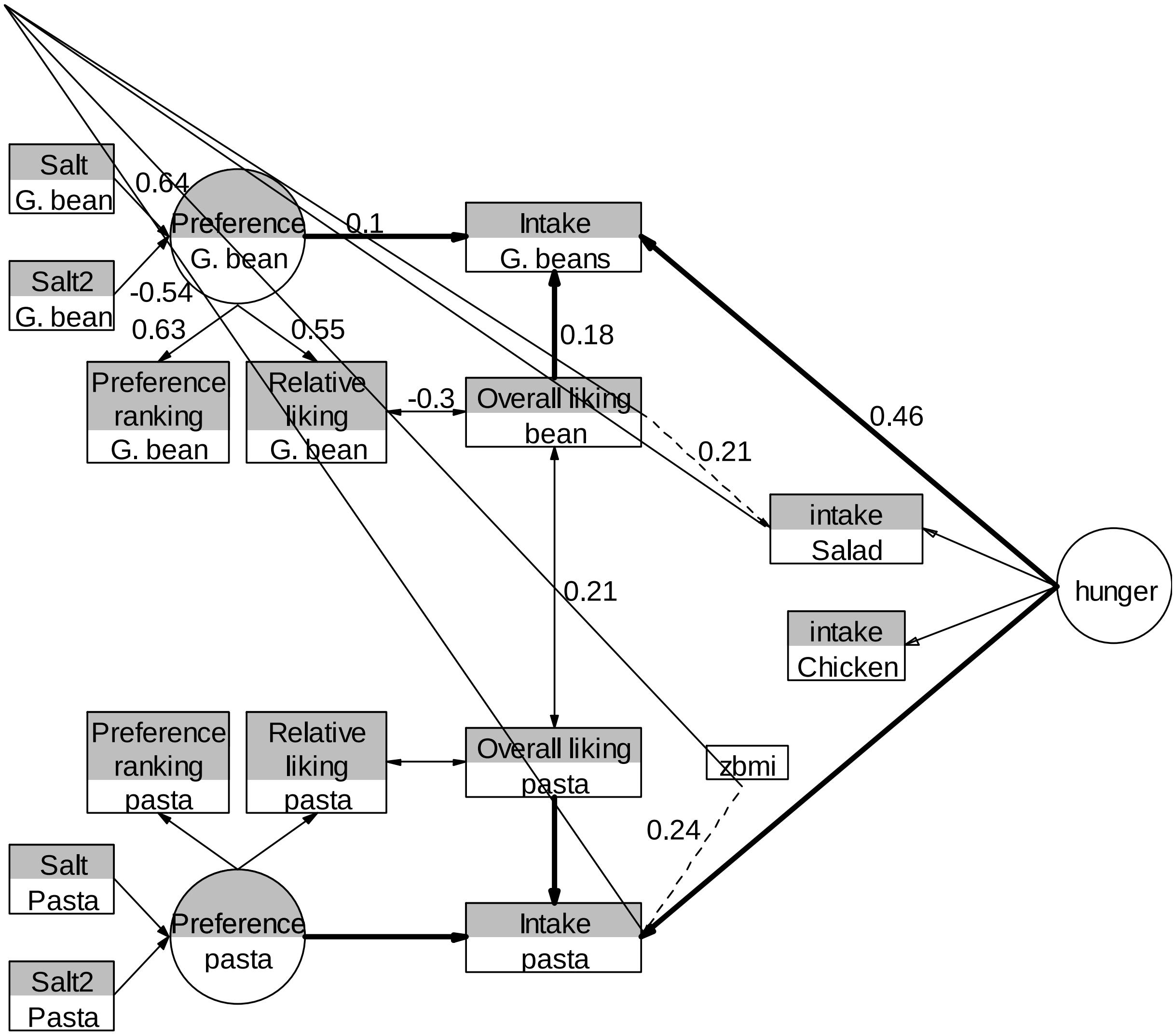

Figure 3. Structural Equation Model illustrating relationships between salt content, green bean and pasta intakes, liking, hunger and z-BMI. The effects concerning green beans are set equal to those concerning pasta and are reported for green beans only. The hunger effect on green beans, pasta, salad and chicken intakes were set to the same value (reported only once). Variables in squares represent measured/observed variables and those in circles represent latent variables. Dotted lines represent effects suggested by the modification indices and solid lines represent assumed effects. Numerical values next to arrows represent path coefficients, significant at $P<0.05$. All fit indices suggest a good fit quality: model chi square=135, df=85, p=0.0005, Tucker-Lewis NNFI=0.93, Bentler CFI=0.95, RMSEA=0.04 with 90% confidence interval [0.03, 0.06].

4. Discussion

The present study aimed at evaluating the impact of salt on children's food intake, taking into account the role of preference. It showed a significant positive effect of salt content on the intake of green bean and pasta, with a different impact for these two foods: a reduction of salt content in green beans decreased their intake by 20.6%, whereas an increase of salt in pasta increased its intake by 23.6%. Moreover, SEM modeling showed as expected that salt modulated food intake through a modification of preference and allowed prioritizing factors affecting target food intake: 1) child's hunger, 2) liking of the food regardless of its salt content and 3) liking for a particular salt content in the food.

In this study, we investigated the effect of salt content on sensory perception (saltiness, preference and liking) and, in parallel, on intake in real life conditions. To date, few studies have compared sensory evaluation and intake data, using the same food varying in its sensory attributes, and with the same subjects. Bellisle and Lucas compared in adults sensory evaluation data and intake of yogurt varying in sugar content and of mashed potato varying in salt content (13, 27). These experiments show that the optimal content for an ingredient in a given food can be over-estimated if only results from sensory evaluation are considered. We drew the same conclusion with children. The minimal salt content to optimize liking scores and preference ranking would be 0.6% added salt for both foods. Considering intake, it seemed possible to suppress salt in pasta as the 0% added salt variant was consumed as much as the 0.6% added salt variant and in a large quantity (134 g). For green beans, further research may explore the impact of a reduction in salt content below the 0.6% added salt content. It may be possible to reveal an intermediate content between 0% and 0.6% added salt which would not impact intake negatively.

Previous studies showed that the perception and acceptability of saltiness might be product-specific (9-11, 28) and that the preference for salty taste cannot be generalized to all foods (10, 29). The present results from intake data are in accordance with these previous findings. This different impact of salt content variation according to the food could be due to a different impact of salt content on the food sensory characteristics (30). For the present foods, measures of taste intensities obtained with a trained panel reported elsewhere revealed that salt content affected saltiness intensity similarly in both foods, but affected other sensory characteristics in green beans only (8). Unsalted green beans were perceived sourer and less fatty than the two other variants. These differences could have negatively impacted green bean intake. One may also suppose that an impairment of the sensory characteristics is more detrimental in a food which is not highly consumed than in a largely consumed food. Altogether, these results suggest possibilities for nutritional improvements concerning salt content in particular in salty foods targeted at children which are easily accepted (e.g., chips, crackers…).

The Structural Equation Modeling method was used to understand better the effect of salt content on intake by providing evidence that it was mediated by preference. This method allowed to take into account and to prioritize each factor affecting green bean and pasta intake: 1) the child’s hunger, 2) his/her overall liking of the food regardless of its salt content and 3) his/her liking for a specific salt content in the given food. In particular, using a model integrating intake data from the whole meal (and not only from the target foods) made it possible to capture the ‘hunger’ effect since intake may vary within and especially between children (31). The present study indeed revealed a wide range of mean intake at individual level over the five meals, from 137 to 520 g. Here, the SEM model could explain 21 % and 26 % of the variance in intake for green beans and for pasta respectively. Hayes and colleagues (9) also used the SEM method and found liking for a series of salted chicken broth to explain 18% of the variance in the intake of high sodium foods (food frequency survey), which is in the same range as the values obtained in the present experiment. This modeling approach help to better understand the significant positive role of salt content among other factors influencing intake.

We showed that z-BMI was specifically related to pasta intake and not to 'hunger' as hypothesized in the initial model. This result should be confirmed with a new independent dataset in order to reach a definitive conclusion. Nevertheless, this effect is in accordance with previous observation in 2-3 year-old children, showing that heavier children tend to consume more pasta (8). It might be supposed that heavier children seek energy-dense foods: the greater the BMI, the greater the intake in order to satisfy energy needs (15).

The results of the present study should be interpreted in the light of its strengths and limitations. The study used familiar foods and took place in the children's usual eating context, with their classmates and caregivers. This context was maintained constant over the study duration. Moreover, intake and liking data were collected from the same group of children. One limitation is related to the fact that it is difficult to generalize the present findings to other foods (other vegetables, other starchy foods or other salty foods), since the salt content effect appears to be food specific. To further understand the role of salt content, it would be particularly interesting to study the impact of low salt contents (i.e. between 0 and 0.6 %) on intake for green beans or for other vegetables, in order to maximize intake while maintaining palatability with a low salt content. The findings of the present study were obtained with a group of children from middle to higher SES and may reflect specific habits of salt use. Therefore it is difficult to generalize them to children from other social background. However, the present findings are consistent with results obtained on younger French children representing a wider range of SES (8).

5. Conclusions

In conclusion, this study showed that in school-age children, suppressing salt decreased green bean intake while increasing salt content increased pasta intake. It also revealed that considering preference to determine an optimal salt content can lead to contents beyond what is necessary for the food to be consumed, in particular for foods which are highly or easily consumed by children such as pasta. Nutritional recommendations urge reduce salt content in food, but salt is important for maintaining food palatability, in particular for vegetables as it could reduce perceived bitterness and/or sourness. Hence, it seems important to adapt the recommendations to the type of foods. Nevertheless, it appears that moderate salt contents are sufficient even in less palatable foods such as vegetables, and could easily be reduced below the current content in highly palatable foods. The present study highlights that in the current context where much of the foods eaten by children are industrially processed and designed to be highly palatable, there are possibilities for nutritional improvement concerning salt content reduction.

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Chapter 4. 'Just a pinch of salt': conditioning vegetable acceptance in toddlers with salt or spice

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In preparation for *Obesity*

The research leading to these results has received funding from the European Community'sSeventh Framework Programme (FP7/2007-2013) under the grant agreement n°245012-HabEat.

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Abstract

Background: Children's vegetable intake is far below the recommended amounts.

Objective: Comparing mere exposure to flavor-flavor learning mechanism, on the increase in acceptance (liking and intake) of a non-familiar vegetable; two unconditioned stimuli were used: salt or a salt-associated spice.

Methods: Toddlers attending six nurseries were allocated to 3 groups: a mere exposure group (ME; 0.2% added salt; n=47), a flavor-flavor learning (FFL) group with salt as an unconditioned stimulus (FFL-Salt, 0.5% added salt; n=54) and a flavor-flavor learning group with a salt-associated spice (nutmeg) as an unconditioned stimulus (FFL-Nutmeg, 0.2% added salt + 0.02% added nutmeg; n=50). A target vegetable (salsify) and a control vegetable (carrots, served at pre- and post-exposure) were used. Toddlers' liking and intake were recorded at pre-, at post-exposure and also across 8 exposures (twice a week for 4 weeks).

Results: All groups increased their intake of the target vegetable from the pre- to the post-exposure, by 64±11 g, 23±11 g and 36±11 g respectively for the ME, FFL-Salt and FFL-Nutmeg groups. This increase in intake was significantly higher in the ME group than in the two FFL groups; no difference in liking was found between the groups from the pre- to the post-exposure. Compared to the ME group which liking and intake increased linearly across the 8 exposures, liking increased linearly for both the FFL-Salt and the FFL-Nutmeg groups, whereas intake followed an inverted U-shape and a U-shape respectively.

Conclusion: The present study revealed that flavor-flavor learning -with salt or a salt-associated spice- was not more efficient than mere exposure to increase the acceptance of a vegetable. Moreover, this study also revealed that presenting vegetables as a starter would be an efficient strategy to make toddlers eat them. In sum, when vegetable are presented as a starter, mere exposure appears to be the wisest choice to increase liking and intake.

Key words: Toddlers; mere exposure; flavor-flavor learning; salt; spice

Introduction

The beneficial health effects of vegetable consumption are well established, and the WHO/FAO report recommends intake to be above 400 g per day and per person [1]. However, vegetable intake is low especially among children. The recent French National Individual Dietary Consumption survey (INCA2 study; 2006-07), shows that the mean daily intake of vegetables -excluding potatoes- reaches only 78 g in children aged 3-14 years [2]. Increasing vegetable intake, in particular in children, is one of the priority nutritional objectives of the French National Nutrition and Health Program ('Programme National Nutrition Santé' or 'PNNS') which has been implemented by the ministry of Health since 2001 [3]. This low consumption among young children, may be due to the presence of aversive tastes, in particular bitterness, and/or to the low energy density they provide [4-6].

With regard to the importance of early childhood in the development of long lasting food habits [7], increasing vegetable intake in early childhood is a matter of preoccupation, and investigators are looking for factors and ways to positively influence children's vegetable liking and intake. Three mechanisms were described in order to explain learning effects in the food domain, that may be applied to increase children's vegetable liking and by extension intake. The first mechanism, "mere exposure" (ME), suggested that the 'mere exposure of the individual to a stimulus enhances his attitude toward it' [8], or as specified by Pliner [9], the 'affective response to a stimulus increases with increasing exposure'. The other two mechanisms, flavor-flavor learning (FFL) and flavor-nutrient learning (FNL) are forms of 'Pavlovian conditioning', and were first described for their importance in acquiring emotional response in animals, and further applied to studies in humans [10]. These associative-conditioning learning are based on pairing a conditioned stimulus (CS) to an unconditioned one (US), inducing a positive shift in affect. The principle is that the positive affective value for the CS brought by the US remains even when the US is removed. In the food domain, the CS is generally an initially novel or neutral flavor/food and the US is a stimulus that triggers a positive response with biological robustness. In the case of FFL the US is a liked flavor; in the case of FNL, the US has positive post-ingestive consequences.

Experiments conducted with 4-to-7-month-old infants revealed the efficiency of mere exposure (ME), for increasing the intake of a novel [11] or an initially disliked vegetable [12]. In older children, ME to vegetables proved its efficiency to increase intake and liking after 14 and 8 exposures, respectively in 2-6 and 5-7 year-old children [13, 14]. A study showed that after 8 exposures, infants aged 4-8 months displayed less negative facial expressions (indicating less dislike) when tasting green beans, if they had been previously exposed to green beans followed one hour later by a peach puree, than if they had been exposed to green beans alone [15]. This suggests a possible association between the sweet taste of peaches and green beans, leading to an increase in liking of green beans, thus a possible FFL effect. In this line, a FFL experiment conducted with 4-6 year-old children associated the sweet taste of dextrose to the taste of a vegetable ranked as ‘just ok’. After 3 days of exposure, a positive shift in preference for the plain taste of the exposed vegetable was observed; intake was not recorded [16]. Both ME and FFL appear to be effective in order to overcome reluctance to eat vegetables. Thus, the present work aimed at comparing the efficiency of both mechanisms in toddlers.

In the case of FFL the majority of studies in children and in adults focusing on vegetables, used a pairing with sweet taste [16, 17]. However, this practice is not usual in France, where mothers add salt, spices or seasonings other than sugar to vegetable preparations for their children, as soon as weaning foods are introduced [18], in limited amounts [19]. A recent study described the sensory characteristics as well as the acceptance of the first foods introduced to infants aged 5-7 months during complementary feeding in France. The results show a greater acceptance of vegetables when salt or salty ingredients were added, compared to plain versions which were perceived as more bitter [20]. Moreover, moderate salt addition in toddlers’ green beans during lunches served in nurseries resulted in an increase in intake [21]. Children are exposed to salty foods early in life, and salt appears to be associated to a higher acceptance of vegetables. Thus, salt could be useful to increase vegetable acceptance, but yet, it has never been used as an US in FFL studies.

With regard to the FFL principle, salt as a US would be used only during the conditioning phase and would be removed afterwards. However, adding salt to vegetables to promote their acceptance could be problematic: repeated exposure to salt might shape preferences toward salty foods [22] and it is known that salt has adverse health effects on the long term. Therefore, different strategies are investigated in order to reduce salt content in foods: the use of salt substitutes (e.g. KCl), but these might be too bitter or too sour tasting for children [23]; the gradual and moderate adaptation to lower sodium levels in food through repeated exposure [24]; the use of an odorant/aroma associated to salty taste in a slightly salted stimulus. This was seen to enhance adults’ perceived saltiness in solutions and in solid foods [25, 26]. This multisensory integration approach, using salt-associated aroma or spices, could be a safe and healthy alternative for salt reduction in children’s food. Thus, it seems worthy to investigate such a strategy to replace part of the salt by a ‘salty-tasting’ spice in a flavor-flavor conditioning, to condition the acceptance of vegetables in toddlers.

The present work aims at comparing the effect of mere exposure to that of flavor-flavor learning mechanism, on the acceptance of a non-familiar vegetable; two unconditioned stimuli were used: salt a lower content of salt with a salt-associated spice.

Participants and methods

Overview. In a between-subject design, one hundred and fifty-one toddlers aged 2-3 years, and attending one of six nurseries in Dijon (France), were assigned to one of three groups of learning (ME, FFL with salt only or FFL with salt and spice). The experiment took place at the toddlers’ usual nursery, two times a week, during a six week period. A vegetable puree was presented warm as a starter at the beginning of the toddlers’ lunch. The week before and the week after the exposure period, pre- and post-tests were conducted with the target vegetable puree and a control vegetable puree. The experimental design is presented in **figure 1**. Acceptance was measured through liking and intake at each eating occasion. Parents signed a written informed consent on behalf of their toddler and received a 10€ voucher for compensating their time to fill in a questionnaire. Each child’s weight and height were reported by the parents according to his/her health records, and z-scores for body mass index (z-BMI) were calculated using the French references [27]. The study took place at the toddlers’ usual nursery from November to December 2010 (figure 1), at the usual lunch time for each nursery (between 11:30 am and 12:00 am). The nurseries and staff were given a present to thank them for their hospitality. The study protocol was approved by the local ethical committee (Comité de Protection des Personnes Est I Bourgogne). The parents, toddlers and nursery staff were blind to the treatment group each nursery was assigned to.

	week 1		week 2		week 3		week 4		week 5		week 6	
			1	2	3	4	5	6	7	8		
Group	Pre-exposure		Exposure phase								Post-exposure	
ME	ME-Salsify	Carrot	ME-Salsify	ME-Salsify	ME-Salsify	ME-Salsify	ME-Salsify	ME-Salsify	ME-Salsify	ME-Salsify	ME-Salsify	Carrot
FFL-S	ME-Salsify	Carrot	FFL-Salt	FFL-Salt	FFL-Salt	FFL-Salt	FFL-Salt	FFL-Salt	FFL-Salt	FFL-Salt	ME-Salsify	Carrot
FFL-N	ME-Salsify	Carrot	FFL-Nutmeg	FFL-Nutmeg	FFL-Nutmeg	FFL-Nutmeg	FFL-Nutmeg	FFL-Nutmeg	FFL-Nutmeg	FFL-Nutmeg	ME-Salsify	Carrot
	Counterbalanced										Counterbalanced	

Figure 1. The experimental design for the three groups of 2-3 year-olds participating in the conditioning study in their usual nursery. At the pre- and the post-exposure, all groups were served the salsify puree following the ME recipe (0.2% salt) and a carrot puree (0.2% salt) in a counterbalanced order. Toddlers were exposed 8 times to either the same salsify puree following the ME recipe (ME, 0.2% salt; n=47), or to the puree prepared with a moderate quantity of salt (FFL-Salt, 0.5% salt; n=54) or to the puree prepared with salt and nutmeg (FFL-Nutmeg, 0.2% salt + 0.02% Nutmeg; n=50).

Study foods.

a) Vegetable and seasoning choices. The target vegetable had to have a low consumption rate and to be hedonically neutral to toddlers. Another criterion was the ability to be supplied in large quantities, and to be appropriate for production constraints. The target flavor/spice had to be known, already tasted and fed to toddlers. Given these constraints, and in order to choose the most appropriate vegetable and spice for conducting the experiment, a questionnaire was designed to investigate frequency of consumption and liking (5-point scale from -2 to +2), for a list of 56 vegetables and of 35 spices/seasonings. It was delivered in nurseries in Dijon, to 229 parents of children from weaning to 42 months, not participating in the main experiment. Seventy-eight questionnaires were returned, from which 33 concerned children 2 years and over. The analysis of the questionnaire revealed that vegetables with low consumption and disliked (-1) to neutral (0) scores of liking were:

chards, Brussels sprouts, salsify, salsify, green/white/red cabbage, green/red pepper and Jerusalem artichoke. Besides, the most common and already tasted spices were: cumin, curry, nutmeg, and ginger. Given the production constraints, the choice was possible between Brussels sprouts or salsify. While the mean (SD) liking score for salsify was neutral (0), that of Brussels sprout was slightly negative (–0.08±1.08). Thus, in order to avoid a rejection by toddlers due to the strong taste of Brussels sprout [28], the choice was taken to use salsify. Two spices suitable to be mixed with salsify, in terms of congruence and taste, were possible: nutmeg and ginger. To choose between both spices a pre-test was conducted as described below. Moreover, the control vegetable chosen for this study was carrots, as it was the most frequently proposed (1 to 3 times a week to 73 % of the toddlers) and liked (1.26 +/- 0.86; mean +/- SD) vegetable by toddlers.

b) Pre-test with adults for spice selection. Informal tastings with few members of the research team were conducted in order to choose the appropriate salt and spice contents to be used. Several contents of salt (0.2, 0.3, 0.4 and 0.5%) were considered for the ME and for the FFL-salt variants. We decided to use 0.5 % added salt in the FFL-salt recipe, as it represents the upper limit recommended in Europe as a final target content for manufactured baby foods [29], and in France for food caterings targeting 0-3 year-olds [30]. From this level, slight decreases were tested to reach the content of 0.2% for the ME variant; an unsalted version was not considered, as a previous study with toddlers revealed its negative effect on vegetable intake [21]. Several contents of spices (0.01, 0.02 and 0.08%) were considered, based on those usually used in manufactured baby foods; after tastings, decision was made to use 0.02%. In order to choose the appropriate spice eliciting the most salty sensation, a sensory session was organized. Students and staff of the Centre des Sciences du

Goût et de l'Alimentation (n=18) were recruited to participate in a sensory evaluation session. All foods were prepared at the laboratory. Unseasoned frozen salsify (Picard®, France) was boiled for 14 min and mashed. Three samples of pureed salsify were prepared: puree + 0.2% salt (S), puree + 0.2% salt + 0.02% ginger (G), or puree + 0.2% salt + 0.02% nutmeg (N). The samples were maintained warm (45 °C) and 50 g were served in 125 ml isotherm covered cups. The participants had to taste the samples and answer three questions: 1) '*describe all it evokes to you*', 2) '*give your perception of its salty taste intensity, from absence (0) to strong perception (9)*' and 3) '*rank the three samples from the most salty to the least salty'.* Mean ±SEM saltiness intensity values were 1.83 ±0.28, 3.22±0.37 and 4.00±0.53 respectively for the samples with salt (S), ginger (G) and nutmeg (N). Both samples with ginger and nutmeg were perceived as saltier than the salt-alone sample (F=16.66; P <0.0001). Saltiness ranking (1=most salty; 3=least salty) was 2.56 ±0.70, 1.83±0.71 and 1.50±0.71 respectively for the S, G and N samples. Both samples with ginger and nutmeg were ranked saltier than the salt-alone sample (F=12.64; P <0.0001). The nutmeg sample was ranked first by 11/18 participants, and the ginger sample by 6/18 participants. Besides, nutmeg was more frequently added to toddlers' foods (48%) than was ginger (21%); thus, nutmeg was chosen to conduct the study.

c) Recipe development and vegetable production. The final recipes were: ME, 0.2% salt; FFL-Salt with 0.5% added salt; and FFL-Nutmeg with, 0.2% added salt + 0.02% added nutmeg. Plain frozen salsify was supplied by the Bonduelle group (Lille-Villeneuve d'Ascq Cedex, France). The recipes (**Table 1**) were developed in our laboratory, as previously described, and the production took place in a food factory (FreshInov, Courmelles, France), accredited to prepare baby foods using appropriate ingredients and processes following baby food industry regulations. The three recipes of pureed salsify were conditioned in a 100 ± 2 g jar with lid and frozen at -20°C until further use. For the three recipes, bacteriological analyses were conducted by the departmental laboratory of analysis and research (Barenton-Bugny, Laon Cedex, France). The nutritional composition was determined by a certified laboratory (INZO, Château Thierry Cedex, France). Unseasoned frozen carrots, used as the control vegetable (Picard®, France) were prepared at the laboratory following the recipe of the ME salsify sample.

Table 1. Recipes (a) and nutritional composition (b) of the three studied pureed salsify.

	ME	**FFL-Salt**	**FFL-Nutmeg**
Recipe (g / 100g)			
Salsify	99.0	98.7	98.98
Salt	0.2	0.5	0.2
Spice	0.0	0.0	0.02
Oil	0.8	0.8	0.8
Nutritional composition (/ 100g)			
Sodium (mg)	88	213	89
Proteins (g)	1.39	1.37	1.38
Total carbohydrates (g)	13.4	13.8	13.7
Total lipids (g)	0.3	0.9	0.2
Energy (kcal)	62	69	62

Sensory description by the trained panel. A sensory description of all study samples (carrots, ME, FFL-Salt and FFL-Nutmeg salsify purees) was conducted by the trained panel of the laboratory, composed of 8 adults aged between 27 and 66 years. They received more than 40 sessions (1-to-1h30) of training on taste description and on the way to use the scoring scales. The references of the *Spectrum*™ intensity scales for descriptive analysis were used to rate the intensity of sweet, salty, sour and bitter tastes [31]. Another ‘Spectrum-like’ scale was developed for fattiness perception. The panelists were asked to score the perceived intensities of each variant monadically on a linear scale, from ‘not perceived’ (left anchor, converted into 0) to ‘very intense’ (right anchor, converted into 10) using the FIZZ software (Biosystèmes, Couternon, France) . Two replications were performed.

Subjects and procedure. The study took place at the toddlers’ usual nursery (November to December 2010; figure 1), at the usual lunch time for each nursery (between 11:30 am and 12:00 am). Experimenters went to each location to get familiarized with the toddlers and the staff before the beginning of the study. Besides, the week before the pre-exposure (October 2010), two training days were organized in each nursery in order for children and staff to get used to the study procedure. From the initial sample of 171 children, 14 (8%) were not enrolled as their parents refused their child’s participation due to allergy suspicions or for other unreported reasons, one left the nursery during the study, and five did not attend the observation days. Thus, a final sample of 151 toddlers was considered for the present analyses. Three groups were set: a mere exposure group (ME; $n_{initial}$=50; n_{final}=47), a flavor-flavor learning group with salt as US (FFL-Salt; $n_{initial}$=64; n_{final}=54) and a flavor-flavor learning group with nutmeg as US (FFL-Nutmeg; $n_{initial}$=57; n_{final}=50). Each group’s final characteristics are depicted in **table 2**; the groups did not differ according to gender [χ^2=2.20; P=0.33], to age (F=0.44; P =0.64), to BMI (F=2.17; P =0.11), or to z-BMI (F =1.67; P=0.19).

Each experimental group was conducted in at least three out of the six nurseries (ME in 3 nurseries; FFL-Salt in 4 nurseries and FFL-Nutmeg in 4 nurseries). Three nurseries were run on Mondays and Thursdays and the three other nurseries were run on Tuesdays and Fridays.

Table 2. The characteristics of toddlers included in the three learning groups.

	ME	**FFL-Salt**	**FFL-Nutmeg**
N	*47*	*54*	*50*
Girls : Boys	17 : 30	27 : 27	24 : 26
Age (months)	27.3 ± 4.7	27.4 ± 3.7	26.7 ± 3.3
BMI	15.3 ± 3.3	16.3 ± 1.4	16.2 ± 1.1
Z-BMI	-0.66 ± 1.4	-0.18 ± 1.1	-0.25 ± 0.9

The sample jars were defrosted and heated in each nursery kitchen and the temperature was recorded (> 63°C when out of the oven or microwave). Each jar was weighed in the kitchen, labeled with the name of a child and served at approximately 45 °C. When all children were sitting, the corresponding jar with the child’s name was given to him/her. Two jars of 100 g of puree each were available per child on each eating occasion. When children stopped eating the purée, jars were removed and post-weighed in the nursery kitchen, out of the toddlers’ sight. So, the amount consumed (g) by each child was calculated. Toddlers were then served their usual lunch without the starter (entrée, dairy product/dessert, bread and water). Besides, as each caregiver was in charge of looking after a small group of 5 to 8 toddlers, and due to their good knowledge of each child’s usual reactions towards foods compared to the experimenters, they were asked to rate how much they thought each child liked the puree while s/he was eating. They used a 5-point scale ranging from -2=’dislikes very much’ to 2=’likes very much’.

Statistical analyses. Statistical analyses were carried out using SAS System for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA). Significance was set at P <0.05, and at P <0.10 for tendency and will be reported as lsmeans ± SEM.

For the sensory description, an ANOVA was performed using SAS GLM procedure for each taste quality and for the 4 purees (carrots, ME, FFL-Salt and FFL-Nutmeg). The model was: taste quality = panelist + puree + panelist x puree + error; 'panelist' and the interaction 'panelist x puree' were considered as random effects.

Two series of analyses were conducted: firstly, analyses investigated the evolution of acceptance of the ME puree from the pre- to the post-exposure for all 3 groups; and secondly other analyses investigated the evolution of acceptance during the 8 days of exposure for each kind of puree.

For the first series of analyses, in order to evaluate the 'group' effect on learning, the evolution of acceptance *from the pre- to the post-exposure* [Δ(post-pre)] was calculated for liking and intake. The number of exposures each child had received during the exposure phase was calculated. It should have been equal to 8 for all toddlers, but due to absences, some toddlers did not receive 8 exposures; this was taken into account in the modeling. However, we checked that the average number of exposures did not differ (F =0.35; P =0.70) between the three groups: ME,6.6; FFL-Salt, 6.9 and FFL-Nutmeg 6.8. ANOVAs were performed using SAS GLM procedure, according to the model: Δ(post-pre) = group + number of exposures + group x number of exposures + error; this was performed for salsify and carrot. No significant interaction between group and number of exposures was found; this effect was removed from the final model. Another model taking into account the child's gender and the interaction between group and gender was tested; as no significant effect was observed either for the gender or for the interaction, these data are not reported further.

The second series of analyses were conducted in order to evaluate the evolution of acceptance of the salsify purees in each group *during the exposure* period. A mixed linear model (SAS Mixed procedure) was used, with “child effect” considered as random. For each group, the exposure number and its quadratic effect were fixed factors and the model considered was: intake (or liking) = exposure number + exposure number^2 + gender + error. Besides, interactions between gender and exposure number and exposure number^2 were tested for each group. Analyses were conducted with or without toddlers for whom Δ(post-pre) was not available (n=3, n=7 and n=7 respectively for the ME, FFL-Salt and FFL-Nutmeg group); no difference was observed, and data for all toddlers were considered for the present analyses.

Moreover, for each group, paired *t*-tests (*t* (df)) were used in order to determine whether the acceptance of the salsify was different between the pre-exposure and the 1st exposure, or between the 8th exposure and the post-exposure; thus allowing to capture the effect of changing the salsify recipe.

Results

Sensory description of the products. Sweetness intensity differed according to the sample tasted, $F(3, 32) = 25.74$; $P<0.0001$: the carrot puree was perceived sweeter (2.48 ±0.15) than the three salsify purees (1.01±0.15, 1.21±0.15 and 0.82±0.15 respectively for the ME, FFL-Salt and FFL-Nutmeg), which were not different from one another. Saltiness intensity also differed according to the sample ($F(3, 32) = 4.33$; $P= 0.01$), as the FFL-Salt was perceived saltier (2.57±0.20) than the carrot puree (1.72±0.20), the ME puree (1.64±0.20), and the FFL-Nutmeg puree (1.87±0.20), which were not different from one another.

No significant effect was observed either on sourness ($F(3, 32) = 0.73$; P=0.54) or on bitterness ($F(3, 32) = 1.97$; P=0.14), or on fattiness intensities ($F(3, 32) = 2.13$; P=0.13).

Pre- to post-exposure difference of acceptance. The mean increase in salsify intake across all groups was 41 g. As it is shown in **figure 2a**, all means were significantly different from zero: all learning groups increased their intake at the post-exposure compared to the pre-exposure. However, the group effect was significant ($F(2, 130)=4.03$; P=0.02: the increase in salsify intake of the ME group was higher (64±11 g) than that in the FFL-Salt group (23±11g; P=0.009), and tended to be higher than in the FFL-Nutmeg group (36±11g; P=0.07). No significant difference was observed between the FFL-Salt and the FFL-Nutmeg groups (P=0.42). Besides, the number of exposures tended to impact this increase in intake ($F(1, 130)=2.83$; P=0.09). Concerning the evolution of the carrot puree intake from the pre- to the post-exposure, the mean increase across all groups was 6 g and was not significantly different from zero. No significant group effect ($F(2, 111)=0.87$; P =0.42) or number of exposure effect were found ($F(1, 111)=20.48$; P =0.49).

Concerning liking, the mean increase of liking for the salsify puree across all groups was equal to 0.70. For the ME and the FFL-Salt groups, the mean increase in liking scores were significantly different from zero (ME: 1.05±0.24; $t\,(39)$ =4.42; P<0.0001; FFL-Salt: 0.69±0.24; $t\,(43)$ =2.92; P=0.005), but not for FFL-Nutmeg group (0.34±0.29; $t\,(35)$ =1.16; P =0.25) as it is shown in **figure 2b**. However, the groups did not differ from one another ($F(2, 113)=1.82$; P=0.16) for the increase in salsify liking. No significant number of exposure effect was found ($F(1, 113)=1.03$; P=0.31). Concerning carrot, the mean increase in liking across all groups was 0.22. The increase in liking of carrot puree tended to be different across groups

(F(2, 107)=2.56; *P*=0.08): it was higher in the FFL-Salt group (0.55±0.19; *P*=0.03) than in the ME and in the FFL- Nutmeg groups (0.05 ±0.22 and -0.06 ±0.21 respectively). The number of exposure had a tendency to impact this increase in carrot liking (F(1, 107)=2.89; *P* =0.09).

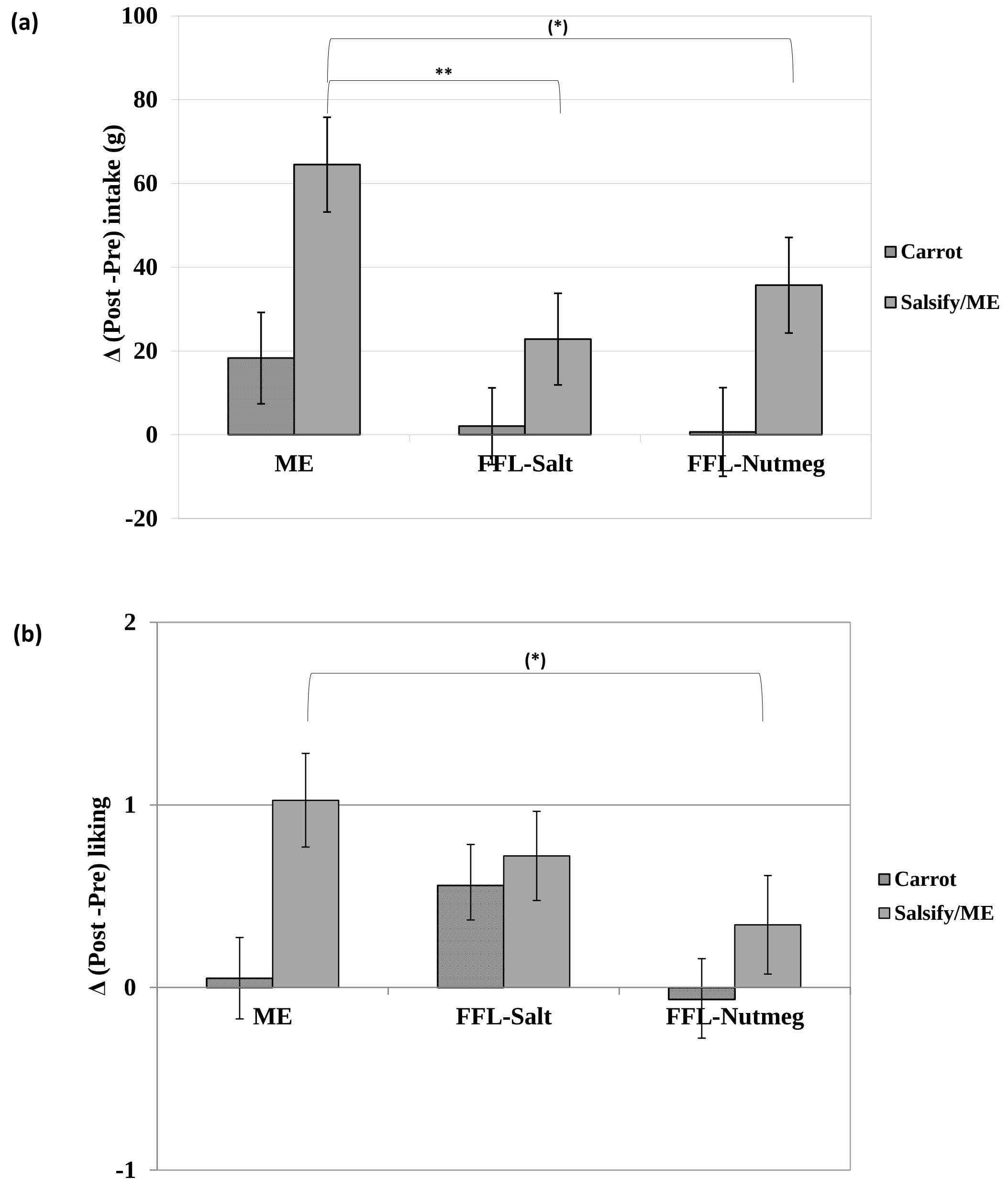

Figure 2. The evolution of intake of (a) and liking for (b) the target vegetable (salsify) and a control vegetable (carrot), assessed as the difference after (post) and before (pre) 8 exposures, for the three learning groups. Results are presented as lsmeans±SEM. (*) represents a tendency at *P* <0.10.

Evolution of acceptance during the exposure period. Concerning intake (**figure 3a**), the effect of exposure number for the ME group was significant (*P* =0.003) and the estimation was positive (4.34 ±1.48 g); the quadratic effect of the exposure number was not significant (*P* =0.46). Thus, the evolution of intake from the 1st to the 8th exposure for the ME group shows a linear increase over time, and toddlers in this group increased their intake by about 4 g per exposure. For the FFL-Salt group, the effect of exposure number was significant (*P* =0.05), its estimation was positive (8.8±4.6 g), and the quadratic effect of the exposure number was also significant (*P* =0.04) but its estimation was negative (-0.9±0.4 g). Thus, the evolution of intake from the 1st to the 8th exposure for the FFL-Salt group was a negative quadratic function over time, depicting an inverted U-shape. For the FFL-Nutmeg group, the effect of exposure number was significant (*P* =0.03), its estimation was negative (-9.9±4.6 g) and the quadratic effect of the exposure number was also significant (*P* =0.01) but its estimation was positive (1.2±0.5 g). Thus, the evolution of intake from the 1st to the 8th exposure for the FFL-Nutmeg group was a positive quadratic function over time, depicting a U-shape.

Concerning the evolution of liking (**figure 3b**), none of the quadratic effect was significant, and the evolution of liking from the 1st to the 8th exposure for all the groups shows a significant linear increase over time and did not differ between groups: for the ME group the estimation of the 0.073 ±0.03 (*P* =0.02), for the FFL-Salt group it was 0.075 ±0.03 (*P* =0.008) and for the FFL-Nutmeg group it was 0.06 ±0.03 (*P* =0.02).

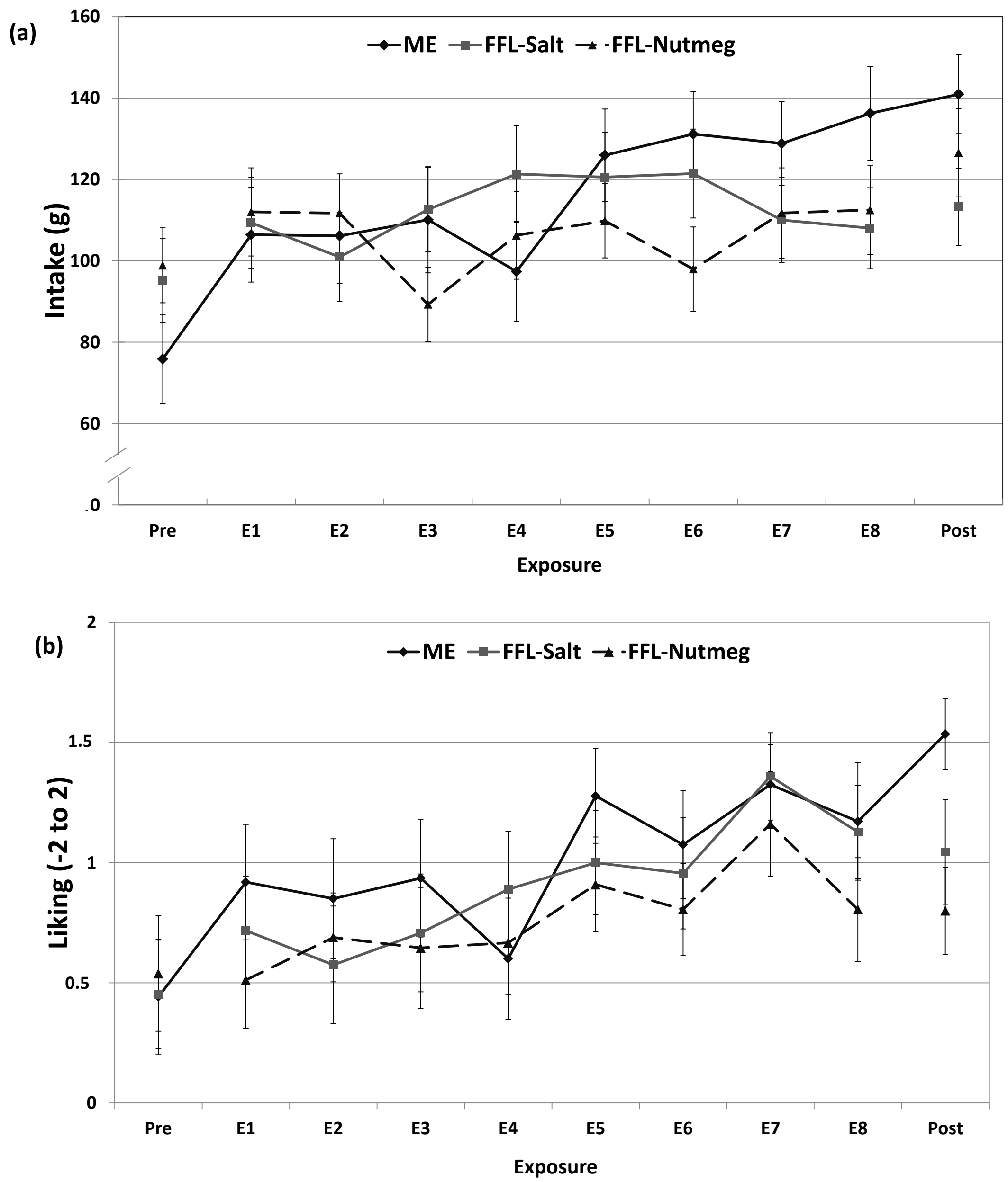

Figure 3. Toddlers’ intake of (a) and liking for (b) the target vegetable puree (salsify) at the pre-exposure, during the 8 exposure and at the post-exposure period. During exposure, toddlers were divided into three group and exposed to different recipes of salsify puree: for the mere exposure group (ME), the puree contained 0.2% salt; for the flavor-flavor learning group with salt (FFL-Salt) with salt it contained 0.5% and the flavor-flavor learning group with nutmeg (FFL-Nutmeg) in contained, 0.2% salt + 0.02% nutmeg. At pre- and post-exposure, all groups received the ME salsify puree. Results are presented as mean ±SEM.

Gender effect on each group’s acceptance during the exposure period. For the ME and the FFL-Nutmeg groups, no effect of gender was observed either on intake (*P*=0.67 and *P*=0.24) or on liking (*P*=0.93 and *P*=0.65). However, for the FFL-Salt group, a significant effect of gender was observed on intake (F(1, 248) =5.41; *P*=0.02), as boys consumed a significantly greater amount of the salsify puree with added salt than did girls (130 ±12 vs. 91±12 g respectively). The interactions between the linear, or the quadratic, effects of the exposure number and gender were not significant. Besides, caregivers had a tendency to score boys as liking the FFL-Salt puree more than girls (1.13 ±0.19 vs. 0.56±0.25 respectively; *P*=0.07); no interaction between the exposure number and gender was found.

Comparison of acceptance at the pre- and 1st exposure. A paired *t-test* compared the intake of salsify puree at the pre-exposure and at the 1st exposure, in particular to evaluate the effect of a change in recipe for the two FFL groups. For the ME group, toddlers significantly increased their intake at the 1st exposure by 25±9 g compared to the pre-exposure (*t* (37) =3.21; *P*=0.002). For the FFL-Salt group the intake at the 1st exposure tended to be higher than at pre-exposure (15±8 g; *t* (46) =1.89; *P*=0.06); this was also observed for the FFL-Nutmeg group (17±9 g; *t* (41) =1.90; *P*=0.06). Thus, changing the recipe from the ME to the FFL-Salt or the FFL-Nutmeg did not affect toddlers’ intake. Concerning liking, a paired *t-test* for the FFL-Salt group showed that liking at the 1st exposure tended to be higher than at the pre-exposure (0.32±0.17; *t* (43) =1.79; *P* =0.07); this was not observed either for the ME (*t* (33) =1.38; *P* =0.17) or for the FFL-Nutmeg group (*t* (34) =1.17; *P* =0.25).

Comparison of acceptance at the 8th and post-exposure. A paired *t-test* compared the intake of salsify puree at the 8th exposure to that of at the post-exposure. No significant effect was observed either for the ME group (t (36) =0.08; P =0.93), the FFL-Salt group (t (44) =0.95; P=0.34) or for the FFL-Nutmeg group (t (39) =0.88; P=0.38). Concerning liking, no significant effect was observed either for the ME group (t (36) =1.42; P=0.16), the FFL-Salt group (t (41) =0.10; P=0.92) or for the FFL-Nutmeg group (t (38) =0.33; P=0.74). Thus, liking and intake did not change when the US (salt or nutmeg) were removed.

Discussion

The present work aims at comparing the effect of mere exposure to that of flavor-flavor learning mechanism, on the acceptance (liking and intake) of a non-familiar vegetable; two unconditioned stimuli were used: salt or a salt-associated spice.

The main results show that learning occurred for all groups as they all increased their intake of the salsify puree from the pre- to the post-exposure. However, learned acceptance (liking and intake) was highest among the mere exposure group, exposed to the salsify with the lowest salt amount and without spice.

Concerning intake during the exposure period, the results can be summarized as follows: for the ME group's intake increased linearly all over the exposure period. For the FFL-salt group, after a first experience with the taste of the 'salty' recipe, the presence of salt increased the intake of the puree for the 3 following exposure occasions, but it seems that afterwards, the taste was not attractive anymore and intake decreased. Finally, for the FFL-Nutmeg group, after a period of a slight decrease, maybe reflecting an 'adaptation' or learning for the taste of nutmeg, the intake of salsify puree increased. Besides, liking increased linearly in all groups during the exposure period. However, the results of the FFL-Salt and FFL-Nutmeg groups highlight a discrepancy between liking and intake during the exposure period, as even if the shapes of intake were different (inverted U-shape and U-shape respectively), liking shows a linear increase over time.

An important highlight should be given to the high intake of the vegetables during this study, as the average individual intake was equivalent to 112 ±2 g. Many factors could explain this high intake. First, the experiment took place in the toddlers’ natural environment/usual nursery, at the time where they are used to have lunch, and with their usual caregiver and peers. Second, the target vegetable was provided as a starter, when children were the most hungry and willing to at least taste it. Third, the target vegetable was given in a pureed form, which might be a suitable form of preparation for this vegetable, as the texture was soft and easy to eat. Finally, salsify is not bitter, as reported by the trained panel, and that might have been of importance in driving toddlers’ intake.

Concerning the ME group where a slight quantity of salt (0.2%) was added to the salsify puree, toddlers significantly increased their intake of the mere salsify recipe by 64±11g from the pre- to the post-exposure. During the exposure phase, the increase in intake was linear, and was equivalent to a 4 additional grams of puree consumed per exposure. Besides, the significant increase in intake observed between the pre- and the 1st exposure was equivalent to 25 ±8 g. This is in line with previous findings of Birch and co-workers [32], who found much of 4-7 month-old infants’ increase in a target food’s intake (banana or peas), to occur between the pre- and the 1st exposure (from 35 to 61 g), compared to that observed across the 10 exposure days of the experiment (from 61 to 69 g).

Thus, also in our study, mere exposure was effective even after a single exposure to the target vegetable, and the increase was in the same amplitude as that observed across the whole exposure phase (27±14 g from the 1^{st} to the 8^{th} exposure).

Concerning the FFL-Salt group, toddlers significantly increased their intake of the mere salsify recipe by 23±11g from the pre- to the post-exposure. During the exposure phase, 0.5% of salt was added to the salsify puree: liking followed a linear increase, whereas intake across the 8 eating occasions followed an inverted U-shape. This result suggests that for such a FFL mechanism with salt as an US, fewer learning sessions may be necessary (e.g. 4 instead of 8). In order to explain the observed shape of intake during exposure, we could assume that the FFL-Salt variant might have been initially as appealing and palatable as the ME variant toddlers were exposed to at the pre-exposure. If we take into account children’s attraction for the salty taste in vegetables [21], we could hypothesize that the increase in salsify intake observed during the first exposures reflected toddlers’ attraction for its salty taste. However, the salty variant was not more consumed than the less salty variant. As the exposures ran, toddlers’ The shape of evolution in intake might be explained by an increased familiarity, followed by a decreased appeal when the product was nor arousing anymore [33], i.e. a boredom effect [34]. An approaching effect was observed in an experiment conducted with sweetened teas in adults: over exposure, subjects became more tired of optimally sweetened teas than of low sweet ones [35]. Alternatively, as it was shown that salt enhances overall food flavors [36], salt may have enhanced flavors of the puree, which may have been too intense for toddlers over repeated exposures, or which may have induced a higher complexity. In addition, the vegetable liking and intake were still high even when salt was removed, indicating the efficiency of this learning procedure. Besides, the FFL-Salt group was the only group where a gender effect was observed (*P*=0.02), with boys consuming greater amounts of the saltier version than girls during the conditioning phase; but no interaction between the exposure number and gender was found. This could be of importance with regard to the greater salt intake observed in children and adult males, and the greater incidence of hypertension observed among adult males [30, 37].

Concerning the results observed for the FFL-Nutmeg group where nutmeg was added, toddlers significantly increased their intake of the mere salsify recipe by 36±11g from the pre- to the post-exposure. During the exposure phase, nutmeg was added to the salsify puree: liking followed a linear increase across the 8 eating occasions and intake followed a U-shape. The initial decrease might reflect a period of adaptation to the flavor, followed by an increase in intake. One might hypothesize that the presence of the spice, endows the puree with a complexity that induced an initial decrease in intake. Afterwards, the child was familiarized to the combination of the spice and puree tastes, and an increase in intake was observed. Besides, the vegetable liking and intake were still high even when nutmeg was removed, indicating the efficiency of this learning procedure. From a practical point of view, while using a spice as an US, caregivers might give up after few trials when the initial decrease is observed, as they could judge the whole group's behavior. For parents, applying this strategy in a personalized way, which is using as a US a salt-associated spice they already have noticed their child likes, could induce an effect on learning. Here, due to the experiment constraints, it was not possible to personalize the spice according to each child's experience with spices.

Conclusions

A ‘pinch’ of salt (0.2 %) was shown to be sufficient to induce a learned acceptance. Using a greater amount of salt (0.5 %) as a conditioning stimulus did not appear to be more efficient. We cannot say if mere exposure to an unsalted variant of the target vegetable (salsify) would have been efficient. However, we previously showed that for green beans, salt suppression induced a decrease in intake among toddlers [21], and a decrease in intake and liking in children’s (8-11 years) [38]. These results could reveal dissociation between the role of salt on intake and its role on learning, which would be worthy to investigate. Moreover, the mere exposure mechanism was more efficient in increasing toddlers’ vegetable liking and intake than was the flavor-flavor learning mechanism, tested either with salt or with nutmeg. Hence, this reveals the high specificity of learning in the food domain: children learn to like and consume the specific variants they are exposed to.

Nevertheless, it appears that a limitation of salt in foods presented to toddlers is possible, but the suppression of salt in vegetables for instance, should be considered cautiously. Besides, the present study revealed that presenting vegetables as a starter would be an efficient strategy to make toddlers eat them, rather than presenting them with the entrée; where they may be in ‘competition’ with other foods. This is line with previous observations of Spill and co-workers [39] in a study conducted with 3-5 year-olds. In sum, under conditions comparable to those used in the present work, that is in particular at the beginning of the meal, when children are hungrier, ME appears to be the wisest choice, as a simple recipe with a small quantity of salt was enough to trigger acceptance.

Acknowledgements

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the grant agreement n°245012-HabEat.

We would like to thank Laurence Depezey and Jean-Yves Favard from the Bonduel group for supplying the salsify; as well as the FreshInov factory for producing the jars. The authors thank the Early Childhood Department of the city of Dijon as well as the nursery directors and caregivers. S. B. thanks the children and their parents who took part in the present study; Christophe Martin for conducting sensory profiling; Vincent De Anfrasio for his help in vegetable production and data collection; Valérie Feyen, Emilie Szleper, Judith Fontaine, Celine Gulluscio, Jennifer Langlois and Françoise Durey are thanked for their assistance with data collection. The authors have no conflict of interest to disclose.

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Chapter 5. The different impact of fat content on toddlers' and adults' food intake

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Submitted

Abstract

Fat in foods is often incriminated in the high prevalence of overweight and obesity. However, the role of fat content on intake and its perception are still a matter of debate. This work evaluates the impact of fat content of a creamy white cheese (CWC), on its intake, sensory characteristics and liking, in children and adults.

The CWC variants were commercially available products with 0, 20 or 40% fat and sweetened with 5% added sugar. 56 toddlers (2-3 years old; 23 ♀-33 ♂) and 51 adults (18-25 years old; 26 ♀-25 ♂) took part in three afternoon snacks (composed of CWC, biscuits, milk and/or water). The same adults scored their liking and sensory descriptors for the three CWCs, in a separate sensory evaluation session. A different trained adult panel of 12 adults provided a taste description of the products.

The untrained adults differentiated the three CWCs according to thickness (P=0.04), creaminess (P<0.0001), dryness (P<0.0001), sourness (P<0.0001) and sweetness (P=0.0003) but not to fattiness. On the contrary the trained panel found differences between the samples according to their fat content. Adults' liking for the creamy white cheese increased with fat content. Toddlers' intake of the CWC was influenced by fat content (P=0.013): the 0% and 20% fat CWCs were more consumed than the 40% one (196±12, 186±12 and 159±11 g respectively). However, fat content had no effect on the adults' CWC intake (P=0.55; 188±6 g on average). Toddlers regulated their energy intake better than adults did.

These results highlight the discrepancy between intake and liking measurements in adults. They also shed light on the poorer ability of adults to maintain their energy intake when fat content varies in a common food contrarily to toddlers.

Keywords: Toddlers; children; adults; food intake; liking; fat.

Highlights:

► Fat content impacts toddler but not adult creamy white cheese weight intake. ► Energy intake decreases with decreasing energy density in toddlers and in adults. ► Toddlers regulate their energy intake better when fat content varies than adults do. ► Discrepancy between intake and liking measurements is observed in adults.

1. Introduction

Fat is the major source of dietary energy for the body. It is necessary for a healthy growth and for many physiological functions (Mattes, 2010). However, the high energy density provided by dietary fat is incriminated in the high prevalence of overweight and obesity (WHO, 2003). Many policies around the world (WHO, 2003) and in France (Hercberg, Chat-Yung & Chauliac, 2008), aim at limiting fat content in the population's diet. Recommendations for children under 2 years old do not advise any limitation for fat intake, due to its importance for an optimal growth and for nervous system and brain development; but from the age of 3 onwards, fat should represent no more than 30 to 35% of energy intake in a diet (Uauy & Dangour, 2009). Thus, many fat-free or fat-reduced products have been developed. However, few data are available on the impact of fat content reduction on food intake in adults and even less in toddlers.

The sensory properties of foods are major determinants of the hedonic response they elicit, and of how much is consumed. Fat is known to play a major role on sensory properties and therefore on eating behavior (Drewnowski & Almiron-Roig, 2010). The high palatability of dietary fat is due to a combination of several orosensory factors such as texture, odor and taste (Drewnowski, 1997; Sørensen, Møller, Flint, Martens & Raben, 2003). The perception of the "fattiness" of a food is primarily based on textural properties, rather than on any conscious identification of the product's fat content (Mela, 1988; Mela, 1990). In adults, liking for a food increases with fat content (Drewnowski & Greenwood, 1983; Vickers & Mullan, 1997). To the best of our knowledge, no study has investigated children's liking for a food according to its fat content.

Concerning weight intake in response to fat content variations in food, no clear effect was found in children. No significant effect of fat content was found on 4-9 days-old newborns' breast milk intake (Woolridge, Baum & Drewett, 1980), nor on 2-5 days-old newborns' or 5-weeks-old infants' formula intake (Chan, Pollitt & Leibel, 1979; Nysenbaum & Smart, 1982). Children aged 2-3 years old consumed the same amount of food when fat content was modified by varying the amount of added butter (+5%) either in green beans or in pasta (Bouhlal, Issanchou & Nicklaus, 2011). Similarly, the intake of macaroni and cheese did not vary in children aged 5-6 years old, after the addition of 7% of butter (Fisher, Liu, Birch & Rolls, 2007). One may wonder whether variations in fat content of greater amplitude would produce a significant difference in intake, and a different effect in adults and children; especially with regard to the discrepancy of previous results concerning this question. Indeed, some *ad libitum* studies did not reveal any effect of fat content on the amount of food consumed neither in adults nor in 5-6-year-old children (Bell & Rolls, 2001; Fisher et al., 2007). On the contrary, a study with adults using a baked pasta entrée (Kral, Roe & Rolls, 2004), and another with 2-5 year-old children using a macaroni and cheese entrée (Leahy, Birch & Rolls, 2008), found participants to consume significantly less weight from the high-fat compared to the low-fat variant. Thus, investigating the response to larger variations in fat content might reveal a different pattern of response in terms of weight intake.

Regarding energy intake, reducing energy density in a food (by manipulating carbohydrate, fat or water content) induces a decrease in energy intake from that food (Rolls, 2009). This decreasing energy intake was observed at a single meal (Kral et al., 2004), during one day (Bell, Castellanos, Pelkman, Thorwart & Rolls, 1998) and over the course of five days (Duncan, Bacon & Weinsier, 1983). This was observed when energy density was altered by varying fat content in a food served *ad libitum* during the course of a meal, in both children (Fisher et al., 2007; Leahy et al., 2008) and adults (Kral & Rolls, 2004).

Thus, two hypotheses can be formulated about the impact of the fat content of a food on its intake. As palatability appears to be important in the short-term control of food intake, one might hypothesize that a decrease in the fat content of a food could induce a decrease in its palatability, thus in its weight intake. As far as energy density is concerned, a decrease in the energy density of a food, associated to a decrease in fat content could induce an increased weight intake in order to 'compensate' for the missing energy.

The objective of the present study was to understand the role of fat content on intake in toddlers and in adults (2-3 and 18-25 years old, respectively), and to determine whether their patterns of response are different in particular regarding the two hypotheses formulated above. Moreover, a measurement of liking was conducted with the adults. Additionally, to investigate the role of fat content on sensory properties, a sensory description of the studied foods was conducted.

2. Participants and Methods

The present study was focused on the impact of fat content on food sensory properties, liking and intake. It was conducted using creamy white cheeses with different fat contents. Three groups of subjects were recruited: 1) a trained adult panel provided a taste description of the foods; 2) untrained adults provided a sensory description of the foods; they scored their liking and their intake of the foods was measured during a snack; 3) and last, for a group of toddlers, intake of the foods was measured during a snack at their usual nursery.

2.1. Participants

2.1.1. Trained adult panel

The panel was composed of 12 adults (6 females and 6 males), aged between 27 and 66. They received eleven 1-to-1h30 sessions of training on taste description and on the way to use the scoring scale.

2.1.2. Adults

Recruitment took place through advertisements and flyers distributed on the University of Burgundy campus. Participants had to be healthy, without any known food allergies and with no previous experience in sensory evaluation. They also had to be used to snacking in the afternoon, and were not to have any intense physical activity before the experimental sessions. The participants were 51 students (26 females; 25 males), and were unaware of the purpose of the study. They were informed that they would participate in three afternoon snacks in order to evaluate the energy need of people of their age group. They were instructed not to eat between their lunch and the test session. All the adults participated in all measurements.

The average age of the participants was 22.0 ± 1.8 years (range: 18 to 25 years), with an average BMI of 22.7 ± 2.7 kg/m² (range: 17.1 to 31.2 kg/m²). The participants received written information and signed a consent form, they received a 20 € compensation for their participation.

2.1.3. Toddlers

All the toddlers attending one of three nurseries in Dijon (France) were proposed to take part in the present experiment: their parents received an information sheet and signed a consent form. The study procedure was conducted according to the guidelines laid down in the Declaration of Helsinki, and approved by a local ethical committee (Comité de Protection des Personnes Est I Bourgogne). All the children participating in the study were used to having an afternoon snack at the nursery. The children's body weight (kg) and height (m) were measured by the nursery doctor, and z-scores for body mass index (z-BMI) were calculated using the French references (Rolland-Cachera, Cole, Sempe, Tichet, Rossignol & Charraud, 1991).

From the initial sample of 67 toddlers recruited, those who were absent at more than one session were excluded from the present analysis (n=11). Thus, data from 56 children (23 girls and 33 boys) were included in the analysis. The average age of this group of children was 29.2 ± 3.4 months (range: 22 to 39 months), with an average BMI of 15.9 ± 1.2 kg/m² (range: 13.8 to 18.9 kg/m²) and an average BMI z-score of -0.2 ± 0.9 (range: -2.2 to 2.0).

2.2. Studied foods

The target food was 'fromage blanc', i.e. creamy white cheese (CWC). This product resembles unsalted cottage cheese and is commonly consumed in France with added sucrose (Daillant & Issanchou, 1993). The CWCs were sweetened by adding 5% sucrose. Three variants of commercial CWCs' were used: 0% (0.3% of fat on total weight; 0.66 kcal/g), 20% (20% of fat on dry weight; 3.3 % of fat on total weight; 0.92 kcal/g) and 40% (40% of fat on dry weight; 8% of fat on total weight; 1.33 kcal/g). The samples were refrigerated at 5°C prior to their presentation to the participants.

2.3. Sensory description of food variants

2.3.1. Trained adult panel

A trained adult panel rated the 'taste' characteristics of the three studied food variants using the FIZZ software (Biosystèmes, Couternon, France). Panel members were screened for their olfactory abilities, their abilities to perceive and identify the basic tastes, and for their capacity to use a linear scale to score intensities. The references of the *Spectrum*™ intensity scales for descriptive analysis were used to describe sweet, salty, sour and bitter tastes (Meilgaard, Civille & Carr, 1991). Another 'Spectrum-like' scale was developed for the perception of fattiness. The panelists were asked to score the perceived intensities of each variant monadically on a linear scale, from 'not perceived' (left anchor) to 'very intense' (right anchor). Two replications were performed.

2.3.2. Adults

The participants attended a sensory analysis session after the last day of food intake evaluation. They were requested to avoid eating and drinking two hours prior to testing. For each of the three variants of creamy white cheese they were asked to rate their perception of sweetness, sourness, creaminess, dryness in the mouth, fattiness and after-taste from absence (0) to strong perception (5), and perceived thickness from 'very fluid' (0) to 'very thick' (5) (CIDIL, 1995) on 6-point scales. They also rated their liking on a linear scale anchored at both anchors with 'I don't like it at all' (left anchor) and 'I like it a lot' (right anchor). The order of presentation was balanced for sensory description and for liking evaluation. The CWCs were served monadically in small plastic cups with lids. Between each sample, participants cleaned their palate with water and/or bread. The data were collected using the FIZZ software (Biosystèmes, Couternon, France).

2.4. Evaluation of intake during afternoon snacks

2.4.1. Toddlers

In a crossover design, the CWCs were served at the three nurseries, every other week during the 3:30 pm afternoon snack. The order of presentation for the three variants was different in each nursery. A first portion of 100 g was served in the child's usual bowl and additional servings of 50 g were available (up to a total of 300 g). Afterwards, children were served up to 18 g of biscuits (4.45 kcal/g) then *ad libitum* milk (0.46 kcal/g) and/or water. All snack items were weighed before and after each serving for each child, in order to evaluate intake.

2.4.2. Adults

A similar design was applied to this group. The snack took place at the laboratory, for three consecutive weeks at 4 pm or 5 pm. The order of presentation was balanced across subjects. The snack items were the same as for the toddlers (CWC, biscuit, milk, water). Adults were first served a 150 g portion and could ask for an additional serving up to a total of 300 g. Then, they were served biscuits, then milk and/or water.

2.5. Statistical analysis

Statistical analyses were carried out using the SAS System for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA). Significance was set at $p < 0.05$. Results are reported as Lsmeans ± SEM. The manufacturers' information was used to convert measured weight intake (g) into energy intake (kcal). Weight and energy intakes were analyzed using a mixed linear model (SAS Mixed procedure), with "child effect" or "adult effect" considered as random. The 'Empirical' option was specified so as to use a sandwich estimator for the variance-covariance matrix of the fixed-effects parameters. The primary factor tested in the model was the fat content. Children's z-BMI or adult's BMI was introduced as a covariate, to evaluate to what extent it might affect intake. Interactions between fat content and z-BMI or BMI were also investigated. Neither the effect of z-BMI on the toddlers' intake and nor the interaction between z-BMI and fat content were significant. Besides, the effect of the adults' BMI on intake was not significant; neither was its interaction with fat content. Therefore, these results are not further reported. The adults (n=5) and toddlers (n=3) who ate more than 95% (285 g) of the CWC during the snack were considered as 'plate cleaners' (Fisher et al., 2007); analyses were conducted both with and without their data. When these data were excluded, it did not change the outcomes; thus, 'plate cleaners' were included in the reported analyses.

The percentage of variation in energy intake (EI) when fat content decreased was calculated individually: % EI = [100 x (EI_0% -EI_40%) / EI_40%]. These percentages were calculated for the CWC energy intake and for the whole snack energy intake, for the toddlers and for the adults. T-tests were used in order to compare these differences between the toddlers and the adults.

For liking data collected with the adults, the grades on the scale were converted into scores from 0 to 10 by measuring the distance between the left anchor and the marks made by the participant. These data were analyzed as intake data using a mixed model, testing for the principal effect of fat content, with adults' BMI as a covariate, and evaluating the fat content x BMI interaction. The effect of the adults' BMI on liking was not significant; neither was its interaction with fat content; so these results are not further reported.

Concerning sensory evaluation data collected with the adults, the grades on the scale were converted into scores as described for liking data. A mixed procedure was conducted to analyze sensory data, with the "adult effect" considered as random. The effect of fat content on each descriptor (sweetness, sourness, thickness, creaminess, dryness in the mouth, fattiness and after-taste) and on liking was investigated; as was the effect of each adult's BMI on liking. For taste sensory data collected with the trained panel, an ANOVA was performed using the SAS GLM procedure for each taste quality with the primary factor tested in the model being the fat content. The model was: taste quality = panelist + fat content + panelist x fat content + Error, where 'panelist' and 'panelist x fat content' were considered as random effects.

3. Results

This study aims at comparing intake data from adults and toddlers in response to fat content variations in a studied food. Fat content and energy density were decreased by almost 50 %: the 40% fat creamy white cheese brought 1.33 kcal/g, whereas the 0% fat variant brought 0.66 kcal/g. The results concerning sensory description and liking will be presented first.

3.1. Sensory description of food variants

3.1.1. Trained adult panel

Sweetness intensity differed according to fat content in the CWC, $F(2, 36) = 4.67$; $p = 0.02$; Fig. 1): the 40% fat CWC was perceived to be sweeter than the 0% fat one. Moreover, the 40% fat CWC was perceived as being less sour than both the 0% and the 20% fat ones ($F(2, 36) = 8.19$; $p = 0.002$; Fig. 1). Concerning fattiness perception, a significant effect of fat content was observed ($F(2, 36) = 4.62$; $p = 0.02$; **Fig. 1**): the 40% fat CWC was perceived as fattier than the 0% fat one. No significant effect of fat content was observed either on saltiness ($F(2, 36) = 0.86$; $p = 0.44$) or on bitterness ($F(2, 36) = 2.54$; $p = 0.10$) intensity.

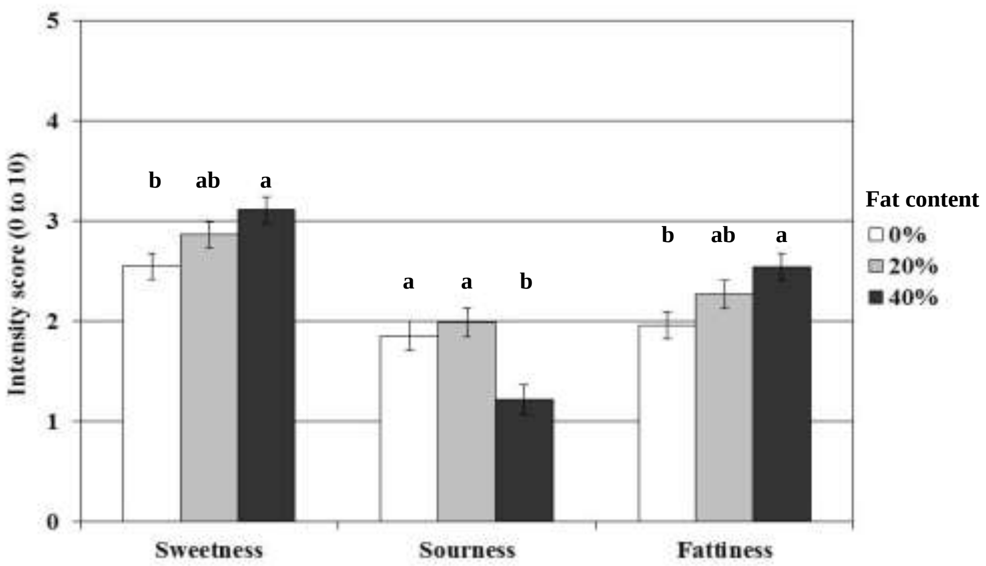

Fig. 1. Sensory description of the creamy white cheeses with varying fat content given by the trained adult panel. Means (lsmeans ± SEM) with the same letter are not significantly different ($p < 0.05$).

3.1.2. Adults

For the untrained adults, sweetness intensity of the CWC was also affected by fat content (F (2, 100) = 8.94; p = 0.0003; Fig. 2): the 40% fat CWC was perceived to be sweeter than the 0% and the 20% fat ones. Concerning sourness intensity a significant effect of fat content was observed (F(2, 100) = 10.78; p < 0.0001; **Fig. 2**), the panel perceived the 40% fat CWC as less sour than both the 0% fat and the 20% fat ones. Concerning the textural properties rated by the adults, a significant effect of fat content was observed on creaminess (F(2, 100) = 18.08; p < 0.0001) and on dryness in the mouth (F(2, 100) = 24.41; p < 0.0001): the three CWCs were distinguished from each other, with the 40% fat CWC perceived as creamier and less dry in the mouth than the 0% and the 20% fat ones. Fat content also had an effect on thickness perception (F(2, 100) = 3.28; p = 0.04), as the 40% and the 20% fat ones were perceived thicker than the 0% fat one. With regard to fattiness perception, fat content had no effect (F(2, 100) = 0.84; p = 0.43; Fig. 2). Aftertaste was not significantly impacted by fat content (F(2, 100) = 2.74; p = 0.06).

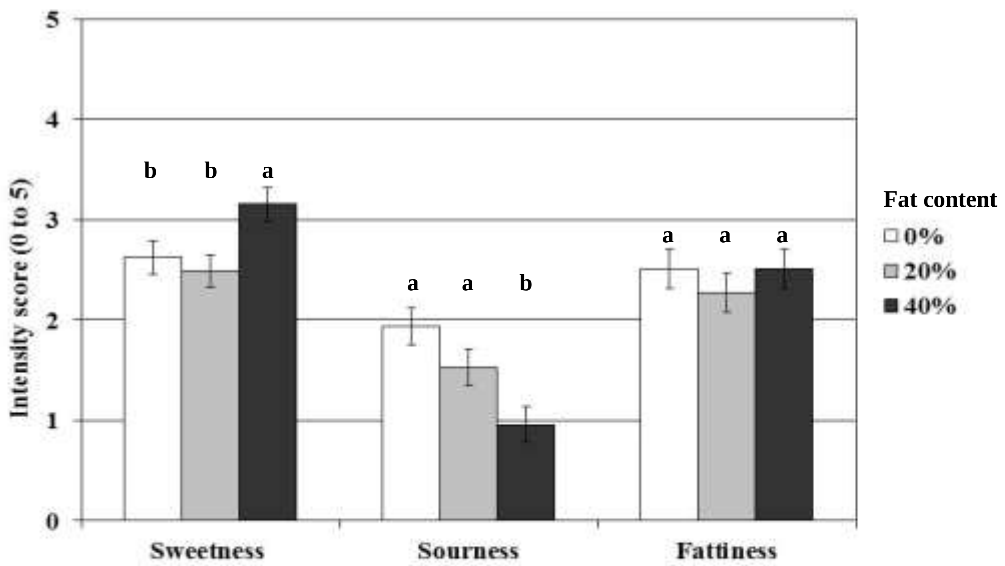

Fig. 2. Sensory description of the creamy white cheeses with varying fat content given by the untrained adult panel. Means (lsmeans ± SEM) with the same letter are not significantly different (p < 0.05).

The CWCs were differently appreciated (F(2, 100) = 10.97; $p < 0.0001$; **Fig. 3**): the higher the fat content, the more the product was liked.

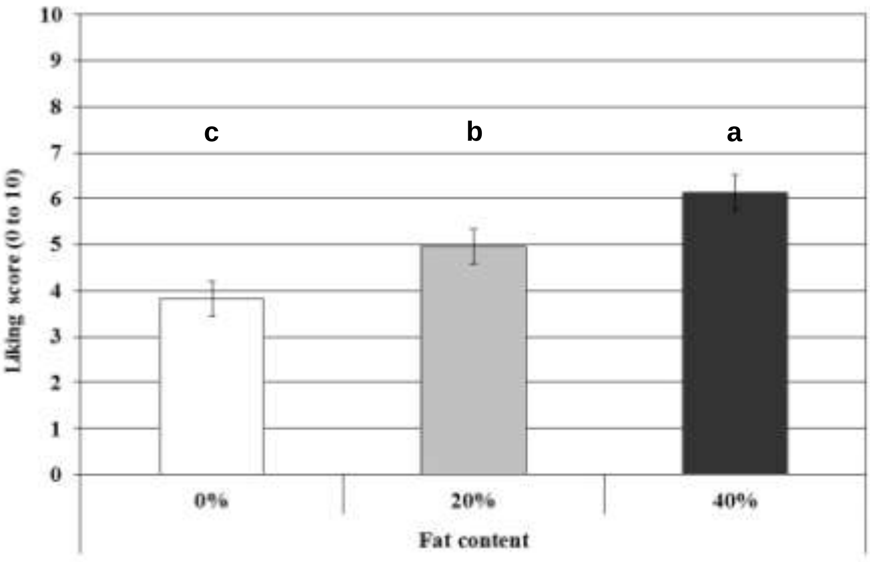

Fig. 3. Liking scores for the creamy white cheese with varying fat content, rated by the untrained adults (n=51). Means (lsmeans ± SEM) with the same letter are not significantly different ($p < 0.05$).

3.2. Impact of CWCs' fat content on weight and energy intakes

3.2.1. Toddlers' and adults' weight intake

The results in toddlers indicated that fat content had a significant effect on CWC weight intake ($F(2, 97) = 4.57$; $p = 0.01$): the 0% fat CWC was more consumed than the 40% fat one, as illustrated in **Fig. 4**.

No effect of fat content on weight intake of biscuits ($F(2, 97) = 0.47$; $p = 0.62$), of milk ($F(2, 96) = 0.49$; $p = 0.61$) or of water ($F(2, 97) = 1.05$; $p = 0.35$) was observed. Globally, fat content had a significant effect on the whole snack weight intake ($F(2, 97) = 5.99$; $p = 0.003$; Fig. 4): snack weight intake was higher when the children ate the 0% fat CWC than when they ate the 40% fat one, in relation with the difference in CWC intake.

Results on adults (Fig. 4) show no significant effect of fat content on weight intake of CWC ($F(2, 100) = 0.26$; $p = 0.77$), of biscuits ($F(2, 100) = 0.28$; $p = 0.76$), of milk ($F(2, 100) = 0.26$; $p = 0.77$) or of water ($F(2, 100) = 0.59$; $p = 0.55$). As a result, no significant effect of fat content was observed on the whole snack intake of adults ($F(2, 9100) = 0.10$; $p = 0.90$).

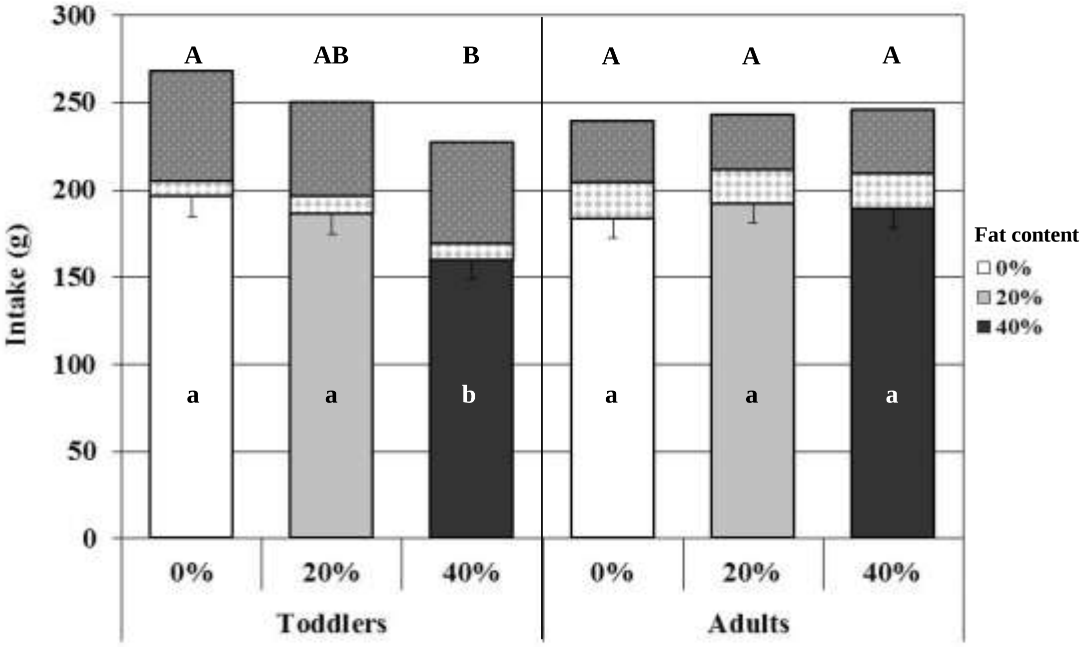

Fig. 4. Weight intake (in grams) by toddlers (n=56) and adults (n=51) in response to creamy white cheeses with varying fat content. Means (lsmeans ± SEM) with the same letter are not significantly different ($p < 0.05$). Lower case letters correspond to the analysis at the food level; Upper case letters correspond to the analysis at the snack level (with: biscuit and milk).

3.2.2. Toddler's and adults' energy intake

The effect of fat content on energy intake from CWC was significant for toddlers and for adults ($F(2, 97) = 25.64$; $p < 0.0001$ and $F(2, 100) = 55.78$; $p < 0.0001$ respectively), as illustrated in **Fig. 5**. A 50% reduction in CWC energy density led to an average 26% decrease in the toddlers' and to a 46% decrease in the adults' energy intake from CWC; these decreases were significantly different ($t = 2.24$, $p = 0.028$).

Likewise, the impact of fat content on the total energy brought by the whole snack was significant for children ($F(2, 97) = 23.40$; $p < 0.0001$) and for adults ($F(2, 100) = 34.72$; $p < 0.0001$). With regard to energy intake from the whole snack, the average percentages of decrease were 26% for toddlers and 33% for adults; they did not differ from each other ($t = 1.46$, $p = 0.15$). The children adjusted their energy intake from the studied food better than adults when fat content varied, but at the snack level did not compensate differently from the adults.

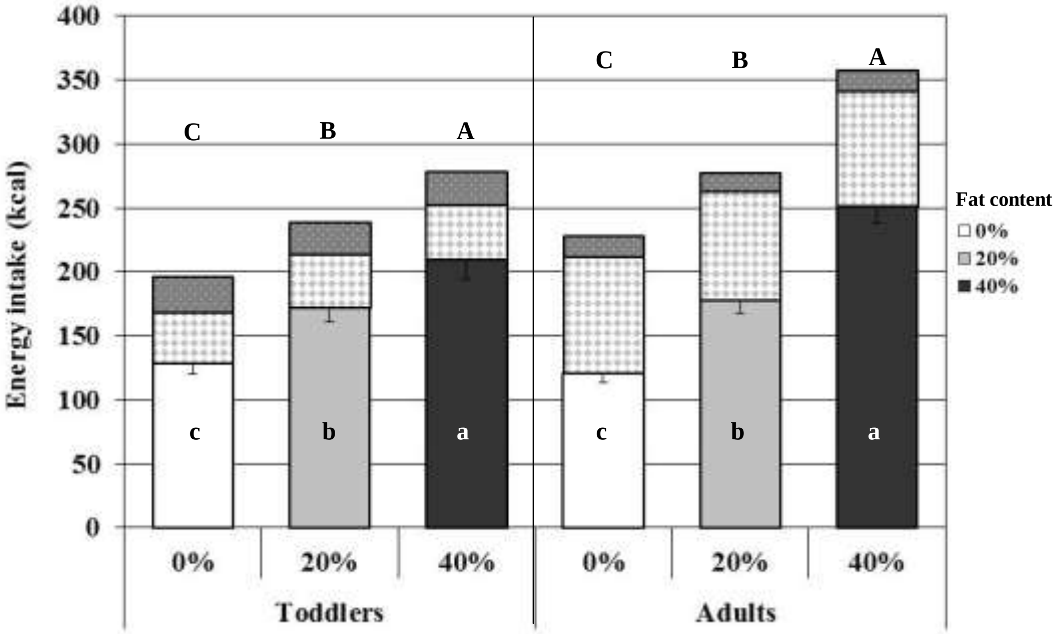

Fig. 5. Energy intake (in kcal) by toddlers (n=56) and adults (n=51), in response to creamy white cheeses with varying fat contents. Means (lsmeans ±SEM) with the same letter are not significantly different ($p < 0.05$). Lower case letters correspond to the analysis at the food level; Upper case letters correspond to the analyses at the snack level (with: biscuit and milk).

4. Discussion

In the present study, we aimed at evaluating the effect of the fat content of commercial creamy white cheeses on their intake by toddlers (2-3 years) and adults (18-25 years). Moreover, a sensory description of the studied foods was provided by the same (untrained) adults (taste and textural properties), who also scored their liking of each variant. A distinct trained adult panel gave a description of the food tastes.

The results showed that fat content had a significant effect on the toddlers', but not on the adults' CWC weight intake: the 40% fat creamy white cheese was less consumed by toddlers than the 0% or 20% fat variants. So, the adults clearly regulated their intake in terms of consumed weight, whereas the toddlers adjusted their energy intake better than the adults (their intake decreased for the most energy-dense variant), although they did not demonstrate perfect compensation either. This is in spite of the fact that adults liked the high-fat CWC better than the fat-free one; and that they were able to distinguish between the samples in term of several sensory attributes (sweetness, sourness, creaminess, dryness in mouth, thickness).

Only the trained adult panel was able to differentiate the high-fat from the fat-free samples according to their fattiness; this group also differentiated their sweetness and their sourness.

Two hypotheses on the impact of fat content on intake were proposed: 1) either the decrease in fat content would result in a decrease in food palatability and thus in a reduction in the weight consumed from the low-fat variant, or 2) the decrease in fat content inducing a decrease in energy density would result in a higher intake of the low-energy density variant in order to 'compensate' for the missing energy.

In the toddlers, our results lean towards the second hypothesis. In fact, the decrease in fat content did not result in a decrease in weight intake, but in an increase. So, the 0% fat creamy white cheese was palatable and appealing enough for toddlers to consume it; as it was consumed in great quantity (about 200g), almost twice the standard individual portion on the market (100g). Unfortunately, with regard to the young age of the children and given the methodological difficulties to conduct sensory evaluation with this age group, no information on their preference or liking for the foods could be collected. This may have provided more insight on the role of liking on weight intake. From the present results, it seems that, in toddlers, fat content is not a direct driver of liking. However, fat may rather contribute to enhance preference for other flavors as demonstrated by conditioning studies (Kern, McPhee, Fisher, Johnson & Birch, 1993).

In the adults, either none of the two hypotheses were verified, or both hypotheses are occurring at a similar level, but their effects counteract each other. The adults did perceive sensory differences between variants of a food, they did prefer the higher fat variant, but when it came to intake they lacked 'unconscious sensitivity' to adjust their intake in response to the variations in fat content and energy density. An explanation for this observation would be the fact that adults might adjust their intake according to their expectancies about the food (Shide & Rolls, 1995). In studies where only the energy density varied while the palatability remained constant, the findings converge in that a constant amount of food is consumed (see Rolls, 2009 for a review). This highlights the strong effect of cognitive factors that might be related to the amount of a given food that should be consumed in order to satisfy hunger (Rolls, 2009).

The present results about weight intake are partially in accordance with former research works. In the present study, comparing a high-fat and a fat-free food, the associated energy density was reduced by 50%; in toddlers this reduction resulted in a significant 23% increase of the fat-free food weight intake. A study with 2-5 year-old children, found a small (6%) but significant increase in macaroni and cheese weight intake in response to a 30% reduction in energy density (Leahy et al., 2008). In another study with toddlers, a 20% reduction in energy density resulted in a 6.5% increase in pasta weight intake, which did not reach significance (Bouhlal et al., 2011). In adults, the similar weight intake of the creamy white cheeses whatever their fat content is not surprising with regard to the literature (Drewnowski, 2000; Rolls & Bell, 1999). In the specific case of fat, this phenomenon was described by several researchers as 'passive over-consumption' or fat hyperphagia (for review see French & Robinson, 2003). High-fat foods are palatable (Drewnowski, 1997), moreover they are not satiating and lead to an excess in energy intake (Drewnowski, 2000; Drewnowski & Almiron-Roig, 2010). The weak satiation elicited by high-fat foods might lead to their passive over-consumption and thus to overweight (Erlanson-Albertsson, 2010). With regard to young children's greater sensitivity to food sensory qualities (Drewnowski, 1997), and in the light of the sensory variations between the creamy white cheeses observed in the present work, the significant effect of fat content variations on weight intake makes sense. Our results reveal that children are able to perceive fat content in foods and to partially adjust their intake during the course of the meal in response to these orosensory signals. In adults this ability is not present anymore, and they maintain the weight or the volume of foods they consume constant. One might hypothesize that adults are less or not sensitive to orosensory signals any more, or that their intake is mostly driven by habits concerning an 'adequate' weight of food to be ingested.

Concerning energy intake, the present work showed that the 50 % reduction in energy density resulted in significant decreases in the toddlers' energy intake from CWC and from the whole snack: 39% and 30% on average, respectively. In a study with 2-5 year-old children, a 30 % reduction in macaroni and cheese energy density resulted in a significant 25% decrease in its energy intake, as well as in a significant 18 % decrease in the total meal energy intake (Leahy et al., 2008). However, in 2-3-year-olds, a 20% decrease in pasta energy density led to a non-significant 12% decrease in energy intake (Bouhlal et al., 2011). It could be hypothesized that, at least in children, minimal amplitude of difference in energy density must be achieved in order to lead to an efficient decrease in energy intake. Our results are in line with former studies using preloads before an *ad libitum* lunch intake, where authors have found children to be better than adults at compensating for variations in energy density (Birch & Deysher, 1986). In general, the compensation or adjustment for energy seems to be efficient in children under five, while this ability decreases between the ages of 7 and 10 (Cecil et al., 2005; Hetherington, Wood & Lyburn, 2000) and also in adulthood (for a review see Benton, 2004). Concerning the results in adults, they are in line with other studies, where a decrease in energy density is not compensated by an increase in weight intake, and leads to a decrease in energy intake (Kral & Rolls, 2004; Rolls, 2009).

Results showed that in adults liking for the creamy white cheeses increased with fat content. This result is in accordance with other works, as the higher the fat content in a food, the higher its hedonic rating (Drewnowski & Greenwood, 1983). Here, liking for the high fat sample was high although these untrained subjects failed to identify fattiness. This result is in accordance with previous works (Drewnowski, 1989; Peryam & Pilgrim, 1957).

Concerning the discrepancy between intake and liking results in adults, previous studies have highlighted such a discrepancy when sugar or salt varied in food (Bellisle, Giachetti & Tournier, 1988; Lucas & Bellisle, 1987). This study draws the same conclusions when fat content varies. Thus, lowering the fat content in creamy white cheese does not prevent its consumption, even if it induces a decrease in liking among adults. These results are encouraging in the perspective of reducing fat content in common foods.

The sensory evaluation of creamy white cheeses revealed the same pattern of ratings of sweetness and sourness for both the trained and the untrained adults: the 40% fat version was perceived as sweeter, and as less sour than the 0% one, although all the samples were prepared with the same amount of sucrose (5%). This phenomenon was described to be related to a synergy between sugar and fat: in dairy products fat enhances perceived sweetness (Tuorila, Sommardahl, Hyvönen, Leporanta & Merimaa, 1993). In another study, fat was shown to contribute to the sweetness scores of the products tested, as the high-fat stimuli were judged sweeter (Drewnowski & Schwartz, 1990). Moreover, when adults were allowed to add as much sugar as they wanted to creamy white cheeses of varying fat content, less sugar was added to high-fat versions compared to other variants (Daillant & Issanchou, 1993). In addition, a previous work using mixtures of sucrose and fat, had shown that perceived sweetness is a strong driver of hedonic ratings, even if subjects fail to estimate a food's fat content (Drewnowski & Greenwood, 1983). Therefore, the observed liking ratings of adults could be due to a 'direct' effect of fat content, or to an indirect effect through perceived sweetness.

Concerning the perception of the creamy white cheeses fat content, only the panel trained to evaluate the fat content of foods was able to identify the 40% fat one as actually fattier than the 0% fat version. The untrained adults failed to taste any difference in fattiness, but did find texture-related differences between the various products. Several studies have pointed to the poor ability of humans to track and identify the increasing fat content of foods (Drewnowski, Shrager, Lipsky, Stellar & Greenwood, 1989; Mela, 1988). In fact, many sensory signals brought by fat interact and contribute to the concept of fat 'taste' (Drewnowski, 1997), which makes it difficult to assess fat content (Drewnowski & Almiron-Roig, 2010). Moreover, preference for fat seems to be independent of the conscious ability to detect or assess the fat content of solid foods (Drewnowski & Almiron-Roig, 2010). This is in line with the present findings in adults.

Although the untrained adults failed to perceive the difference in fat content among the samples, they were able to distinguish the creamy white cheeses according to textural properties such as dryness, creaminess and thickness. In the present work, the higher the fat content, the creamier and less dry in the mouth the product was perceived. These results are in accordance with the existing literature, underlying the role of texture on fat perception in food (Mela, 1988). The fat content is known to enhance the viscosity of the product, and hence its creaminess (Drewnowski et al., 1989). Yet, high fat products are associated with a greater creaminess perception and a lower perception of dryness in the mouth (de Wijk, van Gemert, Terpstra & Wilkinson, 2003).

Finally, we could speculate that the great range of foods found on the market, with varying energy and fat contents but with similar appearance, could be confusing for the consumers. That is especially problematic in the perspective of a regulation of energy intake. This observation is particularly true for fat content, which is not well detected by humans. In adulthood, we can hypothesize that learning about food intake is supposed to be assimilated or acquired by the body and that sensory cues are not integrated during food intake any more, as was the case in childhood. We could also speculate that for toddlers, it is unconsciously more important to be sensitive to the post-ingestive properties of food, in order to satisfy nutritional needs for growth. Understanding how and when expectations about a food's energy density develop during childhood seems therefore to be worth investigating.

5. Conclusions

Modifications of fat content in a food have an impact on sensory perception and liking in adults as well as on weight intake in toddlers. Yet, they do not impact adults' weight intake. Comparing data from toddlers and adults in the present work has shown the greater ability of toddlers to regulate their intake of a food varying in fat content and thus their energy intake at the meal level. Therefore, identifying the 'critical age' at which adjustment ability is lost and trying to find ways to help maintain it would be worth investigating in future studies.

Reducing energy density leads to a decrease in energy intake of the food and also in intake from the same meal. In order to achieve a reduction in energy intake, and meet nutritional recommendations, strategies decreasing food energy density and in particular fat content appear especially promising.

6. Acknowledgements

SB wishes to thank the director and staff of the nurseries, as well as the children and parents who took part in this study; SB also thanks all adult subjects; V. Feyen, J. Fontaine, C. Bernard, are thanked for their assistance with food preparation and data collection. C. Martin and F. Durey are thanked for their assistance with sensory profiling. SB received a PhD grant from the Nutrition, Chemical Food Safety and Consumer Behavior Division of INRA (French National Institute for Agronomical Research) and the Regional Council of Burgundy; research grant from Regional Council of Burgundy to SN (Gustolf).

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Chapter 6. Summary of results and general discussion

The main aim of this PhD thesis was to understand the role of three ingredients: salt, sugar and fat, on children's eating behavior. Excess salt, sugar and fat are targeted by nutritional and health recommendations, which aim is to reduce or limit their levels in foods and to decrease their intakes. However, little was known about the impact of such reductions on children's eating behavior. Such reductions appear to be of a great importance, with regard to the important role of food sensory qualities on preferences and intake, especially in children.

Most of the experiments conducted in the frame of this PhD work took place at the toddlers' and children's real meal setting i.e. nursery or school canteen, during the normally scheduled lunch or snack time. The main findings, by ingredient, and methodological considerations will be discussed in this chapter; as a full discussion concerning each part was provided within each chapter, I will discuss only briefly each topic hereafter. Moreover, implications of the present findings, as well as recommendations for future research and some practical nutritional recommendations will be proposed.

1. Main findings

1.1. Salt impact on toddlers' and children's food intake and preferences

Verification of the hypotheses

Hypothesis 1 ***(chapters 2 & 3)***: *Compared to the usual version of a food, saltier versions would be consumed in a greater amount, and unsalted versions would be less consumed.*

➔ This hypothesis was partially confirmed.

Hypothesis 2 ***(chapters 2 & 3)***: *The effect of salt level variations on intake would be different in a highly consumed food by toddlers and children (e.g. a starchy food), compared to a less consumed food (e.g. vegetables).*

➔ Yes, salt suppression in green beans decreased their intake, while salt increase in pasta increased their intake.

Hypothesis 3 ***(chapters 2 & 3)*****:** *Children aged 8-11 years would display a different pattern of response in terms of intake than would 2-3 year-olds.*

➔ No, both age groups displayed the same pattern of variations in intake in response to salt level variations in green beans and pasta, but the amplitude of responses was different.

Hypothesis 4 ***(chapter 3)*****:** *In children aged 8-11 years old, salt content would impact preference and consequently intake.*

➔ Yes, salt influenced intake through preference. Level of liking for a given level of salt in green beans or pasta ranked third among factors affecting intake, after the child's state of hunger and the level of liking of the food. However, the salty pasta variants were equally liked but the saltier one was more consumed.

Hypothesis 5 ***(chapter 4)***: *Salt may be used as an unconditioned stimulus (or a conditioning agent) in order to increase the acceptance of a vegetable in a flavor-flavor learning experiment.*

➔ Yes, but the flavor-flavor learning mechanism was less efficient than the mere exposure mechanism.

Hypothesis 6 ***(chapter 4)*****:** *A part of the salt used while preparing foods, could be replaced by a congruent salt-associated spice in a flavor-flavor learning experiment.*

➔ Yes, but this other application of the flavor-flavor learning mechanism was less efficient than the mere exposure mechanism.

The main results concerning salt confirm its overall positive effect on food intake in toddlers and children. However, this effect was different according to the food tested:

- Intake of a generally easily and well consumed food by toddlers and children, here pasta, was positively impacted by salt addition, compared to the usual level, whereas no effect of salt suppression on intake was observed;

- Intake of a food generally consumed in small quantities by toddlers and children, here green beans, was negatively impacted by salt suppression compared to the usual level, whereas no effect of salt addition on intake was observed.

These observed effects were food-specific for 8-11 years-old children, as the interaction between salt content and the type of food was significant. Concerning toddlers, this interaction was not significant although the same pattern of response as in older children was observed. Moreover, the amplitude of the effects was different between toddlers and children. Although both decreased their green bean intake when salt was suppressed compared to the usual level, toddlers were more affected than were children ($F(2,106)=10.89$, $p<0.0001$ vs. $F(2,137)=5.65$, $p=0.004$ respectively). In contrast, for pasta, both groups increased their intake when salt was added compared to the usual level, but the effect was less marked in toddlers than it was in children ($F(2,92)=3.19$, $p=0.045$ vs. $F(2,134)=12.26$, $p<0.0001$ respectively). This would suggest that salt suppression in green beans, is more critical in terms of intake for toddlers than it is for 8-11 year-old children, and should be considered more cautiously for this age group. Adding salt to pasta induced a greater increase in its intake in 8-11 year-olds than in toddlers. This would suggest that adding salt to an already well consumed food such as pasta, resulted in a great increase in intake, and that this increase is more important with age, and should be considered cautiously especially when children grow up. For such a liked food, salt addition does not seem necessary.

With regard to the young age of toddlers involved in the first study (*chapter 2*) no investigation of preference for varying levels of salt in foods was possible. However, a sensory evaluation session was conducted with the 8-11 year-old children who participated in the evaluation of intake (*chapter 3*). For those children, the results highlighted the discrepancy between intake and liking measurements. For instance, liking measurements revealed that pasta salted with 0.6 or with 1.2 % salt were equally liked, whereas the saltier variant was consumed in a greater amount than the variants without salt or with 0.6 % added salt. Thus, if we take into account only the liking measurements to define a salt level to be added to children's foods, the level to use would be above the one that is necessary for the food to be consumed, especially if the food is known to be liked by this population.

Therefore, taking into account children liking or preference may lead to salt addition beyond the content which is sufficient for a food to be consumed. That is, the minimal salt content to optimize liking scores and preference ranking would be 0.6 % added salt for both foods (green beans and pasta). As far as intake is concerned, it seemed possible to suppress salt in pasta, as the 0 % added salt variant was consumed as much as the 0.6 % added salt one, and in a large quantity (134 ±6 g). However, concerning green beans, an intermediate content between 0 and 0.6 % added salt may be optimal to trigger intake; this might be worth investigating by further research.

In order to capture better the relative weight of several factors determining green beans and pasta intake, the relationship between the intake (of the two target foods), the preference and liking (for the two target foods), the child's hunger, and the level of salt, was investigated using a Structural Equation Modeling (SEM) approach. This method can deal with multilevel data, and in the case of the present work, data are two-level data: some variables were recorded at the meal level (level-1; intake of each food item); whereas others were recorded at the child level (level-2; liking of green bean or pasta at a given salt content, z-BMI). Modeling food intake by SEM showed as hypothesized that salt influenced intake through preference, and allowed prioritizing factors affecting target food intake: 1) child's hunger, 2) liking of the food regardless of its salt content and 3) liking for a particular salt content in the food.

The role of salt on learning to accept and to consume vegetables was investigated in *Chapter 4*. The results reveal that "*simple is better*" as learning was more efficient with a low salt content than with a high salt content or with a combination of low salt content and a salt-associated spice. But both flavor-flavor learning mechanisms investigated had merits and can be discussed.

Indeed, using salt as unconditioned stimulus was less efficient in increasing vegetable intake, than using a lower salt content. Concerning the FFL with salt (0.5 %), since an increase in intake was observed after about 4 exposures, we may speculate that for a universally used and well liked taste such as salt, only few exposures are necessary to induce learning for 2-3 year-olds. Anyway, the results of our study question the role of salt as an efficient unconditioned stimulus.

Concerning the FFL with nutmeg the assumptions that could be made is that to elicit a greater effect on intake, one or several conditions may have been necessary: firstly, a greater number of exposures; secondly, a higher familiarity with the spice, either through a pre-training (mere exposure) with the flavor of the spice; or through a 'personalization' of the recipe with a spice each child is used to and likes. This last option is certainly the most promising but was not possible to conduct it in a nursery for logistic considerations (see discussion in the 'methodological considerations' section). Using a salt-associated spice could be theoretically efficient on the long run, after an important number of exposures, but parents or caregivers might avoid this strategy due to the relative drop in intake that could be observed during the first presentations. This could be especially true for caregivers who observe the reaction of the whole group of toddlers they are in charge of, and a negative reaction of a small number of toddlers towards such a recipe could induce a conclusion at the group level and an avoidance of this strategy. Although it is the role of parents to 'educate' their child's taste, a significant number of meals are consumed in canteens, and certainly also new vegetables are served in this context; and this has to be taken into account. Regarding parents, what could be advised is to identify a salt-associated spice or seasoning their child likes (e.g. cumin, curry, aromatic plants etc.), and to use it as a conditioning agent in order to increase their child's acceptance of new vegetables.

All things considered, our results confirm the 'direct' sensory effect of salt on food intake and food preference: salt induces a positive effect on intake. However, it was not an efficient mean for learning to occur: it did not prove more efficient than mere exposure to a vegetable to promote its intake. Thus, a dissociation between the role of salt on intake and its role on learning can be highlighted thanks to the present work.

Not surprisingly, we observed a higher intake of pasta compared to green beans among toddlers and children: the intake of green beans averaged 32 ± 2 g and 75 ± 4 g, whereas the intake of pasta averaged 87 ± 4 g and 136 ± 5 g respectively in toddlers and in children. It is worth noting that French recommendations for catering suggest an intake of a maximum of 120 and 100 g of vegetables respectively for toddlers over 18 months to 4 years and children aged 6-12 years old; whereas for starches (pasta, rice and potatoes) the recommended intakes are 120 and 170 g respectively for both age groups. It is stated that *'if the weights,* *appropriate for each age group, are needed they are also sufficient'* (p. 46) (Groupe d'Etude des Marchés de Restauration Collective et de Nutrition, 2011). Thus, in toddlerhood, vegetable consumption is recommended in greater amount than in childhood, whereas the reverse is observed for pasta. One could question the reason for these recommendations. Are they proposed to limit wastes, and/or are they proposed because toddlers need energy, and starchy foods are a good source of energy?

1.2. Sugar impact on toddlers' food intake

Verification of the hypothesis:

Hypothesis 7 *(chapter 2)*: *Toddlers would consume more of the sweeter versions of a food than of the less sweet versions*

➔ No, intake was not affected by the added sucrose content

Hypothesis 8: *This effect would be food-specific, and different for an initially sweetened and an initially unsweetened food*

➔ Yes, but this hypothesis was only partially confirmed: a small amount of sugar in an unsweetened food is favorable to increase intake.

The preference for sweet taste is generally agreed for in the toddlers' population. Moreover, it is widely known that preference impacts intake. Thus, we could speculate that the more the added sucrose, the more the food would be consumed. However, our findings did not support this speculation. Indeed, the intake of a fruit purée served to 2-3 year-olds as part of an afternoon snack, was not affected by added sucrose. Several hypotheses could explain this finding:

- Toddlers did not perceive the difference in sweetness. However, the difference in perceived sweetness between the variants was clearly revealed by the results of the trained sensory panel. So, we could exclude this hypothesis. Toddlers may have perceived the difference in sweetness but did not notice it in the intake situation, as the fruit purée was served at three different occasions every other week, and toddlers did not have the possibility to directly compare them.

In order to verify the last hypothesis, another study not described in detail in this manuscript, was conducted. In this study, an unsweetened food, a creamy white cheese (CWC), was used as the target to measure intake in toddlers aged 2-3 years old (n=50). The results (*appendix 1*) revealed that the unsweetened CWC (0 % of added sucrose) was consumed in a significantly lower amount than the sweetened variants (5, 10 or 15% of added sucrose). However, the three sweetened variants were equally consumed. Thus, it seems that the presence of sucrose is important, but addition of a low amount is enough to trigger intake. In consequence, reduction of added sugar in many processed foods may be possible. Indeed, a sucrose content between 0 and 5% for an initially unsweetened CWC for example, could be sufficient in order to allow an important intake, without affecting liking; actually the average intake of the unsweetened variant was 166 ± 12 g whereas the French recommendations for catering suggest an intake of a 90 to 100 g for toddlers (Groupe d'Etude des Marchés de Restauration Collective et de Nutrition, 2011). Most dairy products available in the French market, such as milk-based desserts (e.g. fruit yogurts, sweetened or flavored yogurts, flavored creamy white cheeses or creamy white cheeses with fruits), have added sugar content ranging from 6 to almost 19% with a median value around 12% (Oqali, 2009). These levels are probably higher than what is strictly needed to satisfy children's appeal for sugar.

1.3. Fat impact on toddlers' and adults' food intake and preferences

Verification of the hypotheses

Hypothesis 9 ***(chapters 2 & 5)***: *As palatability appears to be an important driver of intake, one might hypothesis that a decrease in fat content of a food would induce a decrease in its weight intake.*

➔ No, the variants with a low-fat content were at least as much consumed as the variants with medium fat content (pasta, green beans and CWC), and as much as those with a high fat content (pasta, green beans).

Hypothesis 10 (*chapter 5*): *As far as energy density is concerned, a decrease in a food's energy density, due to a decreased fat content, would induce an increase in its intake in order to 'compensate' for the missing energy.*

➔ Partially confirmed: true for toddlers but not for adults.

Hypothesis 11 (*chapter 5)*: *Toddlers would better regulate their energy intake when fat content varied than would adults.*

➔ Yes, toddlers better regulated their energy intake in response to energy density content, while adults regulated in terms of weight intake.

It was previously reported that the more a food is palatable, the more its intake increases (French & Robinson, 2003; Sørensen et al., 2003). However, the majority of studies focusing on the role of fat content on food intake, conducted with toddlers, children or adults, were based on protocols where the effect of fat was evaluated on the basis of caloric compensation.

The present work was based on a direct quantitative evaluation of the effect of varying fat content in usual familiar foods (green beans, pasta and CWC) and during normal eating occasions (lunch or snack). The first set of results (*Chapter 2*) reveals that varying the content of fat by adding no butter, 2.5 or 5% added butter to pasta or green beans served at lunch, representing a variation of 35 and 30 kcal respectively between the low and the high fat variant, had no impact on toddlers' weight intake. However, when a larger amplitude of variation was tested (67 kcal between the low and the high fat variant) in creamy white cheese (*chapter 5*), toddlers adapted their intake consequently. In such a case, the decrease in fat content inducing a decrease in energy density resulted in a higher weight intake of the low-energy dense variant. This may have been in order to 'compensate' for the missing energy. Thus, the 0% fat CWC was palatable and appealing enough for toddlers to consume it as it was consumed in a great amount (about 200 g).

If we compare toddlers' intake behavior to adults in response to fat content variations in CWC, the adult's intake was not affected by fat content. In terms of energy intake, it could be hypothesized that, at least in toddlers, minimal amplitude of difference in energy density must be achieved in order to lead to an efficient decrease in energy intake. The greater ability of toddlers to regulate their intake of a food varying in fat content, and thus, their energy intake at the meal level could be partially related to the evolution of energy needs for growth. Indeed, as shown in figure 26, the physical development is more important between the ages of 0 and 3 years, and then it slows down and stabilizes with age. Thus, energy needs for growth might be more critical in early childhood than later in childhood or in adulthood. We thus may speculate that toddlers' food intake regulation system is more sensitive to the presence of energy in foods, in order to regulate intake accordingly than that of adults who may rely more on expectations based on previous trials.

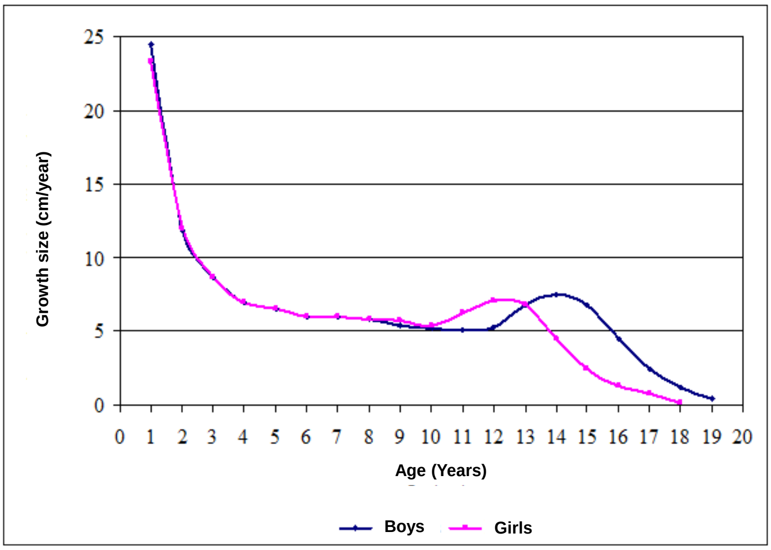

Figure 26. Annual increase in height for age, sex, for French children. Adapted from: Sempé, Pédron & Roy-Pernot, 1979.

2. Methodological considerations and experimental approach

In order to provide with a critical view on the studies conducted and presented during this PhD thesis, the following subsections discuss the methodology and the experimental approach followed for this work. The major limits and strengths of each study were highlighted and discussed in the corresponding chapter. At this point, and in order to give the reader some bases and clues to judge this work factually, an analysis of the different processes surrounding and leading to the decisions made for the design the studies is presented and discussed. This might help improving future researches.

Study foods

We aimed at studying the impact of salt, sugar and fat contents on food preference and food intake. Except for salsify, which was especially chosen for the experiment constraints (see chapter 4), usual foods were used in the studies: green beans, pasta, fruit puree and creamy white cheese. Target foods and ingredient choices were made for practical reasons (as discussed in each chapter). Besides, the variations we applied to each food aimed to be representative of those frequently used by parents or caterings while preparing foods. The aim was to work with 'realistic' variations, in order to be able to easily extrapolate the results to real practical usages. The variations chosen for ingredients reflect those that may be encountered in the foods available in the French market. For example salt level in sausage ranges from 1.3 to 6.9%, in cheeses it ranges from 1 to 2.5%, and in some frozen ready meals, it ranges between 0.98 and 2.5% (AFSSA, 2002). The 5% added butter corresponds to the maximum amount recommended for French caterings (Groupe d'Etude des Marchés de Restauration Collective et de Nutrition, 2011). The quantities of added sucrose, 0, 5, 10 and 15% correspond respectively to 0, 1, 2 or 3 tea spoons of sucrose, added to a jar of a 100 g of fruit puree or CWC and reflect the observed range on the market. Finally, the fat contents in cream white cheese, correspond to those available in the French market, and potentially purchased by parents for their children.
In other respect, a trained adult panel provided ratings of the intensity of the different tastes (saltiness, sweetness, sourness, bitterness, fattiness and umami) for all the foods used during this work. This was of great interest in interpreting some results obtained with preference or intake measures conducted with toddlers and children.

The choice of the procedure

The major objective of this work was to understand the role of salt, sugar and fat on toddlers and children's eating behavior, mainly focusing on food intake. The first decision of importance in this case, was to establish a protocol that was adapted to the toddlers and children. This protocol had to take into account the limited cognitive functions at these ages, i.e. a short attention span as well as limited logical thinking and verbal skills (as it was discussed in chapter 1 part 3). Thus, with regard to our primary aim, we focused on observations of food intake in realistic situations. Eating is an individual action that occurs frequently in a collective environment. So what is more natural than observing food intake in a collective and natural eating environment? As defined by Meiselman Meiselman, 2006: '*a natural eating environment is one that exists in nature without modification, such as the home, restaurant or cafeteria'.* This implies that in the case of toddlers and children, nursery or school canteens are an obvious choice of a natural meal environment. This wass particularly relevant as 63 % of French children, aged 3-17 years old, declare having lunch at the canteen (AFSSA, 2009). Thus, in order to obtain the most 'ecological' data of food intake we chose to ran our experiments in nurseries and schools canteens, in order to maintain the natural socio-affective context toddlers and children are used to (Birch, Zimmerman & Hind, 1980). Indeed, this guarantees that the environment is familiar and that the furniture (chairs, tables, kitchenware etc.) are adapted. Moreover, in such settings children are with their usual pairs and caregivers, which reduce study bias compared to the same study conducted in the laboratory.

The study design

The decision of conducting intake measurement outside the laboratory presents obvious advantages, but it is bounded by many constraints. For an experimenter/researcher, conducting a study outside the laboratory required several reflections while constructing the experimental protocol. Besides taking into account best practice used while conducting a sensory or consumer study in the laboratory, the experimenter had to adapt several steps of the experiment to the context of a 'field experiment'. For instance, food safety and security procedures followed in the laboratory had to be transposed to the field; if it was not already the case. The more known procedures were based on the Hazard Analysis Critical Control Point (HACCP), aiming at ensuring the safety of products (samples) given to the consumers (here, children). For example, if some products used during the experiments were prepared at the laboratory or purchased by the researcher, caution had to be given to the way they were transported to the field to ensure an adequate cold chain, by using cool boxes for example. During the preparation of such protocols the researcher had to be sure that the number of experimenters necessary to conduct the designed experiment, was sufficient to collect the data in an appropriate way. Indeed, the researcher had to be aware that s/he was an *outsider* in this context, and that her/his experiment had to adapt to the context and not the contrary. Thus, for the staff including cooks, caregivers and people in charge of looking after toddlers during the meal, a minimum involvement was required from their part, in order to keep them motivated until the end of the experiment. Indeed, with regard to the capacity of each canteen and the number of children attending a given meal session, the number of experimenters varied. In some of the present studies, 2- to 3 (*chapters 2 & 5*) and up to 8 (*chapters 3 & 4*) experimenters were necessary for a given observation day.

Another important point was the duration and number of interventions to be expected. This had to be as short as possible in order to keep the staff and children motivated all along the study duration. This limited the number of variants or conditions tested during this work. For example, it would have been of interest to test a wider range of salt content variations in the study conducted in chapter 2 (e.g. 0.3 and 0.9% in addition to those already tested), or intermediate sugar variations in the study conducted in *chapter 2* and *appendix 1* (e.g. 2.5 and 7.5% in addition to those already tested) and so on. However, this would have increased the number of interventions, and the nursery and school staffs might have lost interest in participating. In other respects, with regard to the literature a protocol aiming at studying mere exposure or flavor-flavor mechanisms, such as the one conducted in *chapter 4*, requires a minimum of 8 and up to 10 or 15 exposure sessions. However, in France, a 'school-time period' lasts 6 weeks, bounded by two holiday periods. In our case, we could not conduct more than 8 exposures for 4 weeks, surrounded by 2 weeks, one for the pre- and one for the post-test. However, given the results we were able to observe after 8 exposures, we are convinced that this was a sufficient exposure period, and we would not expect other results with one or two additional exposures.

In the same line, it was not possible to conduct a study in older children (8-11 year-olds) at snack time, in order to investigate their intake of a food varying in its sugar content, as it was the case for toddlers, since in France afternoon snacks are not organized in schools.

The 'authorizations' phase, location choice and familiarization

Now that all protocol constraints had been 'resolved', the next step was to recruit the participants. In this PhD work, recruitment was not made at the individual level, but the whole group of toddlers and children with the age range of interest was targeted through the nurseries or schools. In order to recruit in these institutions many steps were followed. First, the authorization of the 'early childhood service', depending on the Dijon City services, in charge of the young infant's education was necessary. After this, we presented our protocol(s) and discussed the feasibility of the study(ies) in the nurseries. At this point, the first challenge was to capture the attention and the interest and to present not only the scientific side of the experiment, but also the practical applications of the expected results. After specifying that the institutions (nurseries or schools) had to welcome children from different socio-economic backgrounds, and due to the confidentiality of such information, we were advised on which specific institution to contact. Besides, in Dijon only few nurseries/schools had a kitchen were the cook was independent for preparing foods; in other places the food was supplied by caterings and in these institutions it was difficult to integrate a part of our protocol and modify foods or menus. As an example, although motivated by our study protocol, the Dijon public school service could not participate. The city central kitchen where food was prepared and supplied to some nurseries and all schools in Dijon, ensured 7000 meals a day. In this context it was impossible for them to prepare a few meals (e.g. 40 a day) corresponding to our experimental meals, in order for us to bring salt/sugar/fat content variations afterwards. Thus, this option was excluded and concerning children (*chapter 3*), we had to conduct the study in a private school.

After the protocol was written and the preliminary authorizations were obtained from the city services and the institutions' directors, the following step was to obtain a validation from the local ethical committee, as it is mandatory according the French regulation for studies with human participants. In the case of the present work, all studies were approved by the local ethical committee (Comité de Protection des Personnes Est I Bourgogne). Moreover, the next step in the 'authorizations' phase, was to obtain the consent of the parents for their minor child to participate. To this aim, they were provided with a written information sheet and delivered a signed consent. The proportion of parents refusing their child to participate was close to zero, and the few cases across the whole work were majority due to suspected or known problems of allergy.

Methodological precautions

Even if the study took place out of the laboratory, the responsibilities in conducting the experiment were the same or even bigger. The experimenters' team was briefed, ahead of the study start, on the protocol, the locations they would attend and the procedure to be followed. In order to avoid biases, it was of importance to explain the procedure for data collection. Indeed, the outcome variable was intake (g), and it was accessed by weighing foods before and after each eating occasion for each child. All leftovers were post-weighed precisely, even those which ended their "journey" under the tables and chairs or on the toddlers' bibs. With regard to the results obtained, where significant effects were observed with a few grams of differences (e.g. a significant difference of green bean intake of 8 g was observed between the 0 and the 0.6% added salt variants); thus, the reliability of data collection cannot be questioned. Moreover, before the beginning of the study, and in order to minimize biases due to our 'unusual' presence, we visited the nurseries and school several times, in order for the toddlers, children and staff to become familiar with each other.

For all the experiments conducted as part of this work, meetings were organized with the teams of each institution, ahead of the study. These meetings had several aims: to introduce the research team and the experimenters who would come to help during the experiment; to explain the aim(s) of the study, its design and protocol to the teams and cooks present in each location. As we aimed at measuring the response to ingredient content variations in terms of intake, the study was designed assuming that the more the food is appreciated the more it will be consumed. For this reason, toddlers and children had to have access to *ad libitum* quantities of the target foods. Thus, one of the major strengths of our work was that the design fitted the nurseries and school we intervened in, as toddlers and children were used to ask for more servings if they wanted so.

One of the important points was to establish the responsibilities of each individual. Indeed, as I mentioned above, the studies were designed in order not to add extra work for the 'local' teams: caregivers were in charge of looking after the toddlers and children as usual, and maintaining calm conditions; in sum, behaving as usual with the group. Moreover, they were instructed neither to exhort nor to urge toddlers or children to eat, as we aimed at measuring their 'natural' intake of the foods we served, without any external influence. Another step was to motivate the cooks and the staff in charge of the meal preparation, and to explain the main goal of our experiment. Indeed, we entered "the cook's kitchen", used their material and somehow we encroached their territory. Luckily, and with a touch of diplomacy and kindness, the presence of the experimenters was welcomed. Besides, in the kitchens, a sufficient number of experimenters was planned in order to prepare all lunch items, and not to add extra work for the cooks and staff. A fun fact is that in a way, and as they told us, they were relieved from part of their cooking duties the days the experiment ran; although their help was precious.

***Advantages and limitations of conducting the studies in nurseries and school*.**

The major advantage of the study strategy we chose was that we were able to approach a great number of toddlers and children at a time. Besides, parents of toddlers attending the 6 nurseries included in the study presented in chapter 4, were from all socio-economic classes (SES), even if the proportions of the different categories differ from the French population (table 13).

Table 13. Comparison between the French population's socioeconomic status (SES) and that of parents of toddlers participating in the study from chapter 6.

SES	Both		Males		Females	
	INSEE[11]	Study	INSEE	Study	INSEE	Study
Farmers	2,0	0,0	2,7	0,0	1,2	0
Craftsmen, shopkeepers, business managers	6,0	2,1	8,2	1,7	3,5	2,4
Frameworks, higher intellectual professions	16,1	33,5	18,8	35,9	13,0	31,2
Intermediate professions	24,9	33,4	22,8	31,6	27,3	35,2
Employees	28,5	18,7	12,8	9,4	45,9	28,0
Laborers	22,6	12,3	34,8	21,4	9,0	3,2
Total	100,0	100,0	100,0	100,0	100,0	100,0
P value of χ^2	<0.0001		<0.0001		<0.0001	
P value of χ^2 (without category 1)	<0.0001		<0.0001		<0.0001	

As the experiment did not imply that the parents had to come to the laboratory, or to be in charge of any task related to the study at home, they were more willing to accept their child's participation. This probably avoids that only children whose parents felt concerned by nutrition and health took part in the studies. However and as a consequence, even if some

[11] INSEE : National Institute of Statistics and Economic Studies – Institut National de la statistique et des études économiques

questions had to be asked, in order for example to characterize the population, a minimum of questions had to be asked in order to have a good and satisfying response rate. Yet, if we consider that we recruited the nursery/school and not the individual children, the response rates we obtained when using questionnaires was more than satisfying: in chapters 2, 3 & 4, the proportion of responses was respectively equivalent to 73% (54 / 74 questionnaires back), 87% (65 / 75 questionnaires back) and 80% (120 / 151 questionnaires back). However, it must be underlined that to get this response rate, some parents were sent the questionnaires twice or even three times, caregivers/teachers were kindly requested to remind them to give the questionnaires back, and we also went to the nurseries several times to see the parents and give them another copy of the questionnaire if necessary.

Few subjects dropped out of the experiments (table 14). The gap between the initial and the final sample for each study was mainly owed to absences due to illnesses. Thus it was necessary to define criteria for considering data from a given child as valid for inclusion in the analyses. For instance, in *chapters 2, 3 and 5*, only those children who participated to at least 2 out of 3 observation days were considered. Moreover, in *chapter 3*, data were removed for some children who attended the sensory analysis session in the laboratory, but who did not complete the criteria for intake data (or vice versa), thus resulting in a greater loss of subjects. In *chapter 4*, due to the longer experimental period, in addition to 'regular' drop outs due to illnesses, there were also some children who moved or changed nursery during the experiment period. The percentages given in this table could be useful to calculate the sample size for future research.

Table 14. Summary of the number of toddlers and children involved in the PhD work.

	Chapter 2			Chapter 3	Chapter 4	Chapter 5	Appendix 1
Ingredient	**Salt**	**Sugar**	**Fat**	**Salt**	**Salt**	**Fat**	**Sugar**
Initial sample	74	74	74	106	171	67	60
Final sample	66	61	69	75	151	56	50
% final/initial	89	82	93	71	88	84	83

Several limits were noted. As the experiments took place with whole groups, the individual responses may have been different if the study had taken place at home, according to what the child was used to be served at home. However, in the context of our experimental design, it was not possible to 'personalize' the recipes. Moreover, to my knowledge, to date no precise questionnaire is available in order to estimate the quantities of salt, sugar or fat parents add to their children's foods. Although we tried to collect these data, only few parents answered accurately the questions, and the exploitation of the results was compromised.

3. General discussion

Detailed discussions on each topic were already presented within each chapter (2-5) and in the section above. In this part, I will consider the results as a whole, discuss and compare the relative effects of salt, sugar and fat on food preference and intake.

Compared to salt, sugar and fat to some extent appeared to be less critical ingredients in driving toddlers' food intake (*chapters 2-5*). Besides, the immediate attractiveness for fat (as judged by intake within a meal) was not verified by the present work (*chapter 2 & adults in chapter 5*), although large variations in fat content elicited a decrease in intake when fat content increased (*toddlers in chapter 5*). It is obvious that compared to fat, sucrose level did not refrain children from consuming neither the sweetened fruit puree (*chapter 2*), nor the sweetened creamy white cheese (*appendix 1*), which were consumed in equal amounts at medium or high levels of added sucrose; whereas the creamy white cheese with the higher fat-content was less consumed than the other variants (*toddlers in chapter 5*). If we reason in terms of pleasantness, we could speculate that the pleasantness induced by the sweet taste was so attractive that it overcame any possible regulation of intake. If we reason in terms of energy density, the 23% decrease in energy density between the 15 and the 5% added sugar creamy white cheeses that induced no change in intake (*appendix 1*), was smaller than the 50% decrease between the 40 and 0% fat creamy white cheeses (*chapter 5*), which resulted in a decrease of the high-fat version intake. We could speculate that if an even sweeter and more energy-dense version had been proposed to toddlers (*appendix 1*), they would have adapted their energy intake as a consequence, by reducing their weight intake.

Although it is difficult to compare the foods used during the present work, as they all differ in affective value, texture and source of energy (sucrose or fat), the table 15 below provides factual data in order to understand the effect of energy density on intake.

Table 15. Difference of energy density (kcal) between target food variants, and its effect on intake.

Chapter	Food (variation)	Considered variants	Δ kcal	Effect on intake
Chapter 2	**Green beans (% butter)**	2.5 - 0	18	NS
		5 - 2.5	17	NS
		5 – 0	35	NS
Chapter 2	**Pasta (% butter)**	2.5 - 0	16	NS
		5 - 2.5	14	NS
		5 - 0	30	NS
Chapter 2	**Fruit puree (% sucrose)**	5 – 0	17	NS
		10– 5	15	NS
		10 – 0	32	NS
Chapter 5	**CWC (% fat)**	20 - 0	26	NS
		40 - 20	41	NS
		40 - 0	67	↗
Appendix 1	**CWC (% sucrose)**	5 – 0	15	↘
		10– 0	29	↘
		15 – 0	42	↘
		10 – 5	15	NS
		15 –5	27	NS
		15 – 10 %	13	NS

NS = Non-significant effect

In their review published in 1997, Blundell and MacDiarmid hypothesized that the processes controlling satiation during the consumption of high-fat foods "*might be too weak or too slow to prevent the intake of a huge amount of energy*"; they also concluded their paper by saying that "*the human psychobiologic predilection to derive pleasure from eating* *allows the environment to overcome physiology*" (Blundell & Macdiarmid, 1997). Our results confirm their conclusion and bring more precisions: this was true in our adult subjects, but this was not always verified in toddlers. This brings us to our main interest on studying eating behavior in toddlers. Indeed, in 1994, Cashdan suggested the existence of a "sensitive period in the first to three years of life during which humans acquire a basic knowledge of what foods are safe to eat" (Cashdan, 1994). At this period, they might also learn the energy and rewarding value of foods, and how much they should consume in order to satisfy their energy needs. Besides, the 'wisdom of the body' theory stating that 'food preferences reflected innate, unlearned 'special appetites' for needed nutrients, including sugar, salt, fat, protein, carbohydrate, and micronutrients" (Birch, 1999), could be lost in between toddlerhood and adulthood, due to the wide availability of, and great exposure to, high-fat / high-sugar foods, which would disrupt the availability to self-regulate intake in response to foods' energy content.

Future research should clarify the 'immediate' role of these ingredients on liking and intake, from their role as drivers for learning to like and to consume foods.

4. Implications

Nutritional policy makers advise a reduction or at least a limitation of salt, sugar and fat quantities in food preparations, for the general population and for children in particular. This section will be presented as an 'advice sheet' or a 'take-home' message, that could be useful for parents, caregivers and nutritional policy makers, in order to improve eating behavior and establish healthy eating habits early in life. My aim is not to spread a scary message about salt, sugar and fat, nor to tell individuals to refrain from consuming tasty foods. It is mainly to give some lines of thought and advices. Indeed, this work brings new insights for the possibilities:

- to reduce levels of salt, sugar and fat in some foods served to toddlers and children;
- to decrease energy intake in toddlers;
- to increase vegetable intake.

Sugar (sucrose) can be decreased at least in foods prepared at home or in the canteens, without affecting toddlers' or children's food intake and preference. However, small quantities of sucrose (about 5 %) can be used to elicit a sweet taste, as the goal is not to remove the entire pleasure accompanying food intake. Hence, the pleasure for consuming the food would remain without the associated energy intake.

Concerning fat, butter for example can be limited while preparing pasta for example, without affecting food intake. For green beans, the same conclusion could be given, but if we take into account their initial low energy density, we could consider that adding some butter could be nutritionally acceptable, especially for young children, who need energy for growth, and for whom, fat intake is not restricted. If for some health-oriented reasons the aim is to reduce energy intake in toddlers or adults, choosing a low-fat or even medium-fat dairy product when purchasing foods would be a wise decision.

Concerning salt, its reduction may be more difficult and should be considered cautiously. If reducing salt while preparing liked foods such as pasta appears feasible without affecting toddlers' or children intake, in vegetables such as green beans this is less obvious. On the one hand, recommendations aim at 'increasing vegetable intake' and on the other hand they aim at 'decreasing salt contents'. However, it seems that from a practical point of view, due to the ability of salt to reduce vegetable bitterness, the recommendations of increasing vegetable intake and decreasing salt content in vegetables, can only co-occur if the used amount in not too low. Although this could be perceived as contravening public health messages, nutritional recommendations should be more precise at this point, and reconsidered for this food category; yet, 'the end justifies the means'. Indeed, this would not be different from the usage frequently observed in some countries, where mothers add sucrose or sweet applesauce to vegetables in order to increase their child's intake. In the case of vegetables, adding a pinch of salt might appear useful to promote their intake.

In addition to using repeated exposure (Birch et al., 1998), other strategies aiming at increasing vegetable intake can be deduced from the present work. One would be to present vegetable as a starter for toddlers, when the child is hungry, and this was also shown to be an effective strategy to increase 3-5 year-olds' vegetable intake (Spill, Birch, Roe & Rolls, 2010). Besides, using a salt-associated spice would be an effective strategy to increase toddlers' vegetable intake, although it was less efficient than a repeated exposure to the vegetable recipe containing a pinch of salt.

5. Perspectives

This PhD work might point to some gaps that could be filled by future researches.

Food-specific effects. This work was conducted on a restricted list of foods, representative of different food categories (vegetables, fruits, dairy products and starches). We observed food-specific effects of ingredient level on intake: in children who reacted differently to salt in green beans and salt in pasta; in toddlers who reacted differently to fat in pasta and in a dairy product; and in toddlers who reacted differently to sugar in a fruit puree and in a dairy product. These observations should be verified by future research, focusing on 'food-specific' effects in order to specify nutritional recommendations.

For instance, as it was reviewed (see part 2) salt masks bitterness, and this might be a key driver for vegetable intake. Thus, investigating the specific effect of salt on various vegetables (more or less bitter), would give more insight on methods to increase vegetable acceptance.

Concerning sugar, it would be worth investigating the acceptance (liking and intake) of different products containing the same amount of sugar. For example: liquids vs. solids; foods of different textures etc.

Concerning fat, investigating the acceptance of different products, with the same amplitude of fat differences (between a low- and a high-fat version) but with different effects on their sensory characteristics would also be of interest. That would give more insight on the limits of fat reduction from a sensory point of view.

Another point to be further investigated concerns the relative effect of the three ingredients on acceptance, for a given individual. In other words, is it easier to remove or reduce, salt, sugar or fat? This was party investigated in chapter 2, however, it could be interesting to test in different foods in order to generalize the findings. Although it would be interesting to compare the response to salt, sugar and fat in a single actual food, it is difficult to find a food where, technically, variations of salt, sugar and fat can be performed. However, it would be possible to look in a given food at variation of fat and sugar, or of fat and salt.

Reducing salt, sugar and fat in toddlers' and children's food. Strategies aiming at gradually reducing salt in foods for example, were experienced among adult population. However, this issue has not yet been investigated in the child population. Although for sugar this seems needful as possibilities of reduction appear possible, this is not the case for salt and vegetables. Indeed, finding an optimal level of added salt in vegetables, while maintaining an appropriate liking and intake would be of interest.

Follow-up for learning study. In order to investigate the long-lasting effect of mere exposure and flavor-flavor learning mechanisms investigated in chapter 4, follow-up studies were conducted at 3 and 6 months after the exposure period. The analyses of these results would bring more insight on the long-term effectiveness of the three tested approaches, on liking and intake. Besides, analyses of the questionnaires filled by the parents, would be of interest in order to explain data in terms of environmental and behavioral factors e.g. food neophobia, global frequency of exposure to and liking for to vegetables and seasonings, child's general attitude towards foods etc. These analyses are on-going.

Sugar and food behavior. The discrepancy between preference and intake in response to salt we observed in 8-11 year-olds, raises questions about a similar pattern in children's response to sugar in food. This could be worth investigating.
Besides, the present work highlighted the fact that a low sucrose level did not prevent toddlers from consuming neither the fruit puree nor the creamy white cheese. As the variants were differentiated by an adult panel, the only hypotheses remaining would be an indifference to sweetness while eating. This possibility is still to be verified.

Environmental factors. As we discussed it during this manuscript, food habits and experience with seasonings and ingredients might shape preferences towards a given level in foods. During this work, we tried to investigate habits of adding salt, sugar and fat at home, in order to explain some intake or preference data we obtained, but this question appears to be hard to explore; mainly due to the lack of a standardized and validated tool to collect this information. To date, a questionnaire (PrefQuest) was developed in our laboratory, but it only assesses recalled liking for sweet, salt, and fat, not actual quantities used (Deglaire et al., in press).

Besides, ingredient usages by caterings and canteen cooks would be interesting to investigate and clarify, as the majority of toddlers and children have lunches and/or snacks at the school canteen/restaurant/cafeteria…

The link between food preferences, food intake and obesity would also be worth investigating further. In the present work, a relation between toddlers' and children's z-BMI and pasta intake was found (*chapter 2 & 3*). We did not observe a systematic link between the majority of ingredient variations and children's z-BMI. However, this relationship in toddlers was shown between z-BMI and the intake of the pasta with the 5% added butter (*chapter 2*). However, the causal connection between sugar and fat contents of foods, preferences, intake and the prevalence of overweight and obesity is understudied. It would be interesting to study these relationships in a child population at risk of overweight and obesity, already overweight/obese, and compare the results with a control population. This could be worth investigating in the perspective of a longitudinal study (Salbe et al., 2004).

Regulation of energy intake. The primary aim of the work conducted during this PhD thesis, was to investigate the impact of food ingredients on food intake considered as a proxy of preference on children. Thus the experiments were not directly designed to investigate energy intake regulation. However, our experiment revealed interesting results on short term regulation of food intake in toddlers vs. adults. It would be worth investigating this question further, using other protocols than pre-load studies. Indeed, in real life, it is not always the case that we eat a preload before a real meal. Thus, measuring the effect of energy density variations within a meal on regulation of daily energy intake would be of interest.

Besides, it would be of interest to further investigate the discrepancy between infants, toddlers, children and adults' energy intake regulation (see figure 26). That would be of interest in order to identify the time frame when this regulation is compromised, and the factors leading to it, in order to address this issue.

Increasing vegetable intake. The strategy we tested in chapter 4, focusing on using a salt-associated spice to increase toddlers' vegetable intake, is worth exploring. Again, and due to the investigation strategy chosen for this PhD work i.e. conducting studies in nurseries with a whole group, it was not possible to adopt an individual recipe for each child, with a vegetable identified as neutral and a spice identified as well liked. But this could be of interest for future research.

6. Conclusions

Food sensory qualities are important drivers of eating behavior and its development. Besides signaling nutrient content, they contribute to the regulation of energy intake as suggested by our results on fat. We could speculate that the great range of foods found on the market, with varying energy and fat contents but with similar appearance, could be confusing for the consumers. That is especially problematic in the perspective of a regulation of energy intake. This observation is particularly true for fat, which is not well detected by humans. Understanding the effects of food sensory qualities on food preference and intake during the first years of life, appears of importance in order to establish long-lasting healthy eating behaviors. This work contributes to a better understanding of salt, sugar and fat impact on toddlers' and children's eating behavior. The present findings would be of interest for parents, nutritional policy makers and caregivers, in order to understand children's eating behavior, and to better adjust their strategies for reducing salt, sugar and fat, decreasing energy intake and increasing vegetable intake.

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Appendices

Appendix 1: *Poster presentation (English)*

Bouhlal S., Bernard C., Issanchou S., Nicklaus S. (2011). "Sugar content impacts food intake in toddlers, but could be reduced" *Appetite 57 (1): S7.* doi:10.1016/j.appet.2011.05.132

Sugar content impacts food intake in toddlers, but could be reduced

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Introduction

Governmental policies aim at reducing the amount of added sugar in foods, due to its supposed contribution to the rising rate of overweight and obesity.

The impact of such a reduction on young children's food intake remains imperfectly known.

Aims

Evaluating 2-3 year-old children's intake of a naturally unsweetened food according to its added sugar content.

Providing a sensory description of the products (taste and textural cues).

Participants

	N	Gender (♀:♂)	Age (years)	BMI ($kg.m^{-2}$)	zBMI
Toddlers	50	26:24	2.2 ±0.3	16.2 ±1.3	-0.17 ±1.08
Untrained adults	31	19:12	22.2 ±1.8	22.4 ±3.1	—

Food

A commercial creamy white cheese (CWC) with 20% fat (dry weight; Jockey, Danone).
Four sugar contents were chosen: *0, 5, 10 or 15%* added sucrose.

Procedure

Sensory description & liking: untrained panel

Thirty-one adults were asked to rate, for each of the four variants of white cheese:

→ their perception of sweetness, sourness, thickness, creaminess, dryness, fattiness and after-taste.

→ their liking

Sensory description : trained panel

Taste intensities were evaluated using Spectrum™ scales. These linear scales have reference points illustrated by taste solutions of known concentrations.

Intake : toddlers

An afternoon snack was served four times to toddlers at their usual nursery, once a week at 3pm.

It was composed consecutively of:

- one of the four variants of the creamy white cheese (*up to 300 g*).
- biscuits, milk and/or water.

Participants were allowed to ask for more cheese if they wanted so.

All foods were weighed before and after each consumption +/-1g.

Results

Sensory description : untrained panel / Sensory description : trained panel

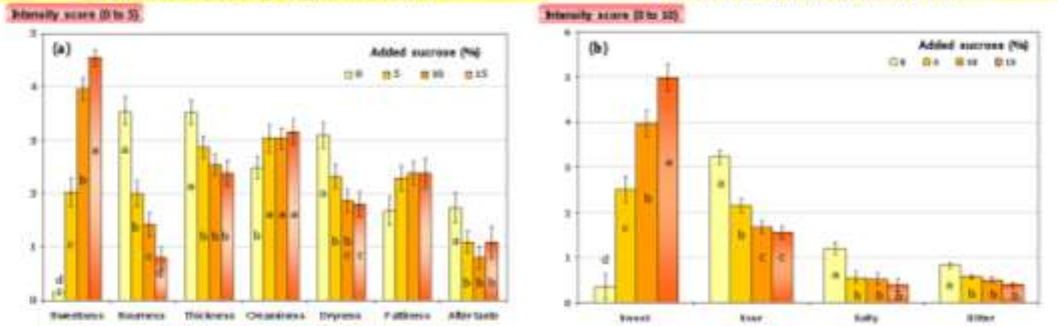

Fig. 1. Sensory description of creamy white cheese with varying added sucrose performed by (a) an untrained adult panel and (b) a trained adult panel (lsmeans ±SEM). Means with the same letter are not significantly different ($P<0.05$).

Intake : toddlers

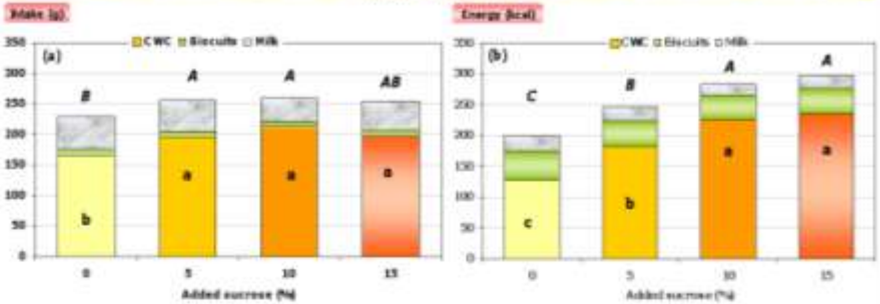

Fig. 3. Impact of added sucrose on (a) intake (g) and (b) energy intake (kcal) (lsmeans ±SEM). Means with the same letter are not significantly different ($P<0.05$). Lower case letters = food level / Upper case letters = snack level.

Liking : untrained panel

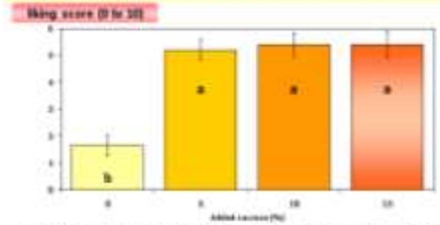

Fig. 2. Liking scores for the creamy white cheese with varying added sucrose (lsmeans ±SEM). Means with the same letter are not significantly different ($P<0.05$).

The unsweetened CWC was less liked than the sweetened ones.

The sensory characteristics of the four CWCs were distinguishable.

The unsweetened CWC was less consumed by 2-3 year-old children than the sweetened variants.

The intake of all sweetened CWCs (5, 10 and 15 %) was similar.

The addition of sucrose in CWC from 0 to 15% was associated to an increase in energy intake at the snack level, with a plateau from the 10% variant.

Conclusions

- The unsweetened CWC was less liked by adults and less consumed by toddlers compared to the sweetened variants.
- Despite their perceived sensory differences, the three sweetened CWCs (5, 10 and 15% added sucrose) were consumed in a same amount by toddlers, and liked equally by adults.
- A sugar content that maximizes liking without affecting intake, may exist between the 0% and the 5% added sucrose level.
- The current observed level of added sugar in commercial dairy products can be high (higher value in French products ~ 14 %). It seems possible to lower this amount without reducing children's food intake.

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12 - 16 July, 2011
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Appendix 2: *Review paper (French)*

Nicklaus S., **Bouhlal S.**, et al. (2010). [Development of fat preferences in children]. Développement des préférences pour les lipides chez l'enfant. *Innovations Agronomiques* 10: 115-124.

Innovations Agronomiques 10 (2010), 115-124

Développement des préférences pour les lipides chez l'enfant

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Résumé

Chez l'enfant de moins de 3 ans, les lipides apportés par l'alimentation jouent un rôle essentiel dans le développement notamment des fonctions cérébrales. Chez l'enfant plus âgé, la part d'énergie apportée par les lipides ne semble pas contribuer au développement de l'obésité, mais la qualité des lipides pourrait être impliquée. La nature des lipides apportés par l'alimentation des enfants dépend beaucoup des aliments choisis et des pratiques culturelles. Comprendre le développement des préférences pour les lipides est donc crucial. Peu de travaux portent sur les préférences de nouveau-nés pour les lipides mais ils semblent indiquer l'absence d'une préférence « innée » pour ces nutriments. Des solutions lipidiques pourraient même être de plus en plus rejetées au cours de la première année. Chez l'enfant (et l'adulte), la teneur en lipides d'un aliment a parfois un effet positif sur son appréciation, avec souvent une teneur optimale, qui dépend de l'aliment. Chez l'enfant, l'augmentation de la teneur en lipides n'est pas associée à une plus forte consommation, indiquant l'absence d'effet à court terme de la teneur en lipides sur la consommation. En revanche, différents travaux mettent en évidence l'effet des lipides dans les apprentissages alimentaires, en lien avec leur apport énergétique, fortement renforçateur des préférences. Enfin, les pratiques éducatives parentales viennent également interférer avec le développement des préférences pour les aliments lipidiques.

Mots-clés : enfant, lipides, préférences, consommation, apprentissage

Abstract: Development of preference for lipids in children

In children below the age of 3 years, lipids from foods play an important role in the development of brain functions. In older children, the percentage of energy from fat does not seem to contribute to the development of obesity, but the quality of fat could be involved. The nature of lipids in children's foods largely depends upon chosen foods and cultural practices. Therefore, understanding the development of preferences for lipids in foods is essential. Newborn preference for lipids was not much studied but it seems that there is no "innate" preference for these nutrients. Lipid solutions could even be more and more rejected during the course of the first year. In children (and adults), the fat content of a food is sometimes associated to a higher liking, very often with an optimal level, which depends upon the considered food. In children, increasing fat content is not associated to a higher short-term intake, indicating the absence of short-term effect of fat content on intake. On the contrary, different studies show an effect of fat on preference learning, in relation with their energetic value, which is a strong reinforcer of preference. Finally, parental educative practices also interact with the development of preference for fatty foods.

Keywords: children, lipids, preference, liking, intake, learning

Introduction

Les lipides dans l'alimentation des enfants ont parfois une image négative auprès de certains parents ou professionnels de santé et de l'enfance, comme d'autres nutriments, par exemple les sucres. A ce sujet, il est parfois difficile de faire la part des faits scientifiques, des idées reçues et des convictions personnelles.

Dans le contexte actuel qui voit la prévalence de l'obésité infantile atteindre 16% en France (Salanave, et al.., 2009), l'enjeu est de taille : et si les lipides contribuaient à la constitution de l'obésité infantile ? Le fait que les préférences et le répertoire alimentaires acquis dès la petite enfance soient très stables jusqu'au début de l'âge adulte souligne l'importance de comprendre les facteurs déterminants la préférence pour les lipides dès l'enfance (Nicklaus et al., 2004 ; Nicklaus et al., 2005).

L'existence d'une préférence pour les lipides présuppose que ces nutriments puissent être perçus par l'organisme. Cette perception des lipides est complexe puisqu'elle est associée à plusieurs composantes sensorielles : une composante texturale, une composante olfactive et peut-être une composante gustative, notamment en terme de détection des acides gras, dont le mécanisme complet est toujours à l'étude chez l'homme (Voir Besnard et al., ce numéro). Les lipides ont un impact important sur la texture des aliments et contribuent à leur appréciation. Ils sont aussi un support d'arômes important et certains acides gras possèdent leur propre composante olfactive (Voir Féron et Guichard, ce numéro). Il semble que chez l'homme, la perception du caractère gras d'un aliment soit difficile à cerner, voire inconsciente (Mela, 1992), et dépende surtout de ses caractéristiques de texture (Bouhlal et al., 2009 ; Drewnowski et Schwartz, 1990 ; Mela, 1990).

Dans tous les cas, la détection sensorielle des lipides dans les aliments, quel que soit le sens ou les sens concernés et que cette détection soit consciente ou non, permet par suite d'apprentissages la détection du caractère énergétique de ces nutriments, et est probablement à rapprocher de l'ajustement de la prise énergétique en fonction de la densité énergétique des aliments.

Nous décrirons d'abord l'origine des lipides dans l'alimentation des enfants, puis nous aborderons brièvement la question du lien entre apports lipidiques alimentaires et étiologie de l'obésité sur la base de synthèses scientifiques récentes. Nous présenterons ensuite les travaux décrivant l'impact de la teneur en lipides sur les préférences et les consommations alimentaires des enfants, moins nombreux que ceux portant sur le lien entre prise alimentaire et obésité. Nous évoquerons ensuite le rôle des lipides dans les apprentissages alimentaires, et dans la régulation de la prise alimentaire. Enfin, l'enfant mangeant toujours au cours de circonstances sociales (avec ses parents au moins), nous évoquerons le rôle des influences parentales dans l'établissement des préférences pour les lipides.

Les lipides dans l'alimentation des enfants : de quoi parle-t-on ?

En matière d'alimentation, la naissance puis l'enfance se caractérisent par des transitions majeures dans les modalités d'apport. L'alimentation se fait d'abord par l'intermédiaire du cordon ombilical, puis l'apport de nutriments se fait par le biais d'un seul aliment, le lait, fourni par la mère ou par des préparations de substitution. Enfin, à partir de la diversification alimentaire, des aliments 'solides' sont introduits progressivement dans le régime de l'enfant, d'abord sous une forme mixée adaptée aux capacités orales de l'enfant, puis sous une forme de plus en plus proche de l'alimentation de l'adulte. Une des conséquences de cette transition alimentaire est que l'enfant doit adapter sa consommation énergétique à des aliments de densité énergétique variable, et apprendre à apprécier ces différents aliments pour éventuellement guider ses choix en fonction de ses besoins et de la « satisfaction » physiologique apportée par chaque aliment. Nous reviendrons sur cet aspect ultérieurement.

Les lipides ainsi apportés dans l'alimentation des enfants sont d'origine et de nature très différentes : lors de la grossesse et de la période d'allaitement, les profils lipidiques dépendent en grande partie de l'alimentation de la mère (Drouillet et al., 2009 ; Kent et al., 2006 ; Mitoulas et al., 2003 ; Xiang et al., 2005). Lors de l'alimentation lactée de substitution et à partir de l'introduction d'aliments solides, les profils lipidiques dépendent de la nature et de la préparation des aliments choisis (Nicklas et al., 2009). Ces choix dépendent en grande partie de la culture alimentaire des parents. Par exemple, les mères italiennes ajoutent de l'huile d'olive aux aliments de diversification de leurs enfants (Greco et al., 1998). Les mères françaises pratiquent probablement des ajouts de matière grasse selon la traditionnelle coupure culturelle beurre/huile entre le nord et le sud de la France ! Une enquête sur les pratiques de diversification menée à Dijon indiquait que parmi les mères qui préparaient les aliments de leur enfant (46% de l'échantillon interrogé), 17% ajoutaient de l'huile, 30% de la crème et 52% du beurre (Maier et al., 2007).

Lors de la période d'alimentation lactée et pendant les trois premières années de vie de l'enfant, différents travaux et recommandations nutritionnelles soulignent la nécessité d'un apport en lipides important, et selon un profil lipidique optimisé (Uauy et Dangour, 2009). Pendant cette période, les restrictions d'apports lipidiques ne sont pas de mise et les aliments « allégés » en lipides ne sont pas adaptés à l'enfant. Par ailleurs, on peut souligner qu'un grand nombre d'aliments riches en lipides sont également riches en sel ou en sucres, autres ingrédients appréciés des enfants et qui peuvent contribuer à rehausser leur appréciation, dont l'intérêt nutritionnel est discuté.

Quel est le lien entre consommation de lipides et développement de l'obésité chez l'enfant ?

La forte densité énergétique des lipides et le stockage corporel de l'énergie sous forme de tissu adipeux ont souvent été rapprochés ; ainsi de nombreux travaux ont cherché à faire le lien entre la part de l'énergie apportée par les lipides et le développement de l'obésité chez l'enfant. Des revues récentes de tels travaux mettent en évidence l'absence de lien systématique entre la part d'énergie apportée par les lipides et l'obésité chez l'enfant (Mace et al., 2006 ; Rodriguez et Moreno, 2006). Néanmoins, certains lipides pourraient être impliqués dans le développement de l'inflammation et de l'obésité chez l'enfant, et un profil de consommation riche en acides gras n-6, saturés et *trans* et pauvre en acides gras n-3 pourrait être délétère (Ailhaud et al., 2007 ; Innis, 2007 ; Robinson et Godfrey, 2008 ; Zimmermann et Aeberli, 2008).

Ainsi, si la quantité totale de lipides ingérés ne peut être directement mise en cause dans l'étiologie de l'obésité chez l'enfant, le rôle de certains acides gras doit être considéré ; soulignant l'importance de considérer les aliments sources de lipides, dont le profil en acide gras peut varier. Cela renforce toute l'importance de tenir compte des préférences des enfants pour différents aliments, dont les profils en acides gras sont variables.

Les préférences pour les lipides dans l'enfance

La préférence pour des solutions d'acides gras a été montrée chez le rat (Tsuruta et al., 1999) et chez la souris (Laugerette et al., 2005). Cette préférence dépendrait essentiellement de leur perception gustative (Laugerette et al., 2005), et notamment pour les acides gras à longues chaînes (>16 carbones). Chez l'homme, la préférence pour les lipides dans les aliments pourrait s'expliquer par leur caractère palatable et par l'amélioration des qualités organoleptiques des aliments dans lesquels ils sont présents ou ajoutés. Chez l'enfant humain, la préférence pour différents types d'acides gras n'a pas été explorée systématiquement. De plus, chez l'homme, les acides gras isolés présentent fréquemment des caractéristiques sensorielles jugées déplaisantes (amertume, irritation, arôme de rance…) qui rendent l'évaluation de leur appréciation difficile. Nous présenterons ici différents travaux explorant la préférence des enfants pour les lipides qui ne distinguent pas généralement la nature des acides gras utilisés. Ces travaux répondent à trois questions :

- quelle est l'influence de la teneur en lipides sur la préférence pour l'aliment ?
- quelle est l'influence de la teneur en lipides sur la consommation de l'aliment ?

- dans quelle mesure les lipides favorisent-ils les apprentissages alimentaires ?

Influence de la teneur en lipides sur les préférences

La réaction des nouveau-nés aux aliments lipidiques a reçu peu d'attention. Lorsque quelques gouttes d'huile de maïs sont présentées à des nouveau-nés aucun effet n'est observé alors que le sucre et la quinine modifient leurs cris, leurs mimiques faciales et le contact des mains avec la bouche (Graillon et al., 1997). Ainsi, on ne peut avancer l'existence d'une préférence « innée » pour le gras, contrairement à la préférence pour la saveur sucrée et au rejet de l'amertume, même si cette question mériterait de plus amples recherches. De plus, des nouveau-nés et des nourrissons de 1 mois consomment autant de lait à teneur élevée en matière grasse que de lait à teneur réduite en matière grasse (Chan et al., 1979 ; Woolridge et al., 1980). En revanche, leurs patterns de succion sont plus longs lors de la consommation du lait le plus gras (Nysenbaum et Smart, 1982).

La préférence pour une solution de lipides (mélange d'huiles alimentaires émulsionnées) a été évaluée relativement à de l'eau, chez les mêmes enfants vus à 3, 6 et 12 mois. Cette préférence se traduit par un indice de consommation de la solution lipidique par rapport à de l'eau, indice qui varie par construction entre 0 et 1, un niveau de 0.5 traduisant l'indifférence comparativement à l'eau (Schwartz et al., 2009). Ces indices de consommation pour des solutions reflétant les cinq saveurs dites primaires et pour le « gras » et pour 3 âges sont représentés sur la Figure 1. On peut comparer ces indices pour un âge donné d'une saveur à l'autre, ou pour une saveur donnée d'un âge à l'autre. Alors qu'on observe une augmentation de la préférence pour les solutions sucrée (lactose) et salée (NaCl) de 3 à 12 mois, on observe une diminution de l'indice de consommation de la solution lipidique entre ces âges, partant d'un niveau reflétant l'indifférence et arrivant à un niveau indiquant le rejet. Toutefois, il est difficile de savoir si cette baisse de l'acceptabilité est due aux lipides ou aux odorants présents dans l'émulsion lipidique présentée aux enfants. En effet, il est impossible dans une telle préparation d'éviter la présence de composants volatils issus de l'oxydation des lipides et chez le nourrisson les évaluations ne peuvent pas se faire avec un pince-nez comme ceci se pratique chez l'adulte.

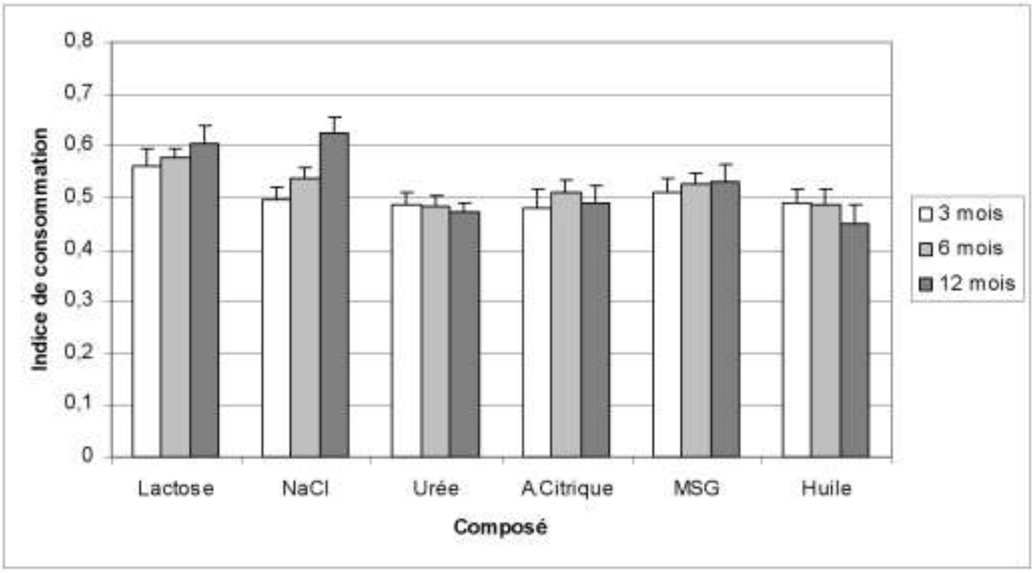

Figure 1 : Indice de consommation (+ Intervalle de confiance à 95%) pour des solutions de différentes saveurs (lactose : sucré ; NaCl : salé ; Urée : amer ; A.Citrique : acide ; MSG : umami ; huile : 'gras') observé chez les mêmes enfants (N=47 à 87) à différents âges (3, 6 et 12 mois). (Source : S. Nicklaus)

De tels résultats montrent une préférence à court terme et en l'absence d'effets post-ingestifs de composés tels que le sucre et le sel, mais ils ne montrent pas une préférence pour des lipides en solution, bien au contraire, alors que le lait consommé au cours de cette période est généralement riche en lipides. Ceci suggère que chez l'enfant, un caractère immédiatement plaisant est perçu pour des stimulations salées et sucrées mais pas pour des stimulations lipidiques. De plus différentes observations conduites chez des enfants plus âgés ou des adultes soulignent la forte appréciation d'aliments riches en lipides (Alexy et al., 2001 ; Cooke et Wardle, 2005 ; Perl et al., 1998 ; Rogers et Emmett, 2002).

Très peu de travaux chez l'enfant ont porté sur une évaluation directe de l'impact de la teneur en matière grasse sur les préférences. Chez l'adulte, certains travaux montrent que des fromages blancs à 40% sont plus appréciés que ceux à 0% de matière grasse (voir Figure 2), mais cela ne prédit pas nécessairement leur consommation, qui peut être identique (Bouhlal et al., 2009 ; Daillant et Issanchou, 1993). Cette observation pourrait dépendre de l'âge. En effet, des aliments riches en matière grasse sont plus appréciés des jeunes adultes que des aliments peu riches en matière grasse, alors que cet effet n'est pas observé chez des sujets plus âgés qui ne distinguent pas les différentes teneurs en matière grasse (Warwick et Schiffman, 1990). De plus, chez l'adulte, les études ne mettent pas systématiquement en évidence une préférence pour les aliments les plus riches en lipides, et la teneur en lipides préférée est souvent différente d'un aliment à l'autre, rendant difficile la généralisation de telles observations (Mela et Marshall, 1992 ; Mela et Saccheti, 1991).

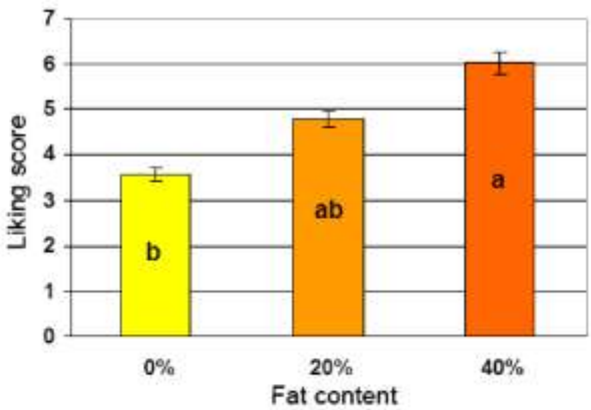

Figure 2 : Note d'appréciation (liking, 1-7, moyenne ± intervalle de confiance à 95%) d'adultes (N=30) pour des fromages blancs de teneur variable en matière grasse (0, 20 et 40%). (Bouhlal et al., 2009)

Influence de la teneur en lipides sur la consommation alimentaire

Dans le contexte actuel du PNNS qui encourage une limitation des apports en sucre, sel et matière grasse dans l'alimentation (Ministère de la Santé, 2006), on peut s'interroger sur l'impact d'une modification de la teneur de ces ingrédients/constituants sur la consommation alimentaire d'enfants. Nous avons abordé cette question en évaluant les consommations d'enfants de 2 à 3 ans vus dans leur contexte habituel de consommation (crèche) (Bouhlal et al., sous presse). Nous avons fait varier la teneur en sucre, sel et matière grasse dans différents aliments et mesuré la consommation de ces différents aliments par les enfants, lors de différents repas espacés de 2 semaines. L'ajout de beurre dans des haricots verts ou des pâtes n'a eu aucun effet significatif sur leur consommation par les enfants, comme représenté Figure 3. Le même travail met en revanche en évidence l'impact de la teneur en sel sur la consommation des mêmes aliments : plus elle est élevée, plus l'aliment est consommé (Bouhlal et al., sous presse). De plus, nous avons observé une relation positive entre la consommation de pâtes et la corpulence des enfants, relation d'autant plus forte que les pâtes étaient riches en matière grasse (beurre) (Bouhlal et al., sous presse).

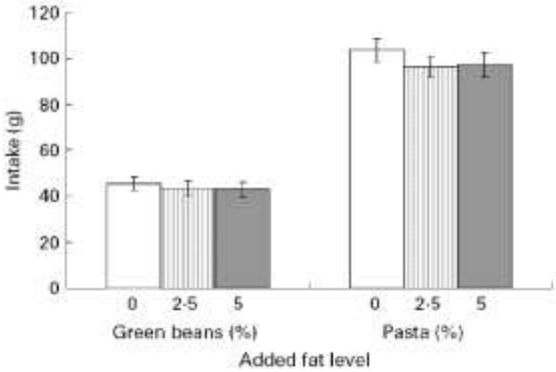

Figure 3 : Consommation (en g) de haricots verts et de pâtes par des enfants de 2 à 3 ans (N=65) en fonction de la teneur en beurre ajouté (0, 2.5 ou 5%). (Bouhlal et al., sous presse).

Dans le même esprit, nous avons observé que dans des fromages blancs, la teneur en matière grasse (0, 20 ou 40%) n'a pas d'impact significatif sur leur consommation, lors de goûters où un seul aliment était consommé, organisés à 2 semaines d'intervalle, chez des enfants ou chez des adultes. Ce résultat est illustré Figure 4.

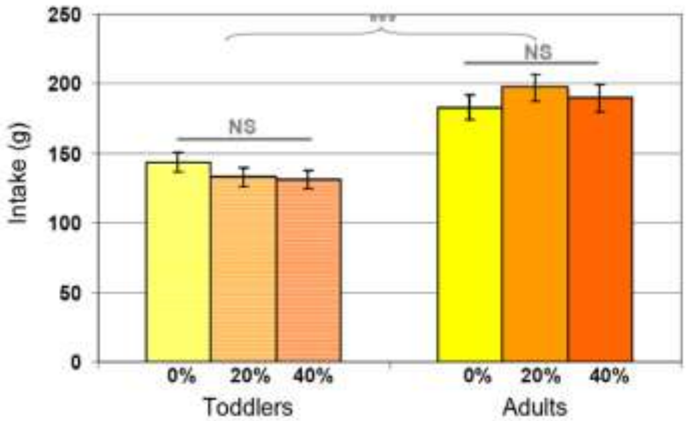

Figure 4 : Consommation (en g) de fromages blancs par des enfants de 2 à 3 ans (N=19) et des adultes de 19 à 25 ans (N=20), en fonction de la teneur en matière grasse (0, 20 ou 40%). (Bouhlal et al., 2009).

Une étude assez similaire conduite aux Etats-Unis a mis en évidence également une absence d'effet de la teneur en matière grasse sur la consommation de l'aliment dans lequel elle variait, mais les repas où l'aliment le moins lipidique était présenté étaient associés à une prise énergétique plus faible (Leahy et al., 2008).

Lipides et apprentissages alimentaires.

Si l'effet de la teneur en matière grasse sur la préférence ou la consommation d'un aliment mérite de plus amples recherches, l'effet de la présence de lipides dans un aliment sur l'apprentissage du « goût » de cet aliment par l'enfant a été plus étudié. En matière de préférences alimentaires, très peu de préférences sont « innées » (au sens observables dès la naissance), à l'exception notable de la préférence pour la saveur sucrée (Nicklaus et Schwartz, 2008). Ces préférences sont acquises sous l'effet des expériences répétées, qui aident l'organisme à se guider en fonction de la récompense physiologique et/ou de l'absence de conséquences négatives consécutives à l'ingestion d'un aliment. Pour qu'un effet d'apprentissage se mette en place, l'aliment doit être ingéré, le voir ne suffit pas (Birch et al., 1987). De plus, la consommation d'une portion d'un aliment est associée à un apprentissage plus marqué que la simple dégustation d'une bouchée (Kern et al., 1993).

Des travaux conduits avec des enfants de 2 à 4 ans montrent ainsi clairement qu'un arôme associé au cours de consommations successives à un aliment de forte densité énergétique (par exemple riche en lipides ou en sucre) est plus apprécié qu'un arôme associé à un aliment de faible densité énergétique (Birch et al., 1990 ; Johnson et al., 1991 ; Kern et al., 1993). Cet effet est d'autant plus important que l'enfant a faim au moment de la présentation de l'aliment. L'effet de cet apprentissage est toujours observé deux mois après la phase d'apprentissage (Kern et al., 1993).

Ce résultat révèle une capacité importante de l'organisme à associer une caractéristique sensorielle *a priori* neutre (l'arôme) à un effet post-ingestif (ici la quantité d'énergie apportée par l'aliment). Ce mécanisme de conditionnement associatif dit « flaveur-nutriment » est très puissant et probablement hérité d'une époque où les ressources alimentaires étaient rares, et où le repérage des sources de nutriments dans l'environnement était essentiel pour la survie individuelle. De nos jours, ce mécanisme puissant est beaucoup moins adapté à un environnement où l'offre alimentaire, surabondante, présente une très forte densité énergétique à moindre coût (Drewnowski, 2003 ; Drewnowski et Darmon, 2005).

Régulation de la prise energétique et consommation de lipides

La sensibilité à la densité énergétique des aliments (et à leur contenu en lipides) peut également être associée à une capacité à ajuster la consommation alimentaire en réponse à la quantité d'énergie ingérée lors d'un repas précédent (Birch et Deysher, 1985). Cette capacité d'ajustement ou de régulation de la prise énergétique semble bien développée chez le jeune enfant, mais chez l'enfant plus âgé, elle pourrait être perturbée par l'environnement alimentaire. Ainsi, la taille des portions perturbe l'ajustement de la prise alimentaire chez l'enfant de 5 ans mais pas chez l'enfant de 3 ans (Rolls et al., 2000). Cette capacité d'ajustement n'est pas spécifique des lipides. Par ailleurs, la consommation d'un aliment pauvre en lipides lors d'un repas n'est pas associée à la sélection ou à la consommation de plus de lipides lors d'un repas consécutif (Birch, 1993).

Interactions sociales et consommation de lipides

Le rôle de certains facteurs de l'environnement, comme par exemple les interactions parents-enfants est à prendre en compte également pour comprendre les déterminants de la formation des préférences pour les lipides (Birch, 1992). Les parents fournissent les aliments à leur enfant mais leur rôle va bien au-delà puisqu'ils incitent l'enfant à manger, le forcent, le récompensent, ou le restreignent parfois. L'effet de ces pratiques n'est pas anodin, notamment pour des aliments plaisants comme les aliments riches en lipides (la situation étant comparable pour les aliments riches en sucre !). Si de telles pratiques peuvent sembler efficaces à court terme, elles le sont moins à long terme et sont même parfois contre-productives. Offrir un aliment en récompense d'un 'bon' comportement ne fait que renforcer la préférence pour cet aliment (Birch et al., 1984). L'imposition de restrictions sélectives sur des aliments particulièrement appréciés ne fait en général que renforcer l'attrait pour le fruit défendu (Fisher et Birch, 1999). De telles pratiques restrictives ont même été associées à l'étiologie de l'obésité de l'enfant (Spruijt-Metz et al., 2006). Des travaux mettent également en évidence une association entre la corpulence des parents et la préférence et la consommation d'aliments riches en lipides de l'enfant (Fisher et Birch, 1995).

Conclusions

En conclusion, il est possible et souhaitable de développer des offres alimentaires adaptées aux besoins du jeune enfant et permettant d'optimiser son statut nutritionnel, notamment lipidique, et plus généralement son développement. Les connaissances des profils lipidiques les plus adaptés doivent encore être renforcées et affinées, en dégageant notamment des optimums pour différents âges critiques du développement. Chez l'enfant plus âgé, la richesse en lipides n'est pas nécessairement un gage de préférence immédiate ou de plus forte consommation, il est donc temps de réfléchir à des formulations 'raisonnables' permettant de satisfaire les besoins nutritionnels et le plaisir de l'enfant. Enfin, éduquons les parents et aidons-les à choisir et offrir des aliments variés à leur enfant, en évitant l'écueil de pratiques ou de discours trop restrictifs.

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Abstract

Background: In France, the National Nutrition and Health Program ("Programme National Nutrition Santé" or "PNNS") recommends to reduce salt, sugar and fat content in foods. However, the extent to which this might impact children's food preference and intake has not been studied.

Objective: The present work aimed at understanding the impact of salt, sugar and fat content variations in foods, on toddlers' and children's food preference and intake.

Methods: Five studies were conducted in toddlers' or children's natural eating environment (nursery and school canteen), at their usually scheduled lunch or snack times. A first study investigated effects of salt variations in two target foods (green beans and pasta), on 2-3 year-olds' intake and on 8-11 year-olds' preference and intake. A second study investigated the effect of mere exposure and flavor-flavor learning with salt or a salt-associated spice, on 2-3 year-olds' acceptance of a vegetable (salsify). A third study investigated, in 2-3 year-olds, the effect of sucrose content on the intake of a naturally sweetened food (fruit puree) and the intake of an unsweetened food (creamy white cheese). A fourth study investigated the effect of fat (butter) variations, on 2-3 year-olds' intake of two target foods (green beans and pasta). The fifth and last study compared the effect of fat level variations in a creamy white cheese on its intake by 2-3 year-olds and by adults.

Results: First, while salt suppression induced a decrease in toddlers' and children's green bean intake, its increase induced an increase in pasta intake in both age groups. Salt impacted intake through preferences. Besides, our results highlighted a dissociation between the direct positive effect of salt on food preference and intake, and its role in learning. Second, concerning sucrose, when comparing no addition and a 5% addition, toddler's intake did not increase in a naturally sweetened food, but it did increase in an unsweetened food; but sucrose contents from 5 to 15% did not modify intake. Third, as far as fat is concerned, suppressing or adding butter did not impact toddlers' green bean or pasta intake. Varying fat content in a creamy white cheese did impact toddlers' but not adults' weight intake, toddlers ate more of the 0% creamy white cheese; low-fat products seem to be well accepted by both age groups. Although energy intake decreased with decreasing energy density in toddlers and in adults, toddlers regulated it better than adults did.

Conclusions: Although, food sensory qualities are important drivers of eating behavior, the present result suggest that reduction of the content of ingredients such as salt, sugar and fat are possible without affecting much of children's intake. Concerning salt, however, its reduction seems more puzzling and should be considered cautiously especially if one aims at increasing vegetable intake

Keywords: Children, salt, sugar, fat, preference, intake, natural setting