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Nutritional interventions addressing food choices and eating behaviour

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ABBREVIATIONS

PCA	Principal component analysis
PC	Principal component
BR	Breakfast
FR	Food record
VAS	Visual analogue scales
GI	Glucose
In	Insulin
Le	Leptin
Gh	Ghrelin
GLP-1	Glucagon-like peptide-1
NEFA	Non esterified fatty acids
FFA	Free fatty acids
LS	Likert Scale
GI	Glycaemic Index
BMI	Body mass index
SD	Standard deviation
SEM	Standard error of the mean
PAL	Physical activity level
HEC	High Energy Carbohydrate
HEP	High Energy Protein
LEC	Low Energy Carbohydrate
LEP	Low Energy Protein
GLM	General Linear Model
iAUC	Incremental area under the curve
RT	Response time
CNS	Central nervous system
fMRI	functional magnetic resonance imaging
ROI	Region of Interest
PANAS	Positive and Negative Affect Schedule
VM	Vending machine
GIP	Gastric inhibitory polypeptide
NPY	Neuropeptide Y
AgRP	Agouti-related protein
POMC	Proopiomelanocortin
CART	Cocaine- and amphetamine-regulated transcript
OXM	Oxyntomodulin
PYY	Peptide YY
PP	Pancreatic polypeptide
CCK	Cholecystokinin

Eating behaviour is generally defined as any response to food-related stimuli resulting in food intake, which is driven by a complex interaction of several factors, such as physiological, sensory, perceptual, cognitive, psychological, and environmental ones. Current research accounts for the wide cross-modulation existing to control appetite between homeostatic and non-homeostatic (reward, emotion, memory, attention, and cognitive) systems via the gut-brain axis. This cross-talk between metabolic and cerebral regulatory systems is coordinated by the brain, which integrates food-related sensory information with satiety signals produced by the gastrointestinal tract to set up a mental representation of food. Those signals allow the evaluation of the reward value of food and the generation of affective responses, determining the regulation of eating behaviour. In order to increase our comprehension of this topic, there is a need for investigations approaching the interaction among homeostatic regulators, reward cues, and motivational signals, within the environment in which this interaction takes place.

On the basis of these considerations and to better understand the complex homeostatic-hedonic system underlying food intake control, the main aims of this Doctoral Thesis were i) to explore the association between the compositional and perceived characteristics of food with nutritional, physiological, behavioural, and psychological variables linked to both the homeostatic and reward-related control of food choices and food intake, and ii) to assess the influence of declaring nutritional and compositional characteristics of foods on food choices, through the enhancement of healthy product availability and nutrition communication at the point-of-choice.

According to these specific objectives, this Doctoral research was divided in two phases. During the first phase, the effect of breakfast on several factors linked to food intake was assessed through a series of nutritional, behavioural, and

neurological studies. Breakfast positively affected appetite control in healthy adults, while consumption of a non-caloric breakfast did not entail nutritional advantages, since it led to a compensatory mechanism in total daily energy intake and hindered the achievement of nutrient intake recommendations. The impact of breakfasts differing in their nutritional and perceptual characteristics needs to be further explored in a shorter time period like, for instance, the morning snacking occasion. This could avoid fasting-like scenarios, while still retaining an effect on factors involved in food intake regulation. In such a way, the sensory, saliency, and reward-related attributes of food that can stimulate food intake might be reduced in favour of physiological and cognitive factors. To deeper investigate the impact of perceptual and cognitive characteristics on food choices, a behavioural intervention addressing food choices and promoting healthy dietary habits, whilst not limiting the freedom of choice of consumers, was carried out during the second phase of this Doctoral research. Enhancing the availability of healthy foods at the point-of-choice increased their purchase, nudging the exposure to healthy foods towards healthy food choices. The communication of product attributes only discouraged selection of less favourable foods, without significantly modifying consumer choices.

In conclusion, the findings of this Doctoral Thesis contribute to shed light on the complex interaction of the several drivers of food choices and eating behaviour. Actually, this Doctoral research yielded useful insights for the implementation of nutrition interventions aiming to promote the consumption of healthy foods and entail eating behaviour improvement by nudging food choices through cognitive aspects and educational approaches.

Chapter 1 - General Introduction

Chapter 1 – General Introduction

1.1. BODY WEIGHT AND HEALTH

Overweight and obesity represent a major threat to global public health, due to their association with both short-term and long-term adverse somatic, psychosocial, and socioeconomic conditions, as claimed by the World Health Organization (WHO, 2011). The health and economic burden of the rising prevalence of overweight and obesity has positioned adiposity among the global non-communicable disease targets to be halted by 2025 (Kontis *et al.*, 2014; WHO, 2013). Actually, the costs of overweight and obesity and their related comorbidities are astounding in terms of healthcare cost, with an obvious need for implementing prevention strategies (Malik *et al.*, 2013b).

In order to better understand overweight and obesity, an explanation of body weight classification for adult subjects is compulsory. Body mass index (BMI), the most extensively used indicator, is calculated by dividing body weight (in kilograms) by the square of the body height (in meters) (Table 1) (National Institute of Health, 1998; WHO, 2000).

Table 1 – Body weight classification for adults (WHO, 2000).

Body weight	BMI (kg/m ²)
Underweight	< 18
Normal weight	18 – 24.9
Overweight	25 – 29.9
Obesity	> 30
Obesity class I	30 – 34.9
Obesity class II	35 – 39.9
Obesity class III	> 40

Compared to normal body weight, greater BMI is associated with a reduction in life expectancy due to a higher risk of all-cause mortality (Flegal *et al.*, 2013; McTigue *et al.*, 2006). Excessive body weight causes approximately 3.4 million

deaths every year, representing one of the five primary causes of death worldwide (Ellulu *et al.*, 2014). In particular, BMI values within the upper limit of normal weight (22.5 to 25 kg/m²) have been associated to the lowest overall mortality risk, with a 30% increase in all-cause mortality with every 5% increase in BMI, in the adult population of both genders (Figure 1) (MacMahon *et al.*, 2009).

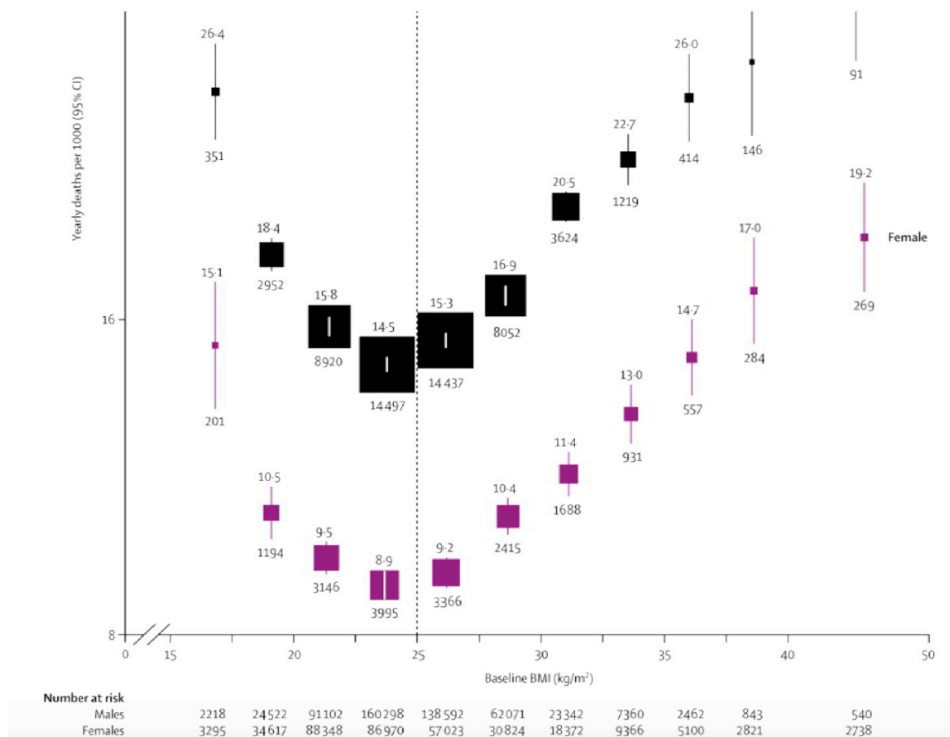


Figure 1 - All-cause mortality versus BMI for each sex.

Floated mortalities are shown above each square and numbers of deaths below. Area of square is inversely proportional to the variance of the log risk. Boundaries of BMI groups are indicated by tick marks. 95% confidence intervals for the floated rates reflect uncertainty in the log risk for each rate (Smith & Smith, 2016).

People with higher BMI are also exposed to an increased risk for multiple comorbidities, such as disability, lipid abnormalities, hypertension, cardiovascular diseases, diabetes, gallbladder disease, kidney-related diseases, osteoarthritis, musculoskeletal disorders, sleep apnoea, stroke, and some types of cancer (Danaei, 2014; De Gonzalez *et al.*, 2010; Field *et al.*, 2001; Hruby & Hu, 2015; Hu, 2009; Kelly *et al.*, 2008; MacMahon *et al.*, 2009; Ng *et al.*, 2014; Ni Mhurchu *et al.*, 2004; Ogden & Flegal, 2010; Singh *et al.*, 2013; Wormser *et al.*, 2011; Zheng *et al.*, 2011).

The complex clinical profile linked to overweight and obesity affects an increasing number of people, reaching more than 2 billion persons throughout the

world (Smith & Smith, 2016). This extremely large prevalence of overweight and obesity depicts the transition from a number of underweight people more than double with respect to obese people, to an epidemic diffusion of obesity worldwide, with the sole exception of some areas of Asia and Africa (Figure 2) (NCD, 2016). Considering the entire world, the mean BMI, standardised for age, increased of approximately 2.4 kg/m^2 over the past forty years, reaching a mean value of 24.2 kg/m^2 and 24.4 kg/m^2 for men and women, respectively, by the end of 2014 (NCD, 2016).

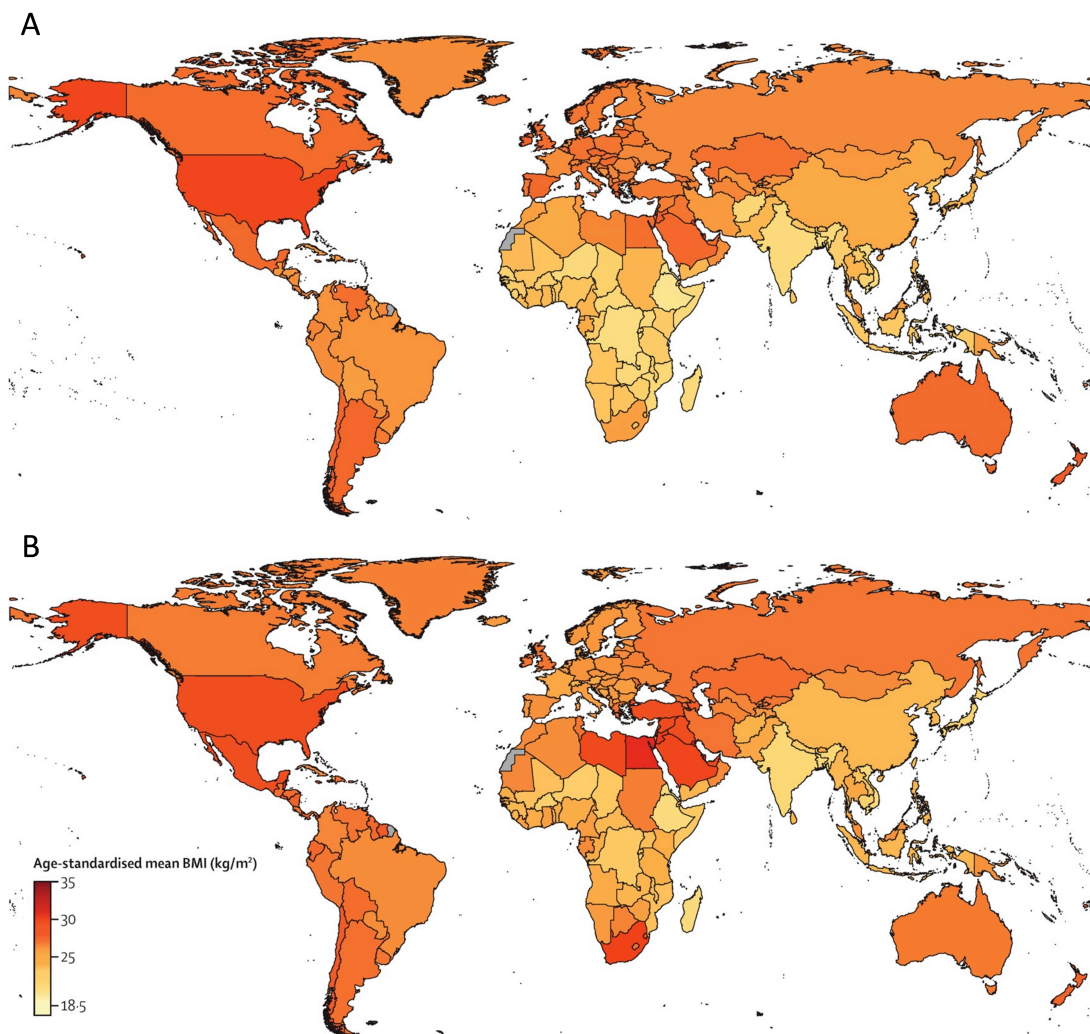


Figure 2 - Age-standardised mean BMI in men (A) and in women (B) by country in 2014 (NCD, 2016).

Over the last four decades, the global obesity prevalence, standardised for age, increased of 7.6% (from 3.2% to 10.8%) in men and of 8.5% (from 6.4% to 14.9%) in women (Figure 3). A BMI higher than 35 kg/m^2 (obesity class II and III) has

been registered in 2.3% and in about 5.0% of the male and female global population, respectively, while the prevalence of morbid obesity is 0.64% in men and 1.6% in women (NCD, 2016). Globally, the prevalence of obesity will reach 18% and 21% by 2025, and the prevalence of severe obesity the 6% and 9% of the population in men and in women, respectively, if the alarming trends registered for the last four decades continue (NCD, 2016).

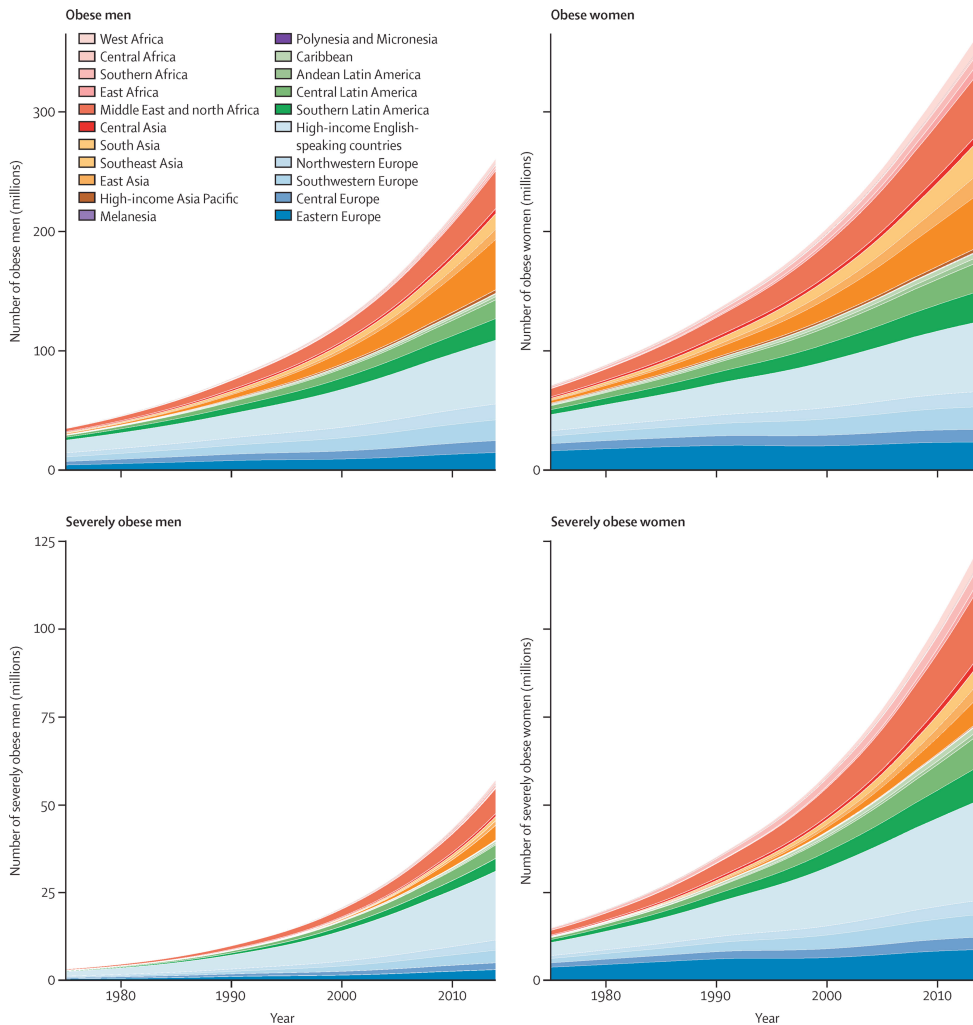


Figure 3 - Trends in the number of obese and severely obese people by world region (NCD, 2016).

1.2. OBESOGENIC ENVIRONMENT

The obesity epidemic that spreads rapidly worldwide in the last decades can be closely associated with the globalization phenomenon and the tragic

transformation of the built environment, involving both food and physical activity surroundings (Cohen, 2008). In fact, globalization has established several macro-level factors, such as global trade liberalization, economic growth, and urbanization, promoting the growth of the obesity incidence (Figure 4) (Malik *et al.*, 2013b).

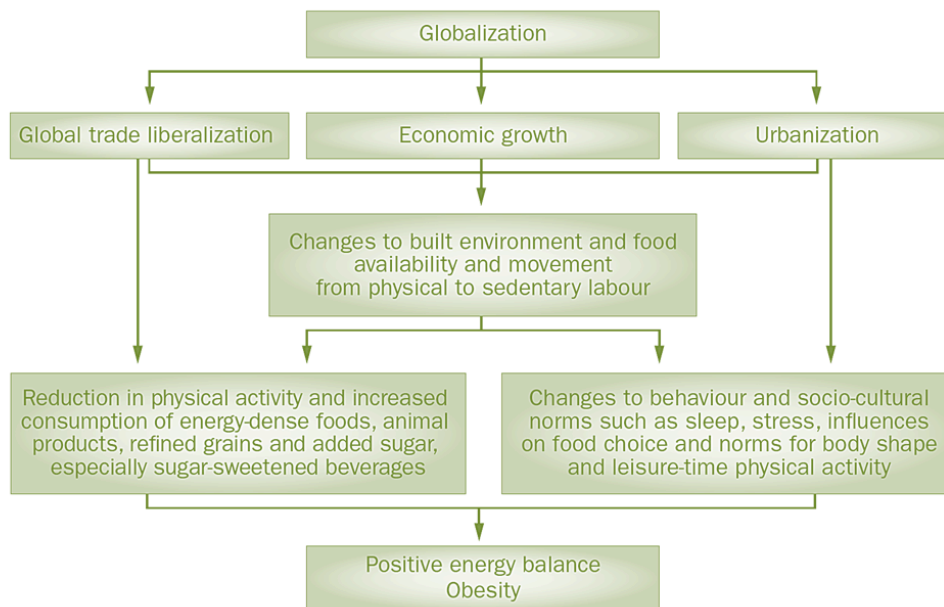


Figure 4 - Schematic representation of the relationship between globalization and obesity (Malik *et al.*, 2013b).

The transformation of the environment has resulted in unhealthy lifestyles and energy unbalance, stuck out for inactivity and extremely high food availability, due to comfort ubiquity and widespread presence of food-related cues (Zheng *et al.*, 2011). The built environment is an extensive surrounding of direct stimuli affecting sensory and perception, and complex stimuli acting on social level (Drewnowski, 2012; Dubowitz *et al.*, 2013). The matched effect of environmental stimuli generates several behavioural responses, building the eating behaviour and encouraging food intake irrespectively of the physiological need of the human organism (Bellisle, 2009). Actually, repeated responses to the food environment, such as food choices, meal circumstances, cooking preferences, and snacking habits, are learned and maintained during the lifespan (Bellisle, 2009).

All these changes in the environment establish a complex interaction of multiple facilitators of weight gain and unhealthy behaviours, such as

socioeconomic factors, which also interact with the genetic predisposition to obesity (Smith & Smith, 2016).

Genetics is a non-modifiable factor and represents the less impacted one at a global population level. Indeed, the many genetic variants identified to be linked with excessive body weight are accountable only for the 1.5% of the inter-individual BMI variation, showing the highest segment of the genetic-related risk population only a 2.7 kg/m² higher BMI compared to the lowest one (Speliotes *et al.*, 2010). Moreover, other miscellaneous weight gain risk factors have been identified, such as the human microbiome, several diseases, and the use of some medicines (Clarke *et al.*, 2012; Smith & Smith, 2016).

Socioeconomic status and education level are well-recognised contributors to the modern obesogenic environment (Zheng *et al.*, 2011). In westernised countries, the socioeconomic status is directly associated with the income. Although higher BMI were more prevalent in higher socioeconomic groups in the past centuries, there was a change in the direction of this trend with low incomes becoming directly related to higher BMI (Drewnowski *et al.*, 2007; Hruby & Hu, 2015; Koh *et al.*, 2012). A similar tendency has also been observed for the educational level, with an increased prevalence of overweight and obesity in the lower educated groups (Drewnowski & Specter, 2004). A lack of nutrition literacy and awareness, and scarce financial means contribute to the exceptional diffusion of cheap, convenient, and nutritionally-poor products, which have clearly been ascribed within the obesogenic contributors (Swinburn *et al.*, 2011).

Overeating and inadequate food choices are nudged by the wide availability, variety, palatability, portion size, and energy density of foodstuffs (Harris *et al.*, 2009; Hill *et al.*, 2008). The thorough analysis of the association between nutritional composition of food and weight gain pointed to simple carbohydrate and fat content as predominant characteristics of products conditioning weight gain (Mozaffarian *et al.*, 2011). Higher BMI has been related to increases in the intakes of energy dense, poor nutritious food, as salty snacks, sweets, and sugar-sweetened beverages, while lower BMI has been correlated to elevated intake of fruits,

vegetables, whole grains, nuts, and yogurt (Malik *et al.*, 2013a; Mozaffarian *et al.*, 2011).

All these dietary factors contribute to increase the daily energy intake, one of the two sides of energy balance. Unfortunately, these energy-rich dietary models are very often combined with a simultaneous reduction in energy expenditure due to a decline in the daily activities of developed societies (Mitchell *et al.*, 2011). The sedentary behaviour associated to the modern lifestyle is characterised by the diffusion of technologies and screen-related activities, such as watching TV, using computer, and playing videogames. These common activities play a very important role in the rise of overweight and obesity prevalence. As an example, a 23% higher risk of weight gain has been observed every 2 additional hours spent in front of a TV screen (Hu *et al.*, 2003).

In a restrictive and not sedentary environment, food intake is controlled by the internal milieu directly through homeostatic processes, and indirectly through the modulation of cognitive and reward-related mechanisms. Contrarily, the homeostatic and the cerebral control mechanisms are hindered in regulating correctly food intakes by the modern obesogenic environment, characterised by a higher availability of nutrients and a lower physical activity level (Figure 5) (Zheng *et al.*, 2011).

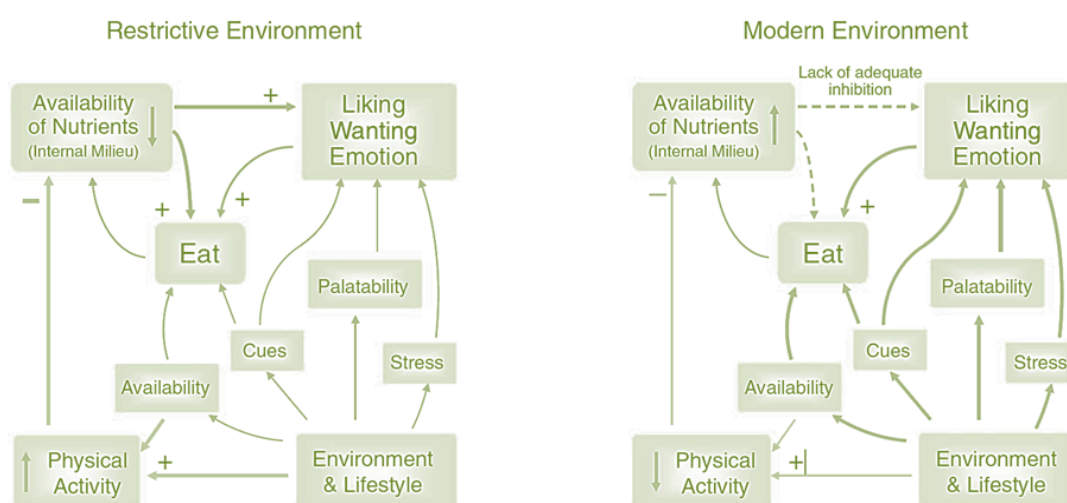


Figure 5 - Schematic diagram showing major factors determining food intake and energy balance in restrictive and modern environments (Zheng *et al.*, 2011).

1.3 ENERGY BALANCE AND EATING BEHAVIOUR

An energy intake that equals energy expenditure represents the optimal condition of energy balance and body weight maintenance. In fact, when energy intake surpasses energy expenditure, a positive energy balance occurs, causing body weight gain. In the current obesogenic environment, energy balance is easily perturbed by several factors shifting this thin equilibrium with infinitesimal daily energy surplus, but prolonged over several years, which results in a long-term development of overweight and obesity (Morgen & Sorensen, 2014; Peters *et al.*, 2002).

The energy balance model, based on thermodynamic principles, is largely accepted to describe body system regulation with the energy balance equation (Figure 6) (Bellisle, 2009). On one side there are various contributors to energy need (e.g. basal metabolism, physical activity, and dietary induced thermogenesis), while on the other side there are contributors to energy intake (e.g. eating patterns and energy and nutrient amounts).

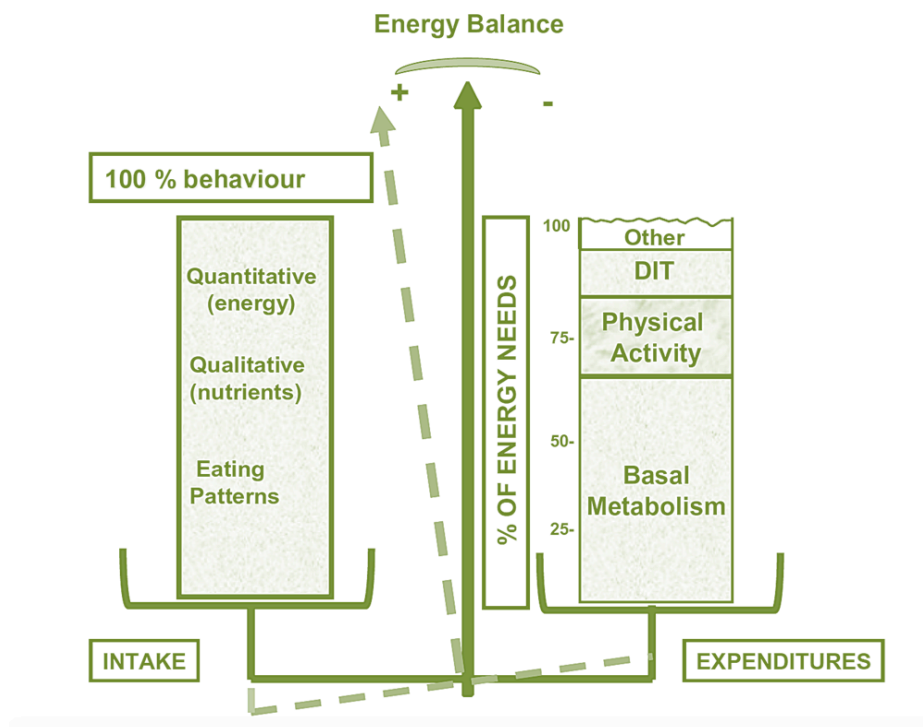


Figure 6 - A schematic representation of the energy balance phenomenon. DIT means “dietary induced thermogenesis” (Bellisle, 2009).

However, although the energy balance model cannot be criticized, the human body is not a mere static regulatory system, but a dynamic multi-component process. When even only one component of this equilibrium is perturbed, a compensatory mechanism occurs to minimise the changes perturbing body homeostasis. If the body is stressed for a prolonged period, these regulatory responses are no longer efficient (Hopkins *et al.*, 2010; King *et al.*, 2007). Understanding whether the positive energy balance occurred in response to a higher energy intake or a lower energy expenditure is not sufficient to explain the complex system underneath healthy body weight management, at least in the current obesogenic environment (Wells & Siervo, 2011; Wells, 2013).

An essential aspect is the metabolic/behavioural double nature of these energy balance components. While energy expenditure is controlled by both metabolic and behavioural factors, contributors to energy input are all behavioural (Bellisle, 2009; Hopkins & Blundell, 2016). In this sense, eating behaviour assumes a pivotal role in the preservation of energy homeostasis and body weight gain. Eating behaviour is generally defined as food-related impulsive or controlled responses to both the availability of nutrients and environmental factors acting to cover the energy and nutritional needs (Bellisle, 2009). Eating behaviour in humans involves several other factors besides physiological regulators, such as social relations, habits, sensory stimulation, preferences, psychological attitudes, and cognitive evaluations, all of them contributing to the definition of body weight (Bellisle, 2009; Berthoud, 2006). The biological/behavioural nature of the compensation mechanism behind energy balance control emphasises the complexity of the processes that drive food choices and eating behaviour. To better explain the complexity of this regulation, the Foresight Obesity Systems Map has been proposed (Figure 7) as a graphical representation of the numerous and inter-related regulators participating in energy balance regulation (Vandenbroeck *et al.*, 2014). Starting from energy balance, the core aspect of body weight, the obesogenic system has been defined as a causal loop model of several factors implied, and of their extremely chaotic interdependencies (Vandenbroeck *et al.*, 2014).

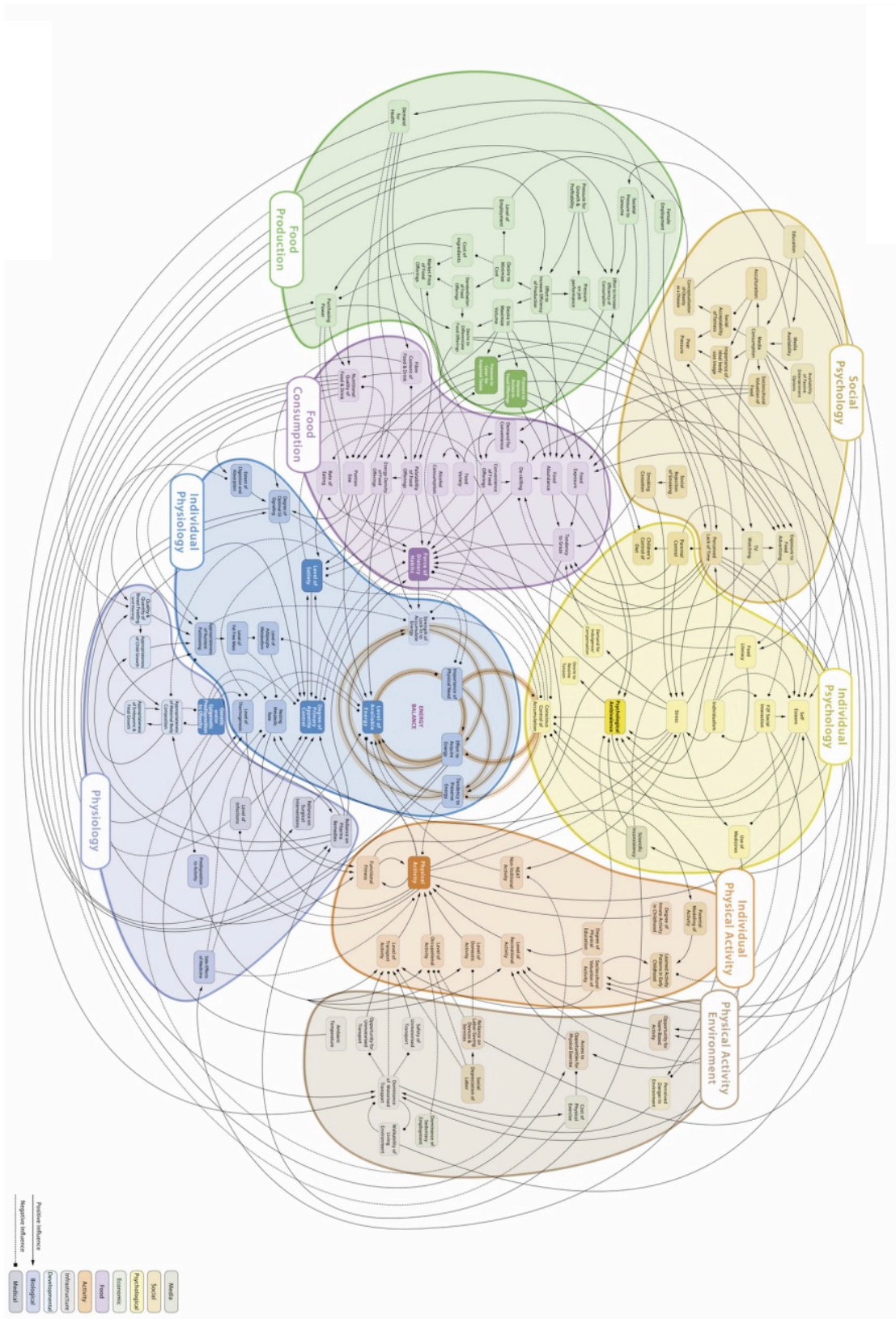


Figure 7 - Foresight Obesity Systems Map (Vandenbroeck et al., 2014).

1.4. SATIETY AND APPETITE CONTROL IN HUMANS

All the modulators of energy balance collaborate to control appetite (Tremblay & Bellisle, 2015). This physiological condition, based on the nutritional status and on food intake, generates hunger and satiety related signals. In particular, hunger results from the internal availability of energy and nutrients, and indicates an expected or actual status of nutritional need. Satiety is instead a status of hunger inhibition. Meal patterns are regulated by hunger signals that elicit the beginning of food intake, and by satiety signals that occur after food intake, determining the extent of time before the next meal takes place (Bellisle, 2009). In such a way, hunger and satiety signals control the number of meals of the day and the meal-to-meal times, while meal size and meal duration are regulated by the mechanism of satiation, which reduces gradually the motivation to eat (Bellisle, 2009).

Not considering behavioural and social restraints, physiological hunger signals and food-related sensory factors determine the beginning of food intake on the basis of body needs (de Castro, 2010). Immediately after the beginning of the eating episode, hormonal, neuronal, sensory, and cognitive factors exert an inhibiting effect resulting in the regulation of meal size and the end of food intake (Tremblay & Bellisle, 2015). At the end of the meal, satiety avoids the forthcoming activation of hunger signals (Tremblay & Bellisle, 2015). This efficient circuit of hunger, satiation, and satiety signals regulates appetite control and equilibrates energy intake with energy needs. Satiation and satiety generate an overlapping cascade of responses to an initial sensory and cognitive perception of food and to post-ingestive and post-absorptive physiological signals (Blundell *et al.*, 1987). This complex interaction of pre- and post-ingestive signals, defined as Satiety Cascade, could be simplified considering satiation, satiety and food choices (Figure 8).

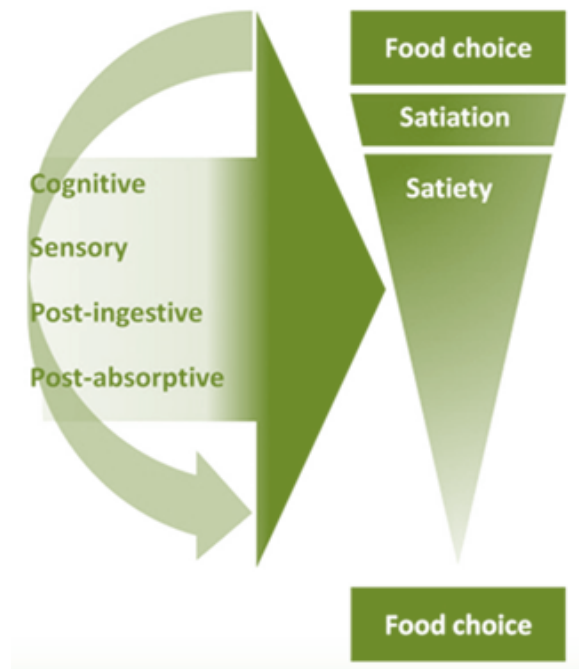


Figure 8 - Satiety cascade framework (Chamberlain *et al.*, 2015).

Nevertheless, cultural, social, and behavioural determinants impact on appetite control, conditioning the frequency, duration, and size of meals, and, thus, the satiety-related mechanisms of food choices and food intake (Tremblay & Bellisle, 2015). Beside these factors, physiological signals play a key role in appetite control and are finely integrated through the peripheral nervous system. Learning, reward, memory, and attention brain centres also contribute to appetite control together with both short- and long-term metabolic signals (Figure 9) (Berthoud, 2007; McCrickerd & Forde, 2016; Toepel *et al.*, 2015).

The first phase of the Satiety Cascade, related to sensory factors, generates a short-term response to food. Sensory cues are strong determinants of eating behaviour since they are the first drivers of the initial experience of consumption. The second step of the Satiety Cascade, however, is related to cognitive responses. Beyond sensory responses, the exposure to food stimuli generates a cognitive activation resulting in the cerebral representation of the food and of its expected satiating power, as well as in a specific attention/distraction during food intake (Bellisle & Dalix, 2001; Bellisle *et al.*, 2004; Brunstrom, 2011; Mitchell & Brunstrom, 2005). After these initial responses, a series of post-ingestion and post-absorption mechanisms elicit middle- and long-term effects on satiation and satiety. While

meal quality impacts on sensory and cognitive factors, meal size, nutrient status and energy balance appear as predominant trainers of appetite regulation after meal consumption, causing the release of several metabolic satiety factors (Tremblay & Bellisle, 2015).

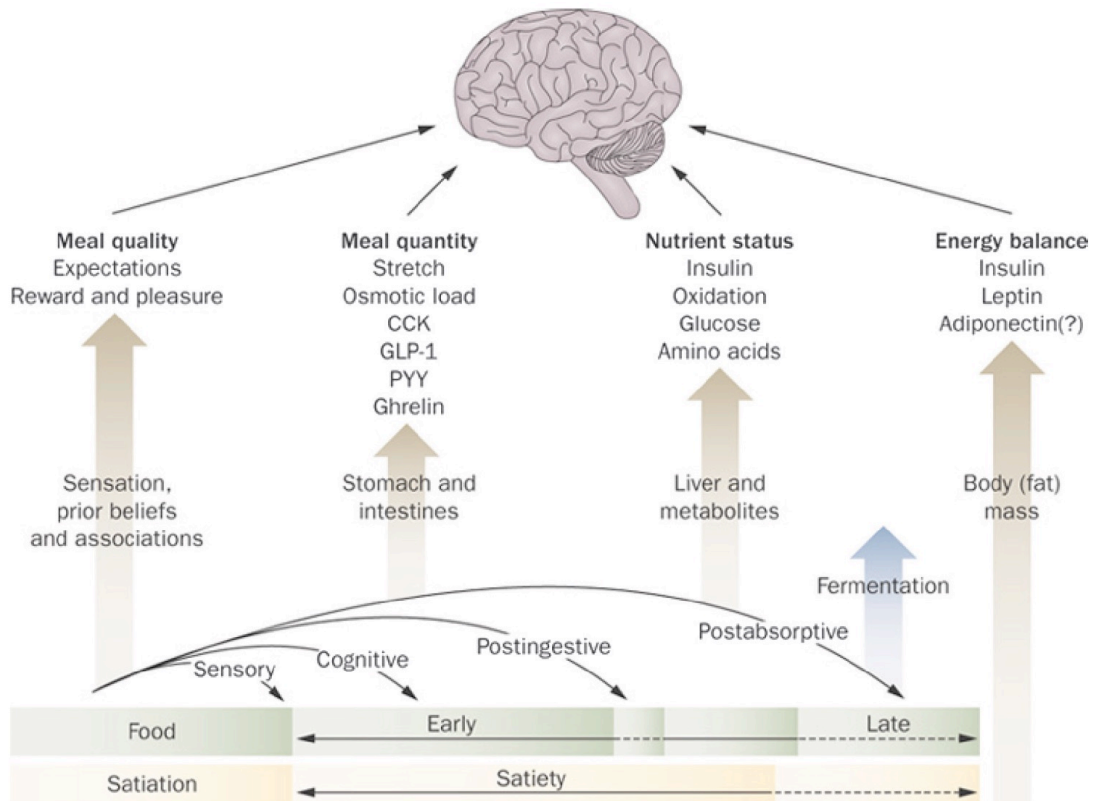


Figure 9 - The Satiety Cascade (Tremblay & Bellisle, 2015).

The association between early sensory perceptions and the post-intake metabolic signals generates a learned cognitive response determining eating behaviour. Indeed, post-ingestive reactions are predicted by the sensory characteristics experienced previously (Sclafani, 1997). This learned integrative process of pre- and post-intake signals impacts on food choices, causing a clear preference for potentially high satiating and nutrient-rich foods, and a sensory-specific habituation to already-known food (Brunstrom, 2007; Hetherington & Havermans, 2013; Rolls *et al.*, 1981). Therefore, it is clear that, besides palatability, sensory signals have a main role in the regulation of food intake, optimising the energy balance process. At the onset of food intake, sensory factors (in particular sight, smell, taste, and texture) stimulate ingestion and the activation of cephalic phase responses, such as salivation, secretion of gastric acid, and release of

gastrointestinal hunger hormones, getting the body ready for nutrients (Feldman & Richardson, 1986; Smeets *et al.*, 2010; Wooley & Wooley, 1973). On the other hand, gastric distension, the release of gastrointestinal satiety hormones, and glycaemia contribute to gradually inhibit the motivation to eat. This condition of early post-ingestive satiation has been reported to be food-specific, with foods characterised by dissimilar perceptual characteristics able to differently stimulate food intake. This promotion of food intake is later interrupted when satiating signals reach a sufficient level to counterbalance sensory and hedonic attractiveness (Hetherington *et al.*, 1989; Tremblay & Bellisle, 2015).

In the post-intake period, the same factors participating in the satiation process act to prolong the meal-to-meal period by promoting satiety (de Castro, 2010). As an example, meal quality and quantity (weight, volume, energy density, and nutrient content) affect both the duration and the intensity of the postprandial satiety (Keller *et al.*, 2013). Moreover, satiety is reinforced by cognitive factors, such as the evaluation of the expected satiety of food, the attention to food throughout the eating period, and the food memory of the previous meals (Bellisle *et al.*, 2004; Cassady *et al.*, 2012; Higgs *et al.*, 2008; Wansink *et al.*, 2005).

Another point worth of attention is the important role held by the brain in appetite control. The brain receives and integrates a myriad of signals and acts as the regulator of all the mechanisms implicated in energy balance and food intake (Berthoud, 2002). In such a way, i) psychological and behavioural events, ii) peripheral, physiological, and metabolic events, and iii) neural and metabolic interactions take place in the brain, where they are finely integrated in order to regulate food intake and to control appetite through a refined cross-talk between the homeostatic and cerebral systems (Figure 10) (Hopkins & Blundell, 2016; Morton *et al.*, 2014).

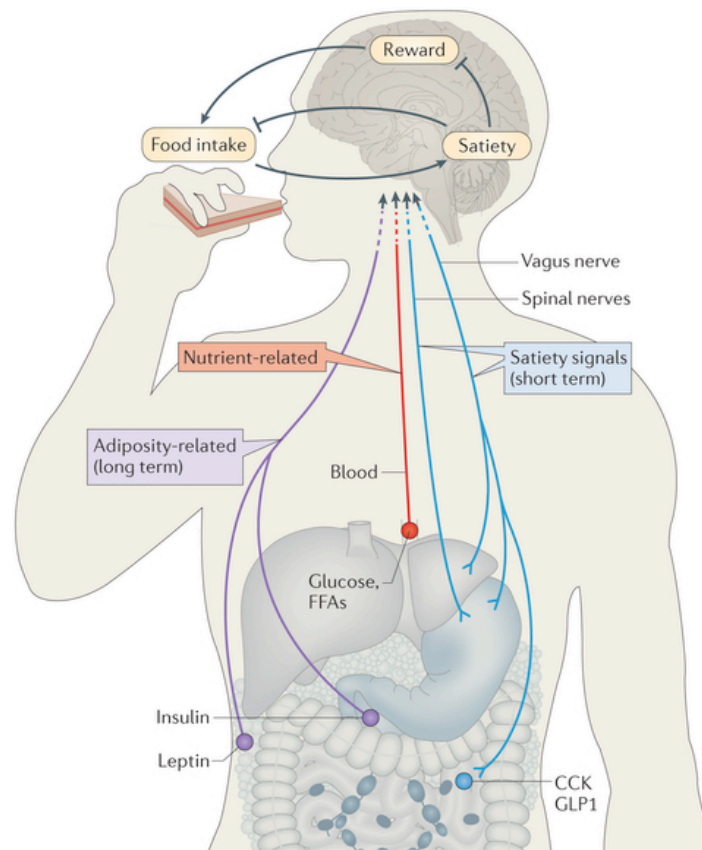


Figure 10 - *The interplay between homeostatic and hedonic circuits influencing food intake and body weight (Morton et al., 2014).*

1.5. FOOD INTAKE REGULATION IN HUMANS

The complex mechanisms taking part in the regulatory system behind satiety regulation and eating behaviour affect also food choices and food intake. In order to simplify the model, the whole system was firstly separated arbitrarily in a dichotomous model with two sub-systems, the homeostatic/physiological and the non-homeostatic/hedonistic one (Figure 11A) (Bellisle, 2009; Yeomans *et al.*, 2004). In such a way, the two mechanisms behind food intake regulation were supposed to be balanced, but well-separated and independent. Contrarily, during the last years, a unique bidirectional model has been hypothesised, integrating the homeostatic control circuit with the hedonic processes and their cognitive, emotional, sensory, and perceptual expressions (Figure 11B) (Munzberg *et al.*, 2016). The network of physiological, sensory, perceptual, cerebral, and psychological factors is

represented by this unique integrative model, gathering all the different drivers involved in the regulation of food intake in an easily understandable scheme (Salem & Dhillo, 2015).

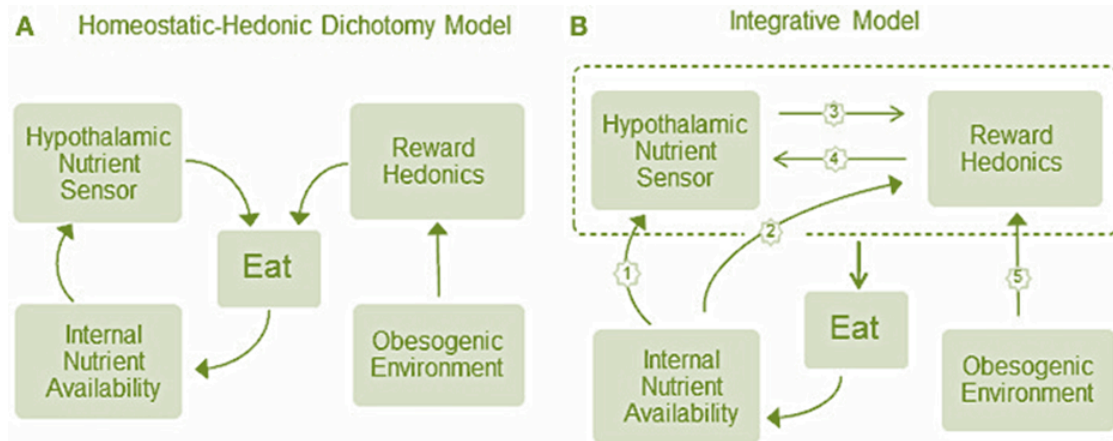


Figure 11 - (A) Dichotomy and (B) integrative models of homeostatic and hedonic control of food intake and regulation of body weight (Munzberg *et al.*, 2016).

In this integrative model (Figure 11B), nutrient availability leading the homeostatic system acts on both the nutrient sensor (pathway 1) and the reward-hedonic center (pathway 2). The cross-talk between homeostatic satiety signals and brain centers (pathway 3 and 4) regulates involuntary food intake. Finally, the obesogenic environment in which all these interactions take place indirectly affects eating behaviour through reward-related regulation (pathway 5) (Lean & Malkova, 2016; Munzberg *et al.*, 2016).

1.5.1. Physiological factors

Numerous inter-connected peripheral hormones are involved in the regulation of food intake (Lean & Malkova, 2016). In fasting conditions, metabolic hormones are secreted to generate hunger signals and guarantee energy needs (Bellisle, 2009). In addition, since meal frequency and timing in humans are generally influenced by social and behavioural factors, an anticipatory secretion of some of these hormones occurs even before the expected time of a habitual meal (Pavlov, 1927; Woods *et al.*, 1974). Indeed, the homeostatic system prepares the

body to receive nutrients, trying to mitigate and avoid extreme metabolic challenges following large amount of food, as in the case of hyperglycaemia (Ahrén & Holst, 2001; Power & Schulkin, 2008; Powley, 1977). This preventive hormonal secretion, called cephalic phase, is inducted by neural signals in “meal-fed individuals” in response to the time of the day and to food stimuli (Figure 12) (Woods, 1972).

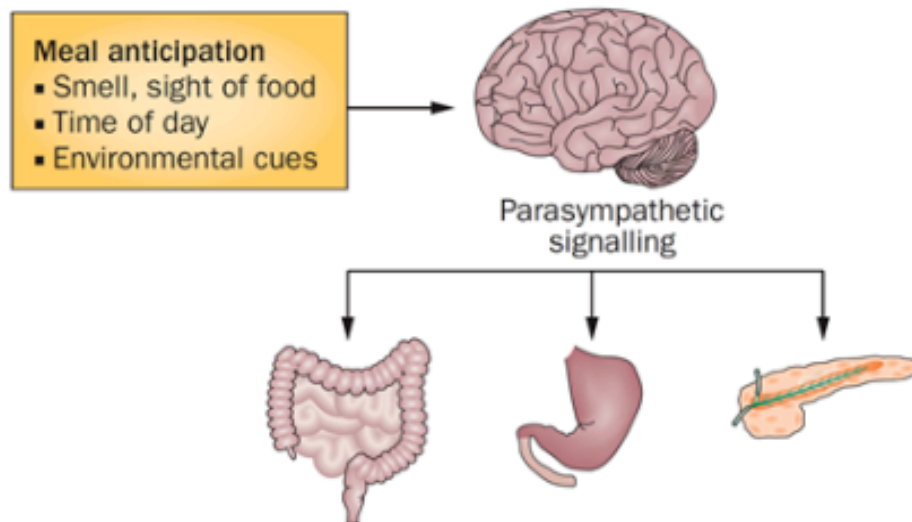


Figure 12 - Meal-anticipatory responses (Begg & Woods, 2013).

After food ingestion, several metabolic signals, short-term hunger and satiety signals, body nutrient status signals, and long-term adiposity signals are released to i) optimise nutrient intake, absorption and assimilation, ii) promote satiation and satiety, and, hence, iii) guarantee energy balance (Begg & Woods, 2013; Carrascosa *et al.*, 2009).

1.5.1.1. Gut hormones

As food intake begins, the gastrointestinal tract releases various hormones involved in food intake regulation, eliciting different physiological responses. Gastrointestinal hormones regulate satiety by improving gut motility, carrying out exocrine actions, influencing the secretion of long-term adiposity signal (i.e. leptin), and modulating the release of neurotransmitters in the brain via the gut-brain axis (Murphy & Bloom, 2006).

The most important gastrointestinal hormones, the site where they origin, and their impact on hunger and satiety are briefly described in Table 2 and reported below.

Table 2 – Gastrointestinal hormones and their effects on hunger and satiety (Vincent et al., 2008).

Gastrointestinal hormone	Embryonic site of origin	Behavioural brain effects	
		Hunger	Satiety
Ghrelin	Stomach (upper foregut)	Increased	Decreased
Pancreatic polypeptide	Foregut and midgut	Decreased	Effect unknown
Cholecystokinin	Foregut and midgut	Decreased	Increased
Gastric inhibitory polypeptide	Hindgut	Effect unknown	Effect unknown
Glucagon-like peptide 1	Hindgut	Decreased	Increased
Oxyntomodulin	Hindgut	Decreased	Increased
Peptide YY	Hindgut	Decreased	Increased

Cholecystokinin (CCK) was the first appetite-related gastrointestinal hormone described to decrease food intake (Gibbs *et al.*, 1973; Lieveise *et al.*, 1995). It is secreted at duodenal and jejunal level in response to food ingestion, with a peak of its plasma levels generally observed in the first quarter-hour after food intake (Buffa *et al.*, 1976; Liddle *et al.*, 1985). CCK exerts its regulatory effects on pancreatic secretion, gallbladder contraction, intestinal motility, and inhibition of gastric emptying (Dufresne *et al.*, 2006).

Ghrelin is a 28-amino acid peptide secreted principally by the stomach (Kojima *et al.*, 1999). It is the only recognised gastrointestinal hormone with an orexigenic effect (Hameed *et al.*, 2009). Ghrelin participates in energy homeostasis by decreasing the meal-to-meal satiety and by promoting the beginning of food intake (Druce *et al.*, 2005; Wren *et al.*, 2001).

The pancreatic polypeptide (peptide PP) belongs to the PP-fold family of proteins, principally produced in the pancreas and only in small quantities in the colon and the rectum (Chaudhri *et al.*, 2008; Hameed *et al.*, 2009). Although its

physiological effect on energy homeostasis is not clear yet, it seems to elicit an anorexigenic influence as a consequence of energy intake, while it is present only in small quantity during fasting (Track *et al.*, 1980).

The peptide YY (PYY) is an anorexigenic 36-amino acid hormone belonging to the PP-fold family of proteins (Tatemoto & Mutt, 1980). PYY is produced by the L-cells of the gastrointestinal tract in proportion to the energy content and nutrient composition of the meal, reaching high circulating concentrations within an hour after food intake (Adrian *et al.*, 1985).

The glucagon-like peptide 1 (GLP-1) is a potent anorexigenic hormone, secreted by L-cells of small and large intestine after food intake. Its concentration in the blood depends on the energy content of the meal and contributes to inhibit additional food intake (Murphy *et al.*, 2006).

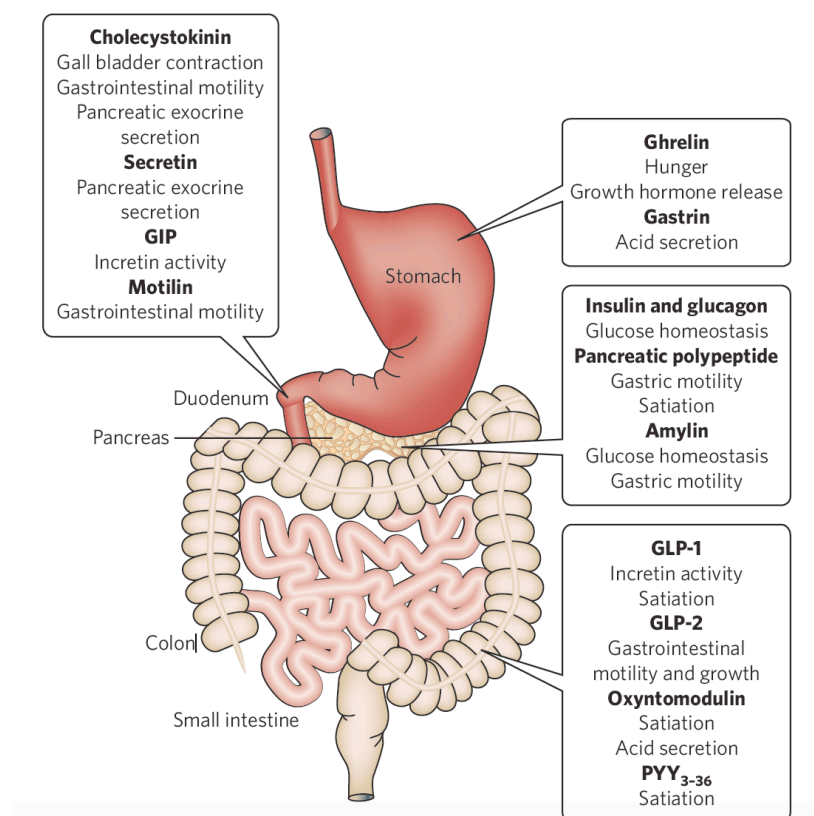


Figure 13 - Schematic diagram of the gastrointestinal tract illustrating where particular gut hormones are concentrated and their major putative functions (Murphy & Bloom, 2006).

The oxyntomodulin (OXM) is a 37-amino acid hormone secreted in the colon. After food intake, it is co-secreted with GLP-1 in proportion to the energy content of the ingested food to elicit an anorexigenic effect (Druce & Bloom, 2006).

The glucose-dependent insulinotropic polypeptide or gastric inhibitory polypeptide (GIP) is a 42-amino acid hormone secreted in the duodenum by K cells as a consequence of food intake. Its physiological effect on food intake regulation and appetite control is related to the induction of insulin secretion (Murphy *et al.*, 2006).

1.5.1.2. Long-term adiposity signals

Energy homeostasis is also controlled through a series of peripheral signals related to the body fat status. Adiposity signals originate from the adipose tissue but also from the pancreas, and their blood concentrations depend on the body fat mass, and not directly on the short-term food intake (Cancello *et al.*, 2004). Within these long-term adiposity signals, leptin and insulin play an essential role in energy homeostasis.

Leptin is a hormone secreted by white adipose tissue. It is also known as the satiety hormone because of its anorexigenic effect. Its concentration in the blood is strictly linked to fat mass, acting as a controller of the body weight, but is also characterised by daily rhythmicity, helping in appetite control. For instance, leptin levels are higher during the late night/early morning (Murphy & Bloom, 2004; Perry & Wang, 2012).

Insulin is secreted by the β -cells of the pancreatic islets and its blood concentration is a consequence of post-prandial glycaemia, but also of body adiposity (Rocha *et al.*, 2011). It shares with leptin the lipostatic role and the appetite regulator function, even though its impact on energy homeostasis is less efficient (Frutos *et al.*, 2007). In addition to food intake control, insulin elicits a stimulating effect on the adipose tissue to synthesise and secrete leptin through the adipo-insular axis feedback circuit (Kieffer & Habener, 2000).

1.5.2. Sensory and perceptual factors

Food choices and intakes can be influenced by how foods are perceived (Buckland *et al.*, 2013; Capaldi *et al.*, 2006; Provencher *et al.*, 2009; Steptoe *et al.*, 1995). The perception of food stimuli is based on several dimensions, like taste, appearance, freshness, satiating capacity, and healthiness (Oakes & Slotterback, 2002; Oakes, 2006; Raghunathan *et al.*, 2006).

Perceptual and sensory properties of food stimuli are used to generate a multifaceted representation of food value before, during, and after eating. In such a way, individual preferences and food choices are mainly conditioned through hedonic signals, while appetite and energy intake are controlled by satiation and satiety (McCrickerd & Forde, 2016). In addition, the sensory properties of food contribute to establish food preferences, both in like and dislike dimensions, a biological learning process maintained for all the lifespan (Sclafani, 2004). Indeed, as far as possible, people opt for food they like, making both palatability and the whole sensory experience appear to be crucial drivers of food choices and eating behaviour (McCrickerd & Forde, 2016).

Several food attributes participate in the complex mechanisms of food evaluation, selection, and ingestion, such as taste, visual, olfactory, tactile, and even auditory characteristics of food. They may affect both the quality (nutritional value) and the quantity (size and duration) of meals (Bellisle, 2009). As an example, food sight and smell passively promote food identification, increase the saliency of food stimuli, anticipate the learned taste and satiety-power, and stimulate the desire-to-eat, even before an eating occasion occurs (McCrickerd & Forde, 2016). During food intake, texture characteristics of food influence the oral phase of ingestion, since the time a food is kept in the mouth, in contact with local chemo-sensory receptors, may positively impact on the satiating-power beliefs and anticipatory physiological responses. Furthermore, retro-nasal sensations and taste intensity impact on meal size as well as on post-ingestion satiation and post-absorptive satiety (McCrickerd & Forde, 2016).

1.5.3. Cerebral factors

The central nervous system (CNS) regulates appetite and body weight following a series of intricate interconnections involving cortical and subcortical systems, as well as multiple cognitive and affective processes, holding a key role in merging both external and internal signals to modulate energy homeostasis (Farr *et al.*, 2016). However, together with homeostatic processes, the reward, emotion/memory, attention, and cognitive control systems also participate in the regulation of the brain circuitry controlling food intake in humans (Figure 14) (Farr *et al.*, 2016).

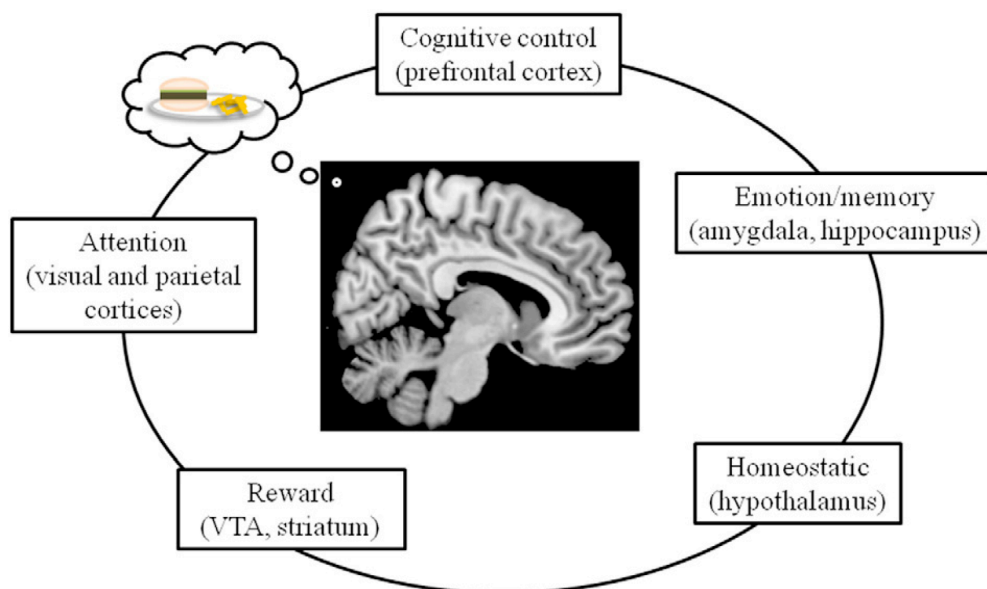


Figure 14 - Control of eating in human brain (Farr *et al.*, 2016).

Current research accounts for the wide cross-modulation existing to control appetite between homeostatic and non-homeostatic (reward, emotion/memory, attention, and cognitive control) systems via the gut-brain axis. A schematic representation of the gut-brain axis and the appetite-related and homeostatic brain areas that are modulated by eating are represented in Figure 15 (Francis & Eldeghaidy, 2015).

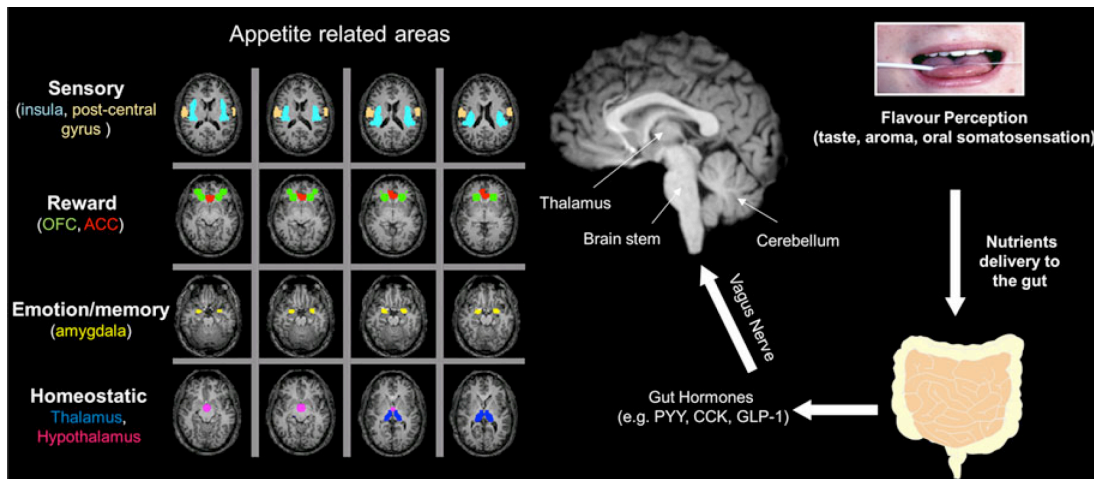


Figure 15 - Gut–brain axis and appetite and homeostatic brain (Francis & Eldeghaidy, 2015).

1.5.3.1. Homeostatic brain system

The homeostatic brain system involved in food intake regulation is mainly controlled by a network of hypothalamic neurons. In particular, the arcuate nucleus of the hypothalamus regulates appetite, increasing energy expenditure and decreasing appetite by expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), and decreasing energy expenditure and increasing appetite through expressing agouti-related protein (AgRP) and neuropeptide Y (NPY) (Schwartz & Porte, 2005). Additional orexigenic and anorexigenic neurons placed in other hypothalamic nuclei connect with the neurons of the arcuate nucleus that take part in the control food intake (Chan & Mantzoros, 2001; Mantzoros, 2001; Stieg *et al.*, 2015).

The neurons of the hypothalamus are inhibited or excited by peripheral hormonal signals, which provide feedback to the brain to control energy balance. For instance, the blood concentrations of leptin are increased as a consequence of higher levels of body fat, reducing energy intake and intensifying energy expenditure through the inhibition of AgRP/NPY neurons and the stimulation of POMC/CART neurons. On the contrary, if the body presents low fat levels, food intake is stimulated and energy expenditure is reduced by lower concentrations of

circulating leptin. Similarly, the hypothalamic activity and energy homeostasis are regulated by other peripheral hormones such as insulin, ghrelin, adiponectin, amylin, GLP-1, and irisin (Table 3) (Farr *et al.*, 2016).

Table 3 – Hormones involved in hunger and satiety (Austin & Marks, 2008).

Sensation	Hormone	Primary location of production	Action
Hunger	<i>Hypothalamus</i>		
	NPY	Medial arcuate nucleus (also widespread in CNS)	Stimulating feeding and antagonizing POMC actions
	AgRP	Medial arcuate nucleus	Stimulating feeding
	<i>Peripheral peptides</i>		
	Ghrelin	Stomach	Stimulating feeding by increasing NPY/AgRP and antagonizing leptin effects
Satiety	<i>Hypothalamus</i>		
	POMC	Arcuate nucleus	Inhibiting feeding, stimulating basal metabolic rate, and altering nutrient partitioning
	CART	Arcuate nucleus	Inhibiting feeding
	<i>Peripheral peptides</i>		
	CCK	Duodenum, jejunum	Inhibiting feeding and stimulating pancreatic secretion, gall bladder contraction, intestinal motility, and inhibition of gastric motility
	PYY	Ileum, colon, rectum	Inhibiting feeding by inhibition of NPY and stimulation of POMC
	PP	Endocrine pancreas	Inhibiting feeding
	OXM	Distal ileum and colon	Inhibiting gastric acid secretion, decreasing gastric emptying, and decreasing pancreatic enzyme secretion
	GLP-1	Distal ileum and colon	Delaying gastric emptying, stimulating glucose-dependent insulin secretion, inhibiting glucagon secretion, and stimulating somatostatin secretion
	GIP	Stomach, duodenum, jejunum	Glucose-dependent insulin secretion, induction of β cell proliferation, promotion of energy storage, enhancement of bone formation
	Insulin	Endocrine pancreas	Inhibiting feeding
	Leptin	Adipose tissue	Inhibiting NPY and AgRP and Stimulating POMC and CART
	Adiponectin	Adipose tissue	Inhibiting feeding

Despite the pivotal role of the hypothalamus in the homeostatic regulation of food intake, this phenomenon is affected, at brain level, by a multitude of other components. Indeed, the hypothalamus also collects external signals and is interconnected with mechanisms of the reward and emotion/memory systems. It also communicates with the cognitive control and other cortical areas through the thalamus (Farr *et al.*, 2016).

1.5.3.2. Reward system

Foods are naturally rewarding stimuli, since they are able to impact on the reward mechanisms of the human brain. Alterations in the signaling of the reward system have been pointed out as one of the main causes behind obesity (Figlewicz, 2003; García-García *et al.*, 2014; Gosnell & Levine, 2009; Kelley *et al.*, 2015; King, 2013; Murray *et al.*, 2014; Small, 2009; Stice *et al.*, 2013). Reward pathways are composed of dopaminergic neurons originating in the substantia nigra and the ventral tegmental area of the midbrain, and projecting across the whole brain. Some brain areas, such as the nucleus accumbens, the striatum, and the orbitofrontal cortex can be activated in response to visual food cues and have a central role in the reception and integration of dopaminergic signals to produce response actions (Burger & Stice, 2011; Dileone *et al.*, 2012; García-García *et al.*, 2014; Volkow *et al.*, 2013; Ziauddeen *et al.*, 2015). Hyper-responsivity to food stimuli can be triggered by exposure to highly rewarding foodstuffs, which entails food seeking processes and higher food intakes (Figure 16) (Farr *et al.*, 2016). Repeated exposure to highly rewarding foodstuffs can provoke the disconnection between the reward responsivity to food and food consumption, leading to increase food intake to reach the expected reward. On the other hand, a natural hyposensitivity to rewards also enhances food intake since higher amounts of foods, mainly highly caloric or high in fat, are required to reach the same level of reward (Figure 16) (Farr *et al.*, 2016).

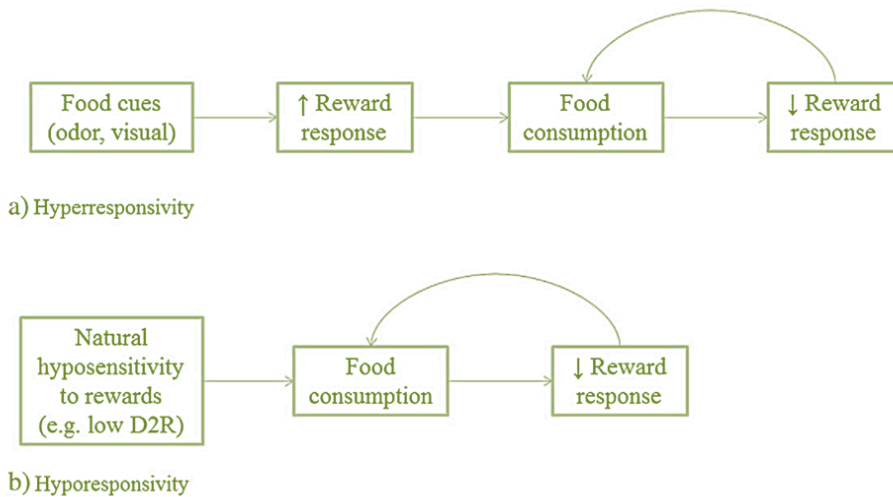


Figure 16 - Reward response to food consumption (Farr et al., 2016).

1.5.3.3. Emotion systems

Emotions modulate appetite deeply. The primary brain area controlling appetite and food intake in response to emotional stimuli is the amygdala, which is activated in presence of food cues (O'Doherty *et al.*, 2002; Small *et al.*, 2008). Short-term emotions, like anger and joy, promote appetite and entail poor food choices in comparison to emotions like fear and sadness (Macht, 1999), while long-term emotional status like depression or anxiety are considered to be usual comorbidities of obese subjects and are associated with poor diet quality (Novick *et al.*, 2005; Potenza, 2014; Roberts *et al.*, 2003).

1.5.3.4. Memory systems

Regulation of the memory system, which may also be involved in the dysfunctionality of eating behaviour, relies on the hippocampus and parahippocampal formations. The hippocampus collects food-related inputs from several brain areas such as the arcuate nucleus of the hypothalamus, the orbitofrontal cortex, and the insula (Rolls, 2008; Smith, 2000; Wang *et al.*, 2008). Moreover, the hippocampus can be modulated by peripheral hormones, like ghrelin and leptin, to control eating (Carlini *et al.*, 2004; Farr *et al.*, 2006; Oomura *et al.*,

2006). Despite traditional meal timings are regulated by circadian rhythms, memory and personal experiences can frequently prevail over them (Parent *et al.*, 2014). A poorer nutritional quality of the diet, associated with increases in food consumption, can be the result of a decreased functionality of the hippocampus (Martin & Davidson, 2014; Parent *et al.*, 2014).

1.5.3.5. Attention system

Human attention is focused on those things having a higher saliency, importance, or value, which are estimated on an individual basis. In terms of brain activity, attention-related modulation of neural reactivity has been associated with cortical areas, mainly the visual and parietal cortices and some frontal cortex regions (Corbetta *et al.*, 2002; Corbetta & Shulman, 2002). In the case of normal weight subjects, trends linked to overeating and weight gain have been observed in those subjects paying more attention to food cues, while an impaired food-related attention has been described in obese subjects, who attend more to foods than normal weight people (Doolan *et al.*, 2015; Leland & Pineda, 2006; Wade & Lowes, 2002).

1.5.3.6. Cognitive control system

The cognitive control network relies mainly on the prefrontal cortex, in particular in the inferior frontal cortex, the cingulate cortex, the dorsolateral prefrontal cortex, and the pre-supplementary motor area (Aron, 2011). Cognitive control is based on executive functions, in particular inhibiting responses believed to be inappropriate. For instance, when it comes to eating behaviour, cognitive control is in charge of refusing unhealthy food choices (like salty-snacks or a piece of cake) even under hunger episodes (Farr *et al.*, 2016).

1.5.4. Psychological factors

Food choices and food intake are also conditioned by some psychological factors. It is worth noting that these psychological factors are idiosyncratic responses to environmental stimuli and not eating disorders (Bellisle, 2009). Although their impact on food intake is normally low in healthy eaters, psychological attitudes can elicit a higher disturbing effect on eating behaviour even in healthy persons not presenting a recognised eating disorder (Bellisle, 2009).

Emotionality, externality, and disinhibition are the three most frequently observed psychological dimensions involved in eating behaviour. In particular, the emotionality attitude is a component of emotion generated in response to food during eating condition of positive or negative affect; the externality is the vulnerability to eat in response to environmental stimuli; the disinhibition is the lack of rationality during food choices and eating occasions. Thus, these psychological attitudes can negatively impact body weight regulation, acting like potential obesogenic factors (Bellisle, 2009). While emotionality acts on a personal level, disinhibition and external eating are usually observed during social eating occasion such as parties, celebrations, gala dinners, and other social activities involving food. If these circumstances are not recurrent, energy balance and body weight are not threatened, but if they have a sufficiently high frequency, they can negatively impact eating habits and, in the most critical circumstances, can even bring to eating disorders (Bellisle, 2009).

Stress is another psychological factor proved to have a deleterious impact on food intake control. Stress-related responses to food could be both anorexogenic and orexogenic. Indeed, stress usually inhibits food intake in non-restrained individuals while, in highly restrained persons, it promotes overeating, in particular of high palatability, high sugar, and high fat foodstuffs (Cartwright *et al.*, 2003; Oliver & Wardle, 1999; Wardle *et al.*, 2000). In addition, stress seems to negatively condition body weight, promoting obesity and abdominal fat deposition, by disturbing the hormonal regulation of appetite (Bellisle, 2009).

Chapter 2 - Aims of the Doctoral Thesis



Chapter 2 – Aims of the Doctoral Thesis

Eating behaviour is generally defined as any response to food-related stimuli resulting in food intake. The regulation of food choice and food intake is driven by a complex interaction of several factors that have not been well understood, yet. Both "internal" and "external" factors have been identified among the mechanisms ascribable to eating behaviour and food intake regulation, as food intake is not only controlled by body homeostasis but, in humans, it is also affected by sensory, psychological, cognitive, and environmental signals. Despite many insights about cause-and-effect relationships within those variables have been provided, only few salient mechanisms have been well established so far. In order to increase our comprehension of this topic, the scientific challenge is combining these different fields and integrating existing knowledge with new observations. In this framework, there is a need for investigations approaching the interaction among homeostatic regulators, reward cues, and motivational signals, within the environment in which this interaction takes place.

On the basis of all these considerations, the main objective of this Doctoral Thesis was to investigate the impact of food composition and food perception on food choices and eating behaviour, to better understand the complex homeostatic-hedonic system underlying food intake control.

To reach this general aim, two specific targets were set:

1. To explore the association between the compositional and perceived characteristics of food with nutritional, physiological, behavioural, and psychological variables linked to both the homeostatic and reward-related control of food choices and food intake.

2. To assess the influence of declaring the nutritional and compositional characteristics of foods on food choices, through the enhancement of healthy product availability and nutrition communication at the point-of-choice.

According to these specific objectives, the research was divided in two phases. During the first part of the present Doctoral Thesis, a randomised, crossover, controlled trial, with four experimental conditions consisting in four different breakfast meals was carried out, in collaboration with the Department of Neuroscience of the University of Parma. This first intervention has been divided into a series of studies on aspects ascribable to the multi-factor interactions existing between breakfast consumption and food intake, with specific focus on:

- Cognitive and hedonic perceptual attributes of food, in Study 1: *Food perception at lunchtime does not depend on the nutritional and perceived characteristics of breakfast* (Chapter 3.1.).
- Physiological responses and eating behaviour, in Study 2: *Effects of four different breakfast meals on satiety-related sensations, metabolic responses and food choices and intake later in the day* (Chapter 3.2.).
- Sustained and selective attention to food stimuli, in Study 3: *Effects of four different breakfast meals on cognitive performance at lunchtime* (Chapter 3.3.).
- Saliency of food stimuli and neural correlates associated with food choices, in Study 4: *Effects of four different breakfast meals on brain activation at lunchtime* (Chapter 3.4.).

Finally, during the last phase of this Doctoral Thesis, a behavioural intervention addressing food choices and promoting healthy dietary patterns whilst not limiting the freedom of choice of consumers was performed in collaboration with the Department of Economics of the University of Parma. This part was strongly influenced, in its design, by the results obtained in the first part of this Thesis, and is described in Study 5: *How to improve food choices through vending machine: the importance of healthy food availability and consumers' awareness* (Chapter 3.5.).

Chapter 3 - Studies selected for the Doctoral Thesis

3.1. STUDY 1

***Food perception at lunchtime does not
depend on the nutritional and perceived
characteristics of breakfast***

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3.1.1. Introduction

Eating behaviour and appetite control are influenced by a number of different factors, such as metabolic, sensory, cognitive, and environmental stimuli (Blundell *et al.*, 2010). Within this complex system of food intake regulation, breakfast has been ascribed as an important meal able to affect appetite control, promoting satiety and reducing hunger during the morning (Clayton & James, 2016). Thus, breakfast consumption has been related to overall health and nutritional advantages, as breakfast eaters have been reported to maintain healthier eating patterns at subsequent meal, to be less predisposed to overweight and obesity, and to have a lower BMI (Marangoni *et al.*, 2009; Rosato *et al.*, 2016; Timlin *et al.*, 2008). However, not all breakfast meals equally contribute to nutritional adequacy and appetite regulation. A healthy breakfast has been proposed as the one able to provide adequate amounts of energy and nutrients, as well as to satisfy sensory preferences and cultural traditions (O'Neil *et al.*, 2014). In fact, breakfast meals composed of healthy and tasty foods might extend the favourable impact of breakfast consumption to both homeostatic and reward systems, by increasing the hedonic value of food and extending the feelings of satiety and satisfaction arising from food intake. These factors may be affected by different nutritional characteristics of foods, such as dietary fibre, proteins, fats, and carbohydrates with different glycaemic index (GI) (Bornet *et al.*, 2007; Rebello *et al.*, 2013).

Educational programs favouring healthy dietary habits and aiming at enhancing the nutritional value of breakfast meals are of critical importance, but hedonistic aspects of food have to be fully considered. Food choice is a very complex, poorly understood process that relies on a wide array of product characteristics, comprising both sensory and non-sensory aspects (Jaeger & Rose, 2008; Killgore & Yurgelun-Todd, 2006). Sensory associations such as taste, smell, appearance, and texture are major features affecting food choice (Köster, 2009). Non-sensory characteristics of food, such as consumption context (i.e. breakfast, lunch, dinner, etc.), healthiness perception, perceived satiety, and previous

experiences of consumption, may also contribute to food choice regulation (Birch *et al.*, 1984; Buckland *et al.*, 2015; Hoogeveen *et al.*, 2016; Miller & Cassady, 2012). Healthiness perception of food affects eating behaviour and can be determined by the perceived energy content and nutritional profile of food products (Carels *et al.*, 2006; Oakes & Slotterback, 2004; Provencher *et al.*, 2009). Similarly, beliefs about the appetitive and satiating value of foods are supported by energy density and nutrient composition (Buckland *et al.*, 2015; Larkin & Martin, 2016). In addition, recognition of previous experiences influences consumers' preferences regarding food choice (Oeusoonthornwattana & Shanks, 2010; Thoma & Williams, 2013). In this sense, small shifts, rather than severe changes, towards more appetitive and healthier food choices might have a greater and more sustained effect on the nutritional adequacy of breakfast meals.

Visual food cues are widely used to evaluate the factors behind appetitive responses and healthiness perception of foodstuffs (Larkin & Martin, 2016; van der Laan *et al.*, 2011). Food images represent major sensory inputs allowing the estimation of the palatability, satiety, energy content, and healthiness of foods (Blechert *et al.*, 2014; Buckland *et al.*, 2015; Jensen *et al.*, 2016; Larkin & Martin, 2016). They can be considered surrogate stimuli linked to the hedonic and homeostatic responses to food intake and can be thus themselves rewarding (Dagher, 2012). Therefore, the food picture viewing appraisal is thought to be a good tool for the evaluation of eating preferences and food-related perceived characteristics (Blechert *et al.*, 2014).

The influence in food perception of largely different breakfast meals have been widely addressed (Holt *et al.*, 1999; Stubbs *et al.*, 1996), but the effect of minor changes in breakfast composition on perceived attributes of food throughout the day has been scarcely investigated to date (Berti *et al.*, 2015). Moreover, the effective contribution of breakfast meals differently composed and perceived to the perceptual judgement of foods at lunchtime remains elusive. In this context, intervention studies able to shed light on these topics may be extremely helpful for promoting adequate eating behaviours. This work aimed at studying the impact of breakfast meals differing just for a single food item on food perception, both at

breakfast and at lunchtime. It also delved into the relation between subjective perceptual attributes and objective energetic and compositional characteristics of food.

3.1.2. Material and Methods

3.1.2.1. Food stimuli definition and evaluation

This preliminary phase of the study aimed at validating a series of perceptual dimensions useful to categorise foodstuffs, and at defining a group of food image stimuli to be used in the second phase. Thirty volunteers were enrolled after giving their informed consent. Participants were all men, native Italian speakers, aged 20-30 years, had a BMI estimated from self-reported weight and height ranged between normal values (BMI > 20.0 and < 25.0 kg/m²), and had normal or corrected-to-normal vision. Volunteers were individually tested in a sound-attenuated and dimly illuminated room, and were comfortably sat in front of a computer monitor (22" LCD screen, with 1920x1080 pixel resolution and 60 Hz refresh rate). The eye-to-screen distance was about 57 cm. Following precise instructions displayed on the monitor, participants performed a rating task on food perception. A total of 65 food images were used as stimuli during the test. Stimuli represented a variety of common Italian foods, easily recognisable and generally consumed by the Italian population at lunchtime. A single foodstuff was depicted in each image, in a state characteristic of a typical consumption (ready-to-eat; i.e., pasta with sauce already cooked and not raw pasta). Foods were represented in both standard and large portions, in plates, bowls or trays. Visual stimuli were digitised colour photographs of equal size, standardised on a white background, and harmonised for brightness and contrast conditions. Volunteers were asked to evaluate each stimulus on five perceiving dimensions: *healthy*, *palatable*, *satiating*, *energizing*, and *caloric* attribute, and were instructed to express their evaluation through a 7-point Likert Scale (LS) using a computer keyboard. *Energizing* and *caloric* attributes were both present in the evaluation, despite their correspondence

to the same objective characteristic, since the term caloric could be perceived as less healthy with respect to energizing. Perceptual attributes were presented singularly in a random order. Within each dimension, visual stimuli were randomly displayed at the centre of the screen for 2000 ms or until volunteer's response. An example of the task is showed in Figure 17. The experiment was programmed in the Matrix Laboratory software (MATLAB®, the MathWorks® Inc., Natick, Massachusetts), which was also used to collect data.

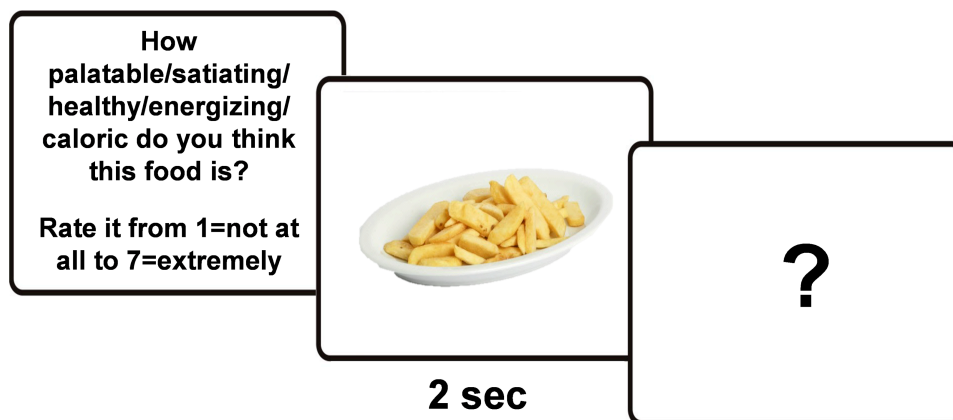


Figure 17 - Schematic representation of the procedure for the food image rating task. Perceptual attribute were presented singularly and in random order. The visual stimulus was displayed until volunteer's response, for a maximum of 2000 ms.

Based on its nutritional composition, each food stimulus was classified into a nutritional category using a 2 (high/low energy) x 2 (carbohydrate/protein-based) scheme: High Energy Carbohydrate (HEC), High Energy Protein (HEP), Low Energy Carbohydrate (LEC), and Low Energy Protein (LEP).

3.1.2.2. Breakfast meals

To explore the perceptual characteristics of a breakfast and its impact on food perception at the subsequent meal, four different breakfast models were defined. Three of the meals were breakfasts commonly consumed in Italy differing just for a single "cereal-based chocolate-containing" food item. These three test

breakfasts (BRs) were composed of an apple and semi-skimmed milk, and white bread with chocolate hazelnut spread (BR1), or muesli with chocolate and nuts (BR2), or puffed chocolate rice (BR3). The control meal consisted of decaffeinated tea -with no sugar and no milk- (BR4).

The three test BRs were iso-caloric (about 330 kcal/1.38 MJ), while BR4 was a non-caloric meal, representing fasting conditions. The three iso-energetic meals were carefully assembled to provide about 15% of the estimated mean total daily energy intake for an adult population, according to the Italian guidelines (Cialfa *et al.*, 2007; SINU, 2014). In addition, they were similar for protein and fibre contents but different in carbohydrates, lipids, and GI. Breakfasts were also hypothesised to be different for the cognitive perception of healthiness of the cereal-based chocolate-containing products, being muesli thought as healthy while bread with spread and puffed rice as not so healthy. BR1 had the same total carbohydrate, sugar and lipid content and the same GI as BR2, while the same healthiness perception as BR3. BR2 had the same total carbohydrate, sugar and lipid content and the same GI as BR1, but a higher healthiness perception than the other two experimental breakfasts. BR3 had the same healthiness perception as BR1, but lower lipid and higher sugar amounts, and a higher GI than the other two experimental breakfasts.

The nutrient composition of each breakfast was calculated summing the nutritional values of each food item based on nutritional information from food label or from the food database of the European Institute of Oncology (Gnagnarella *et al.*, 2008), collected for each commercial product or generic foodstuffs, respectively. The GI value of each food item was obtained from published data (Foster-Powell *et al.*, 2002; Scazzina *et al.*, 2016) and calculated using the formula for mixed meals (Wolever *et al.*, 2006). More details on composition and nutritional content of breakfast meals are provided in Table 4.

Table 4 - Composition and nutritional content of breakfast meals.

Breakfast meal	Energy (kcal/MJ)	Protein (g)	Lipid (g)	Carbs (g)	Sugars (g)	GI
Breakfast meal 1 (BR1) Semi-skimmed milk (125 mL) Apple (100 g) Chocolate hazelnut spread (15 g) White bread (45 g)	331.5/1.4	9.5	11.2	48.5	30.6	45
Breakfast meal 2 (BR2) Semi-skimmed milk (150 mL) Apple (150 g) Muesli chocolate and nuts (40 g)	328.1/1.4	9.4	11.1	48.3	33.8	47
Breakfast meal 3 (BR3) Semi-skimmed milk (150 mL) Apple (150 g) Puffed chocolate rice (50 g)	331.0/1.4	8.2	4.2	66.9	43.4	57
Breakfast meal 4 (BR4) Tea (decaffeinated) (125 mL)	-	-	-	-	-	-

3.1.2.3. Breakfast meal evaluation

Fifteen subjects were recruited in the second phase of the study, after providing their informed consent. Volunteers were all healthy males, aged 20-30 years, had a normal (BMI estimated from self-reported weight and height ranged between 20.0 and 25.0 kg/m²) and stable body weight in the three previous months (less than $\pm 5\%$ change), and had normal or corrected-to-normal vision. Eligible participants were also screened for their dietary habits, resulting in a study population of omnivorous, habitual breakfast eaters, with no food allergy. Volunteers were involved in a randomised, crossover, controlled trial, with four experimental conditions corresponding with the four BRs. On four different days, participants were asked to consume an assigned breakfast and they were instructed to express a hedonic rating of the assigned breakfast through a 7-point LS at two time points: before - visual rating in fasting condition - and 15 minutes after breakfast consumption. Each breakfast was evaluated on the same five perceiving dimensions used in the first phase of the present study: *healthy*, *palatable*, *satiating*, *energizing*, and *caloric* attribute.

3.1.2.4. Food stimuli evaluation at lunchtime

Four hours after breakfast, volunteers were asked to perform a rating task on food perception. Twenty food images (Figure 18) out of the overall set of 65 initial ones were selected as stimuli for the second phase of the study. Foods were chosen to ensure that each was easily recognisable and consistently identifiable. Food pictures were selected to be homogeneous with respect to nutritional category (5 HEC foods, 5 HEP foods, 5 LEC foods, and 5 LEP foods), and to be matched for lexical frequency ($F_{3,16}=0.6$, $p=0.6$), word length ($F_{3,16}=0.8$, $p=0.5$), and syllable number ($F_{3,16}=0.7$, $p=0.5$). Except for the stimuli, apparatus and procedure were the same as in the first phase rating task (as explained in paragraph 3.1.2.1. Food stimuli definition and evaluation).



Figure 18 - Food images selected as stimuli for the rating task in the second phase of the experiment. HEC, High Energy Carbohydrate; HEP, High Energy Protein; LEC, Low Energy Carbohydrate; LEP, Low Energy Protein. 21, potato dumplings; 27, lasagna, tray; 38, chips; 44, pizza; 20, ice cream; 33, sandwich; 41, fish coated in crumbs; 48, meat balls; 8, chocolate pudding; 22, hamburger; 3, biscuits; 30, bread wholegrain; 39, pasta with vegetables; 46, polenta (porridge made with corn flour and salted water); 51, rice; 65, yogurt; 25, salad; 49, Parma ham; 7, bresaola, arugula and parmesan cheese; 63, boiled egg.

3.1.2.5. Statistical analysis

Data were presented as mean \pm standard error of the mean (SEM) of thirty independent measurements and of fifteen independent measurements, for the first and second phase, respectively. A difference was considered significant at $p < 0.05$. The statistical analysis was performed with the Statistical Package for Social Sciences software (IBM SPSS® Statistics, Version 22.0. IBM Corp., Chicago, IL).

Principal Component Analysis (PCA) was carried out with varimax rotation to describe the perceived characteristics of foods through the five perceiving dimension (*healthy, palatable, satiating, energizing* and *caloric* attribute) in both food image rating tasks. Data of breakfast meal evaluations were analysed for each perceiving dimension by 1) repeated measurement General Linear Model (GLM) (breakfast, time, and breakfast x time) using Greenhouse–Geisser correction if epsilon was lesser than 0.75 or Huynh–Feldt correction if epsilon was greater than 0.75, and Bonferroni *post-hoc* tests for multiple comparisons, and 2) paired-sample *t*-test, for comparison within the same group before and after breakfast meal consumption. The effect of breakfast meals during lunchtime on the perceiving dimension of food stimuli was analysed by repeated measurement GLM, using Bonferroni *post-hoc* tests for multiple comparisons among groups.

3.1.3. Results

3.1.3.1. Efficacy of food perceptual attributes to characterise food images

A total of 65 visual stimuli, representative of an omnivorous diet and comprising food items that may be consumed throughout the day, served to categorise foods according to five perceptual attributes. Food description was achieved by using multivariate PCA (Figure 19). Two principal components (PCs) explained 92.5% of the total variability observed. Almost 54% of the variability was explained by the first PC. PC1 correlated positively with *energizing, palatable*, and

satiating variables (Figure 19A). PC2, accounting for 38.6% of the total variability, had high negative component loading from the *healthy* feature, while it had positive load from the *caloric* attribute (Figure 19A). According to this categorisation based on appetitive and heuristic judgments, food palatability was associated with satiating and energizing/caloric dimensions. Interestingly, these interlinked food-related variables were judged to be opposed to the healthiness dimension. The perception of the calorie content (*caloric*) was not perfectly correlated to the attributed energy value (*energizing*) of the foodstuffs. As highlighted by PCA results (Figure 19B), different foodstuffs were distinguished for these perceptual attributes. These perceptual dimensions assisting food characterisation served to further categorise different breakfast meals attending to their perceived attributes.

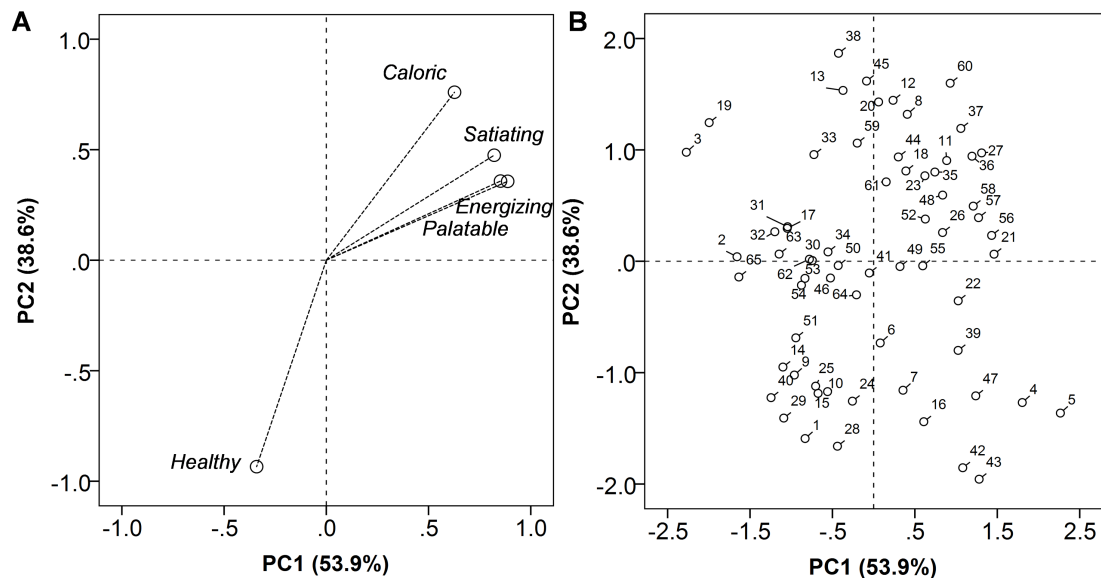


Figure 19 - Biplots (loadings -A- and scores -B-) obtained from the PCA with varimax of the five perceptual attributes (palatable, satiating, healthy, energizing, and caloric) for the 65 food visual stimuli. 1,watermelon; 2,cereal bars; 3,biscuits; 4,steak; 5,steak with vegetable; 6,bresaola; 7,bresaola, arugula and parmesan cheese; 8, chocolate pudding; 9,caprese salad; 10,caprese salad with oil; 11,breaded cutlet and chips; 12,nuggets; 13,tart with jam; 14,beans; 15,kidney beans; 16,spelt; 17,focaccia; 18,fried fish; 19,smoothie; 20,ice cream; 21,potato dumplings; 22,hamburger; 23,hamburger and chips; 24,fish salad ; 25,salad; 26,rolls; 27,lasagna; 28,fruit cocktail; 29,apple; 30,bread wholegrain; 31,bread white, sliced; 32,bread white, commercially prepared (sandwich type); 33,sandwich; 34,sandwich with vegetables; 35,parmigiana; 36,pasta with tomato cooked in the oven; 37,pasta with cream cooked in the oven ; 38,chips; 39,pasta with vegetables; 40,pear; 41,fish, coated in crumbs; 42,fish, grilled; 43,fish, grilled with lemon; 44,pizza; 45,pizza with chips; 46,polenta (porridge made with corn flour and salted water); 47,chicken, grilled; 48,meat balls; 49,parma ham; 50,apple puree; 51,rice; 52,cold cut; 53,spaghetti with oil; 54,spaghetti with oil and basil; 55,spaghetti with tomato sauce; 56,stew; 57,pork knuckle; 58,pork knuckle and chips; 59,apple strudel; 60,tiramisu; 61,vegetable savoury pie; 62,boiled egg; 63,boiled egg and vegetable; 64,stuffed vegetables; 65,yogurt.

Later, a subset of 20 visual stimuli was selected according to their energy (high or low) and nutrient composition (carbohydrates or proteins) (HEC, HEP, LEC, and LEP). These 20 food images were representative of the whole set of 65 visual stimuli, as confirmed by a new PCA performed with these selected visual stimuli and matching the loads and scores registered for the PCA carried out for the whole set of stimuli (Figure 20). This subset of 20 food images was further used to assess the impact of different breakfasts on food perception at lunchtime.

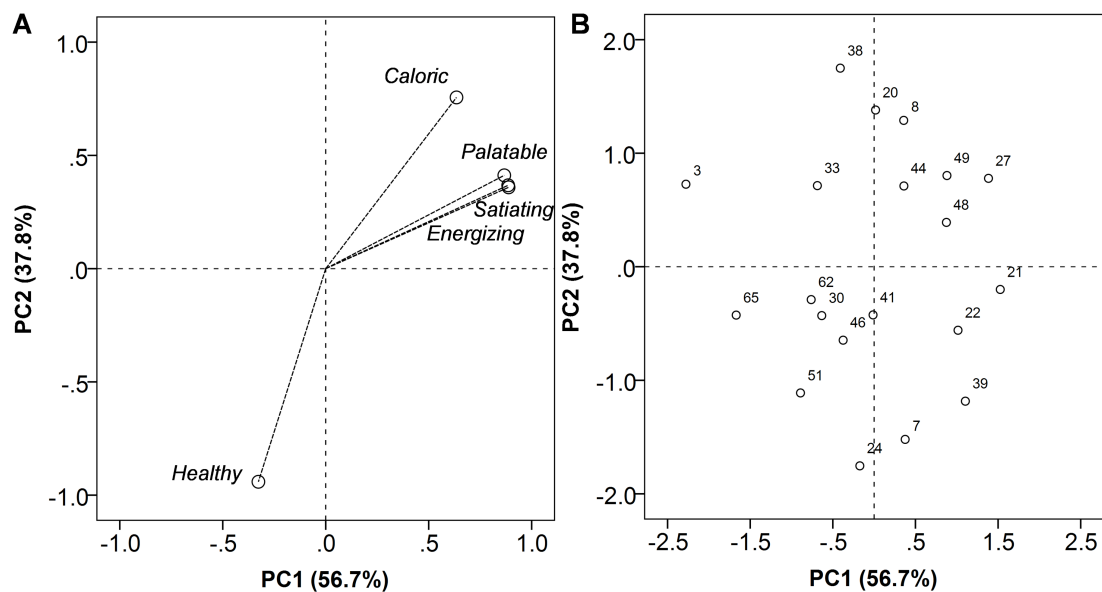


Figure 20 - Biplots (loadings -A- and scores -B-) obtained from the PCA of the five perceptual attributes (palatable, satiating, healthy, energizing, and caloric) for the 20 food visual stimuli (out of the overall set of 65 initial ones) selected for the second phase of the experiment. HEC, High Energy Carbohydrate; HEP, High Energy Protein; LEC, Low Energy Carbohydrate; LEP, Low Energy Protein. 21, potato dumplings; 27, lasagna, tray; 38, chips; 44, pizza; 20, ice cream; 33, sandwich; 41, fish coated in crumbs; 48, meat balls; 8, chocolate pudding; 22, hamburger; 3, biscuits; 30, bread wholegrain; 39, pasta with vegetables; 46, polenta (porridge made with corn flour and salted water); 51, rice; 65, yogurt; 25, salad; 49, parma ham; 7, bresaola, arugula and parmesan cheese; 63, boiled egg.

3.1.3.2. Breakfast perception before and after consumption of different breakfasts

To explore the association between compositional and perceived characteristics of breakfast meals with perceptual variables, four different breakfasts were compared. The five perceptual attributes affecting food choice were evaluated before and after the consumption of three nutritional-balanced breakfasts having different GI and hypothetically endowed with different health

beliefs (Figure 21). A non-caloric control breakfast mimicking fasting conditions was also used (BR4). There were main effects of breakfast for all the perceptual attributes ($p < 0.01$ for *healthy* and $p < 0.001$ for the rest of attributes), while there were no significant main effects of time and the interaction of breakfast x time ($p > 0.05$ for all the attributes).

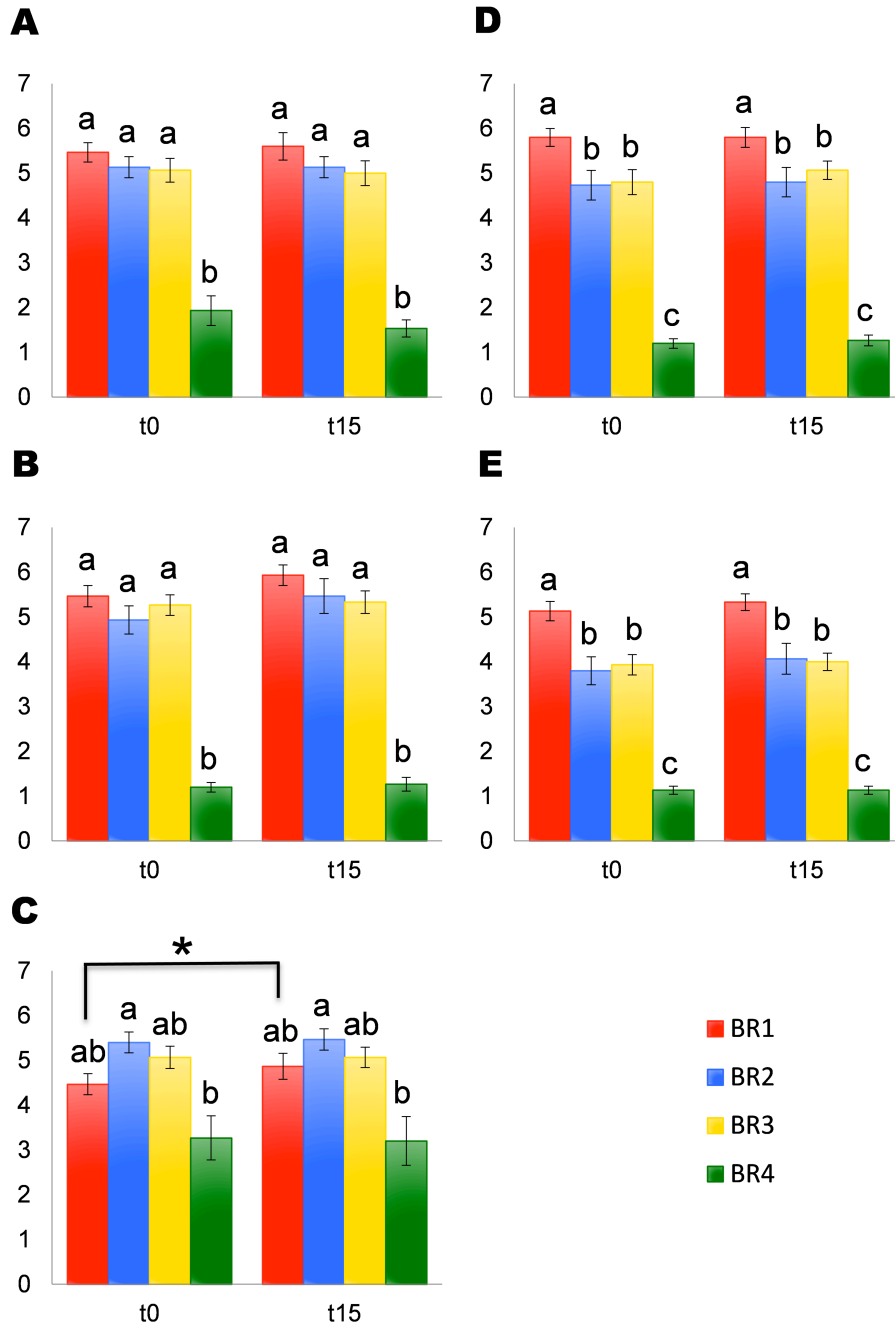


Figure 21 - Palatable (A), satiating (B), healthy (C), energizing (D), and caloric (E) perception for the four breakfasts evaluated before (t0) and after (t15) meal consumption. Values are means ($n=15$) \pm SEM. Different letters in the same grouped bars indicate statistically significant differences ($p < 0.05$) among breakfast meals. * indicates statistically significant differences ($p < 0.05$) within the same breakfast before and after meal consumption.

Before being consumed, the three iso-caloric, chocolate-containing breakfasts (BR1, BR2, and BR3) showed similar values for the *palatable* and *satiating* perceptual attributes, in contrast to the control breakfast (BR4), which had very low values for these parameters (Figure 21A and 21B). Breakfast meal healthiness was reported to be statistically different between BR2 and BR4, while BR1 and BR3 presented the same values for the *healthy* variable than the previous breakfasts (Figure 21C). Hedonic rating of the breakfasts for the *energizing* and *caloric* attributes pointed out that the breakfast containing white bread and chocolate hazelnut spread (BR1) was perceived as more energetic and caloric than breakfasts containing muesli with chocolate and nuts (BR2) and chocolate rice puffs (BR3) (Figure 21D and 21E). Therefore, taking into account the hedonic rating for the five perception responses, all the breakfast meals were perceived as different. It should also be mentioned that, although energizing and caloric attributes followed a common trend, ratings for *energizing* were higher than those attributed to *caloric* (Figure 21D and 21E).

Breakfast consumption did not change the perceptual evaluation registered before consumption among the four breakfast meals (Figure 21). Nevertheless, consumption of the breakfast containing white bread and chocolate hazelnut spread (BR1) led to a statistically significant change in the perception of its healthiness, raising the *healthy* value for this breakfast meal (Figure 21C).

3.1.3.3. Impact of different breakfasts on the perception of foods at lunchtime

The influence of breakfast intake on food perception was evaluated at lunchtime by using the food image viewing appraisal previously reported (Figure 22). The perception of 20 images representing foodstuffs suitable for lunchtime did not vary after the consumption of differently composed and perceived breakfasts ($p > 0.05$ for the five perceptual attributes). In this sense, the hedonic rating of lunch items was not affected by the type of breakfast or the simple consumption of a cup of decaffeinated tea. Similarly, when the outputs for the 20 foodstuffs were

grouped by nutritional category according to their energy and nutrient contents (HEC, HEP, LEC, and LEP), the emotional/cognitive responses to the visual food cues did not change among breakfasts (Figure 22). Only one statistically significant effect was observed for the *satiating* perception of HEP foods (Figure 22B), being this food category judged as poorly satiating after consumption of BR1 with respect to BR3.

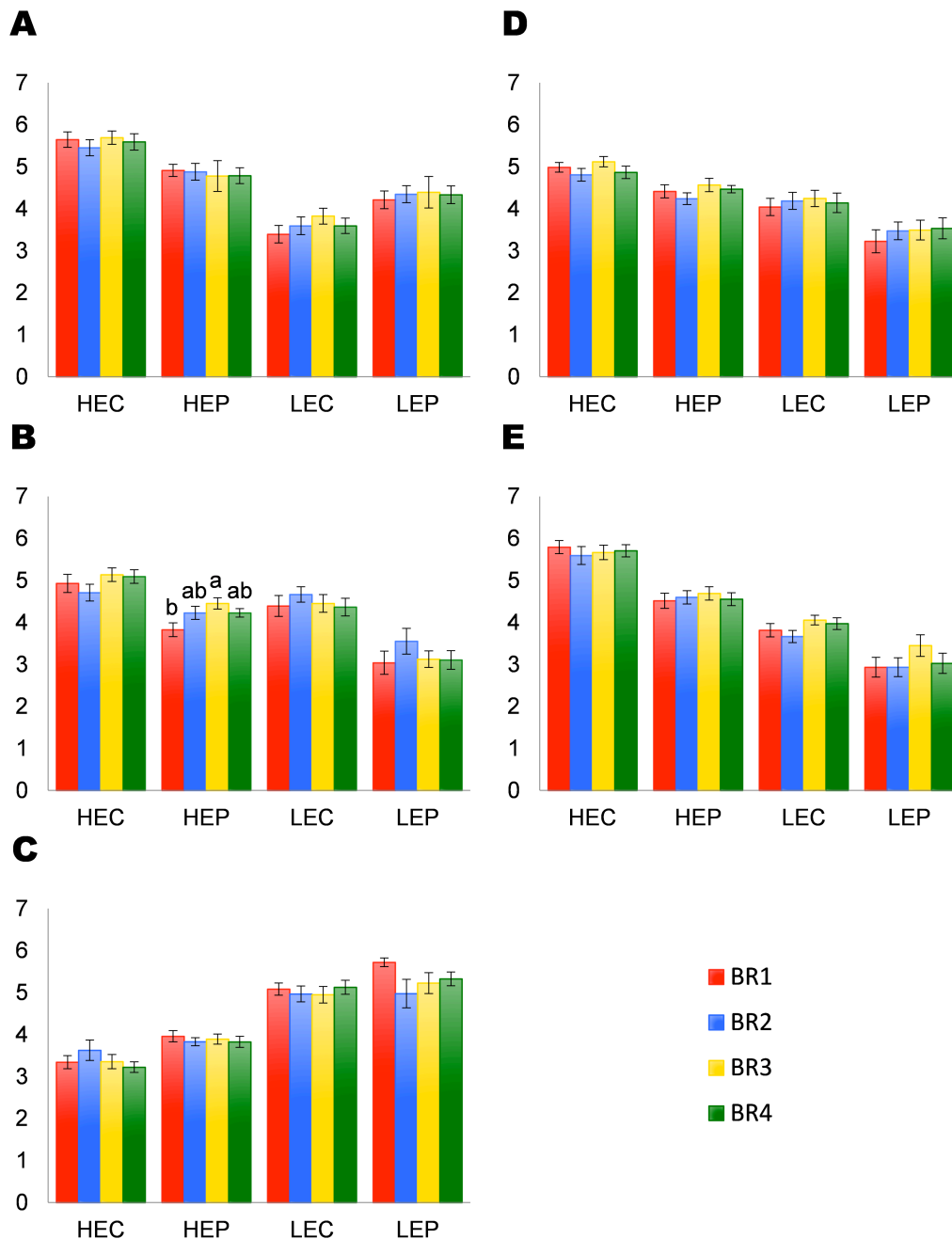


Figure 22 - Palatable (A), satiating (B), healthy (C), energizing (D), and caloric (E) perception for the four nutritional categories (HEC, HEP, LEC, and LEP) by the four breakfasts. Values are means ($n=15$) \pm SEM. Different letters in the same grouped bars indicate statistically significant differences ($p < 0.05$) among breakfast meals. HEC, High Energy Carbohydrate; HEP, High Energy Protein; LEC, Low Energy Carbohydrate; LEP, Low Energy Protein.

The formation of nutritional groups by energy and nutrient content (HEC, HEP, LEC, and LEP) was also used to better understand the relation among the different perceptual attributes and types of breakfasts at lunchtime, by using multivariate PCA (Figure 23). Two PC explained the 97.8% of the total variability, with the first PC accounting for the 50.8% and the second PC for the remaining 47.0%. PC1 was mainly loaded from *satiating*, *caloric* and *energizing* attributes (Figure 23A). PC2 had positive loads from *palatable* and negative loads from *healthy* dimensions (Figure 23A). Individual scores for the perception attributes of each nutritional category after consuming different breakfast meals confirmed the lack of effect of the type of breakfast, but they provided interesting outputs dealing with the perceptive characteristics of the four nutritional categories (Figure 23B). In this sense, highly energetic foods (HEC and HEP) were perceived as such by volunteers, they being also judged as palatable (Figure 23B). Low energetic products (LEC and LEP) were considered to be healthier than those at high energy content (HEC and HEP). Carbohydrate-rich food products (HEC and LEC) were valued as satiating and energetic/caloric, while protein-rich ones (HEP and LEP) were perceived as less satiating, mainly those at low energy content (LEP) (Figure 23B).

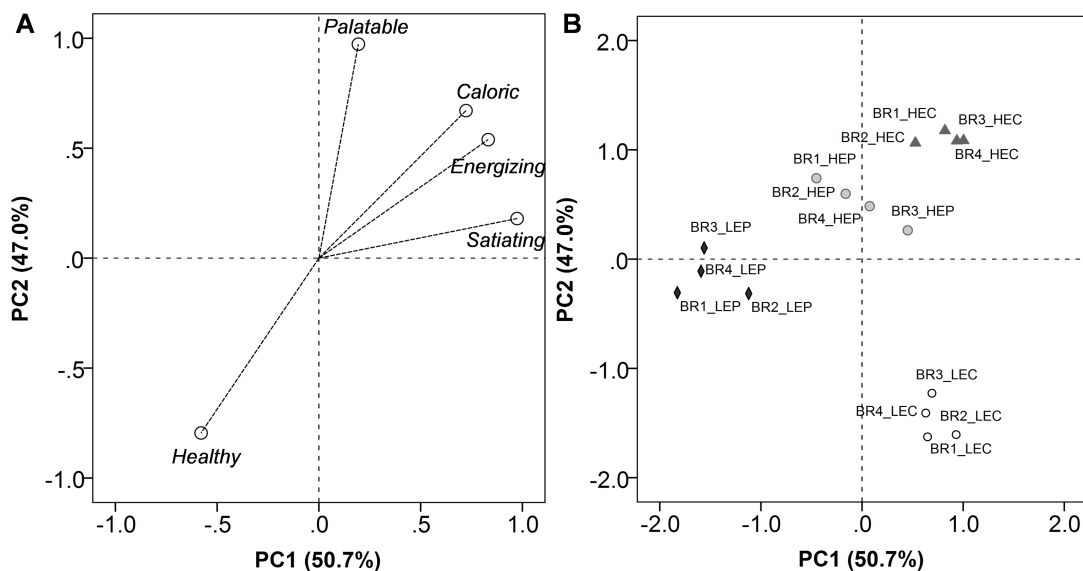


Figure 23 - Biplots (loadings -A- and scores -B-) obtained from the PCA of the five perceptual attributes (palatable, satiating, healthy, energizing, and caloric) for the four nutritional categories (HEC, HEP, LEC, and LEP) by breakfast meal.

BR, Breakfast; HEC, High Energy Carbohydrate; HEP, High Energy Protein; LEC, Low Energy Carbohydrate; LEP, Low Energy Protein.

3.1.4. Discussion

Breakfast consumption is a key dietary habit that may condition consumers' nutritional and health status (Marangoni *et al.*, 2009; Rosato *et al.*, 2016; Timlin *et al.*, 2008). The present work aimed at studying the impact of different compositions of breakfast on food perception at lunch. It also served to reappraise the importance of breakfast on food choices throughout the day. In particular, we attempted to investigate the effect of balanced breakfasts differing just for a single food item on widely recognised food perceptual attributes (Blechert *et al.*, 2014; Buckland *et al.*, 2015; Larkin & Martin, 2016). Although the effect of different breakfast models have been extensively assessed (Holt *et al.*, 1999; Stubbs *et al.*, 1996), the influence in food perception of small changes in breakfast composition has been barely tackled so far.

The food image viewing method has been recognised as a useful tool for the assessment of perceptual and behavioural preferences (Blechert *et al.*, 2014). When labelling foods, general population tends to use dichotomous categorisations like healthy/unhealthy or good/bad (Carels *et al.*, 2007; Rozin *et al.*, 1996). However, the heuristic recognition of food images based on dichotomous variables presents carry-over effects on the appraisal of perceived nutritional content of food (Oakes & Slotterback, 2001). For instance, estimations of fat and caloric content may be biased by healthiness perceptions of foods (Carels *et al.*, 2006; Carels *et al.*, 2007). Therefore, to overcome the limitations that dichotomous judgment may have on food perception, five food-related subjective perceptual attributes were used, selected according to consumer contextual and experimental knowledge on food perception, and in line with previous research (Blechert *et al.*, 2014; Buckland *et al.*, 2015; Jensen *et al.*, 2016; Larkin & Martin, 2016). On the other hand, the visual food cues, used as stimuli to ascertain whether a set of five perceptual variables was able to properly characterise different foodstuffs in a rating test, were representative of an Italian omnivorous diet, and comprised food items that may be consumed throughout the day. Rating of perceptual attributes succeeded in categorising the perceived properties of the foods displayed in the visual cues (Figure 19B).

Interestingly, healthiness and appetitive beliefs were almost diametrically opposite (Figure 19A). As previous studies have reported, healthy foods are judged as less tasty, underlying that healthiness and taste are inversely and implicitly perceived (Huang & Wu, 2016; Raghunathan *et al.*, 2006). It should also be noted that *energizing* and *caloric* subjective attributes were differently perceived despite they correspond to the same objective attribute -energy content- as hypothesized. This may account for the lack of adequate nutritional literacy in the young population. A good grouping by energy content, but not by nutrient categorisation, was achieved when a subset of food images was chosen (Figure 20). However, the use of a shorter number of visual stimuli at lunchtime was extremely helpful in outperforming time constraints and increasing volunteer welfare.

Assessment of breakfast perception succeeded to discriminate experimental meals against control breakfast. As expected, the perceived satiation and palatability of the three experimental breakfasts (BR1, BR2, and BR3) was superior to the breakfast restricted to a cup of tea (BR4) (Figure 21A and 21B). Interestingly, no clear statistical differences were found between the perceived healthiness of two breakfasts (BR1 and BR3) and the control one (BR4). A likely explanation may be found in the high inter-individual variability observed for the healthiness estimation of the tea-based breakfast (see the high deviation bar at BR4, Figure 21C). This variation on an individual level could be determined by differences of volunteers' knowledge on the widely-reported health benefits of tea (Rodriguez-Mateos *et al.*, 2014), assimilated by a part of the general population (Oke *et al.*, 2016). The lack of differences among BR1, BR2, and BR3 on the perception of their healthy characteristics is also a point worth mentioning. It may be based on the fact that associations of different ingredients can modify the perceived health value of foods (Oakes, 2004). While chocolate-based products can be endowed with negative health beliefs (Oakes, 2004), apples and milk may be perceived as nutritious/healthy foods (Larkin & Martin, 2016; Oakes, 2004). In this sense, despite the hypothesised differences on the healthiness perception of the cereal-based chocolate-containing products consumed in the experimental breakfast meals (for instance, between the white bread with spread -BR1- and the muesli -BR2-), they

were all perceived as equivalent within a balanced meal. Therefore, the presence of an apple and milk in the experimental breakfasts may have counteracted the negative values hypothetically attributed to the different cereal-based chocolate-containing products.

The evaluation of perceived attributes of breakfasts showed differences for the *energizing* and *caloric* attributes among the three experimental breakfasts, despite they were iso-caloric and equally perceived as healthy (Figure 21C, 21D, and 21E). Estimations on energy/caloric content were likely conditioned by the healthiness perception of the different cereal-based chocolate-containing products present in each breakfast. Actually, previous works have indicated that healthiness/unhealthiness perception of foods condition the energy/calorie estimations expressed by volunteers, with those perceived as healthy rated to provide far fewer calories than the ones perceived as unhealthy (Carels *et al.*, 2006; Carels *et al.*, 2007; Larkin & Martin, 2016; Rozin *et al.*, 1996). This may be one of the reasons behind the inaccurate report of the energy/caloric content of the three iso-caloric breakfasts. On the other hand, subjective attributes related to breakfast perception were not affected by objective compositional differences of breakfasts in terms of carbohydrates, lipids, and GI. These results were not in agreement with Buckland and co-workers (2015), who noted that perceived satiety values were related to lower fat and higher protein, among other factors. These discrepancies may be due to the different methodologies used and to the fact that most of them evaluated single items instead of whole breakfast meals (Buckland *et al.*, 2015). Actually, in accordance with our results, satiety induced by breakfast did not change on the basis of different macronutrient compositions when seven breakfast meals consisting of a standard drink and different cereal-based products were evaluated (Berti *et al.*, 2015).

The perception of 20 foodstuffs at lunchtime was not affected by the type of breakfast consumed. Similarly, appetitive sensations did not change among breakfasts when food images were classified according to their energy and nutrient content (Figures 22 and 23). Although the four breakfasts were different in terms of perceived attributes, energy content, and nutritional composition, responses to

food pictures were the same 4 h after breakfast consumption, independently of the type of meal. This fact may account for the effect that short-term food deprivation has for food evaluation (Blechert *et al.*, 2014). While postprandial metabolic responses likely changed as a result of the different carbohydrate content and GI of the breakfasts (Geliebter *et al.*, 2015; Samra & Anderson, 2007), this would not be enough to provoke different perceptual ratings of foods at lunchtime. Regardless of the type of breakfast, a fasting-like effect at 4 h after meal consumption could have occurred, hence limiting the effects of breakfasts on food perception (Geliebter *et al.*, 2015; Samra & Anderson, 2007). With respect to the associations found between perceived characteristics and macronutrient content at lunchtime (Figure 23), some of them were not in line with previous research (Buckland *et al.*, 2015; Stubbs *et al.*, 1996). However, they matched the heuristic judgments of volunteers for the same products under fasting conditions (Figure 20). Therefore, differences among various works can be related to the different population of study, methodologies, and visual cues used. Overall, this research provided valuable insights in terms of food categorization by perceived characteristics, contributing to better explaining the relation between subjective food perception attributes and objective energy and nutrient values.

This study presented some limitations that could be tackled in future research. Assessment of breakfast perception was based on the perceptual attributes used for appraising single food items. This may represent a confounding factor since the perceived characteristics of foods may change when evaluated within a meal or in concomitance with other ingredients (Larkin & Martin, 2016; Oakes, 2004; Oakes & Slotterback, 2004). Nonetheless, it was not feasible testing a large set of complete breakfast meal-related images beside the individual visual cues used for lunch-related foodstuffs in order to evaluate meal-linked perceptual attributes. Secondly, perception rating at lunchtime was based on food images instead of exposure to real food, which may have led to different rating of subjective perceived characteristics (Buckland *et al.*, 2015). Thirdly, subject homogeneity (healthy young men) limits the generalizability of the study outcomes, but helped to draw conclusions on the effects that small differences at breakfast

may have on food perception. As a last point, the study of shorter periods of time between breakfast and lunch, avoiding fasting-like scenarios (Berti *et al.*, 2015), may have enhanced the effects of different breakfasts on food perception.

3.1.5. Conclusions

The present work contributed to better understand the impact of nutritionally balanced breakfast meals differing just for a single food item on food perception, both at breakfast and at lunchtime. It strengthened the knowledge on the relation between subjective perceptual attributes and objective energy value and nutrient characteristics. When subjects evaluated the perceived attributes of breakfasts differing for nutritional composition, they judged breakfast healthiness as a whole, while they rated breakfast energy/caloric content on the basis of single food items. In the context of nutritionally balanced breakfast meals, negatively-perceived health properties of single breakfasts items may no longer possess unhealthful characteristics when associated with food perceived as healthy, like an apple. In this sense, the association of different ingredients can modify the perceived health value of foods. Heuristic judgment of food items at lunchtime did not change following consumption of different breakfasts. These outcomes would underpin the need for snacking between breakfast and lunch times, which may avoid fasting-like status nullifying the putative effects of breakfasts differently composed and perceived. Further works aiming at validating real intake of foodstuffs belonging to different nutritional categories after consumption of different breakfasts are guaranteed.

3.2. STUDY 2

Effects of four different breakfast meals on satiety-related sensations, metabolic responses and food choices and intake later in the day

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3.2.1. Introduction

A large body of evidence shows that regular breakfast consumption is associated with a better health status (Marangoni *et al.*, 2009; O'Neil *et al.*; Williams, 2014). Nevertheless, a high prevalence of breakfast skipping is observed worldwide, with differences among age groups and populations (Rampersaud *et al.*, 2005). The misperception of cutting calories seems to be a relevant contributor among the reasons behind the decision of not having breakfast, so that breakfast skipping is often used as a wrong strategy to control body weight (Astbury *et al.*, 2011). On the contrary, breakfast consumers seem to be less predisposed to overweight and obesity, generally showing a lower BMI than breakfast skippers (Purslow *et al.*, 2008; Timlin *et al.*, 2008). This can be partially ascribable to a redistribution of daily energy intake, so that more energy is consumed at breakfast and less energy is consumed later in the day (Purslow *et al.*, 2008).

Epidemiological evidence also showed favourable effects of breakfast, and some of its components (e.g. whole-grain), on the risk of many chronic diseases, like cardiovascular diseases and type 2 diabetes, through a reduction of the main risk factors, including lipid profile, impaired glucose tolerance and overweight or obesity (Cahill *et al.*, 2013; Kleemola *et al.*, 1999; Kochar *et al.*, 2007).

In addition, breakfast, based on its nutrient composition, can deeply affect the overall quality of the diet. For example, regularly having breakfast seems to mean higher intake of fibre and calcium and lower intake of fats and total calories (Fayet-Moore *et al.*, 2016), whereas skipping breakfast can reduce the probability to meet the recommended dietary allowances of many micronutrients (Nicklas *et al.*, 1998; Williams, 2007) and this can provide a further explanation of the protective effect of breakfast consumption on the risk of chronic diseases. In general, the optimal breakfast model includes carbohydrates with different GI, as well as proteins and fats, with different effects on satiety. In this context, it has been proposed that consumption of low GI foods at breakfast may have an impact on food intake in the remaining of the day, probably through their ability to increase satiety (Bornet *et al.*, 2007; Livesey, 2005; Rebello *et al.*, 2013). Moreover, the

combination of different food items may be a valuable strategy for reaching a considerable intake of micronutrients (Marangoni *et al.*, 2009).

However, although breakfast seems to be positively associated with a healthy eating patterns and better food choices throughout the day, this association is complicated by the complex interaction of several factors. Food intake is not only affected by the body homeostasis, but also by environmental signals, like cultural and social habits, lifestyle, etc. These parameters evoke reward-related and motivational signals influencing our daily eating choices. Most of the theories on the regulation of food intake propose two parallel systems interacting with food consumption: homeostatic and hedonic (Lutter & Nestler, 2009; Saper *et al.*, 2002). The former allows adequate nutrition by increasing the motivation to eat following depletion of energy stores. This system includes hunger, satiety and appetite mechanisms, as well as the levels of systemic mediators like leptin and ghrelin, commonly used by the brain to collect information about peripheral energy levels (Klok *et al.*, 2007). As a result, a dysfunction in components of the homeostatic system may result in a positive energy balance that inevitably leads to weight gain in the long term. On the other side, the hedonic or reward-related system represents one of the most potent determinants of the feeding process and can result in caloric intake exceeding requirements by increasing the desire to consume highly palatable foods (Kenny, 2011). The cross-talk between metabolic and emotional-cognitive regulatory systems determines food intake (Volkow *et al.*, 2011), and the coordinator of these processes is the brain, integrating food-related sensory information with satiety signals produced by the gastrointestinal tract to set up a mental representation of the food. Those signals allow the evaluation of the reward value of food, the regulation of feeding behaviours, and the generation of affective responses (Li *et al.*, 2012). For all these reasons, there is an increasing interest on motivational and decisional aspects of food choices, eating behaviours and how they are influenced by food characteristics.

In this framework, the present study aimed at exploring the association between the compositional and perceived characteristics of breakfast meals with nutritional, biochemical, and physiological variables, trying to better understand

homeostatic-hedonic system underlying food intake control. It was hypothesised that the best breakfast meal is the one able to generate the greater satiety in the postprandial status, the best adiposity regulator profile and glycaemic metabolism, as well as the best hedonistic and reward-related perceptions. To this purpose, we tested the effect of breakfast meals different for nutritional values and perceptual characteristics, on i) appetite and satiety perception, ii) metabolic profile, iii) food choices at subsequent meal, and iv) compensatory responses to foods consumed later in the day.

3.2.2. Material and Methods

3.2.2.1. Participants

Volunteers were recruited through advertisement posted in all Departments of the University of Parma. Eligible participants were healthy males, aged >18 and < 30 years, with normal (BMI > 20.0 and < 25.0 kg/m²) and stable body weight (less than ±5% change) in the three months prior the study, no smokers, not taking medication, and with a moderate-active lifestyle. In addition, volunteers were habitual breakfast eaters, not following a specific diet, and had no food allergy.

Fifteen healthy young men adults participated in the study (mean ± standard deviation (SD): age 24.1 ± 2.1 years; BMI 23.4 ± 1.6 kg/m²; PAL 1.6 ± 0.1). All volunteers gave an informed written consent prior to their enrolment.

3.2.2.2. Study design and procedures

This study was a randomised, four-arms, crossover and controlled trial, with four experimental conditions consisting of different breakfast models. Volunteers consumed each one of the four breakfast meals throughout four different weeks, separated by at least one-week washout. Breakfasts were assigned in a random and counterbalanced order. Participants were instructed to have the assigned breakfast

every morning around 8.00 a.m., and to record their food and drink daily consumption. They were asked to maintain their usual eating habits – except for breakfast – and their normal lifestyle. Each Wednesday of the experimental weeks was chosen as test day. On the evening before the experimental day, participants were instructed to have a free-choice standard dinner and to avoid strenuous activities. Volunteers arrived fasting to the hospital at 07.30 a.m. of the test day. Appetite and satiety ratings, and blood samples were assessed and drawn before (baseline measures after 12-h fast) and up to 4 hours after breakfast consumption, between 8.00 and 12.00 a.m. After completion of these tests, an *ad libitum* standard lunch was served. The breakfast period lasted 15 minutes, while the lunch lasted 30/60 minutes with participants left eating alone. Study design and schedule of experimental procedures are reported in Figure 24.

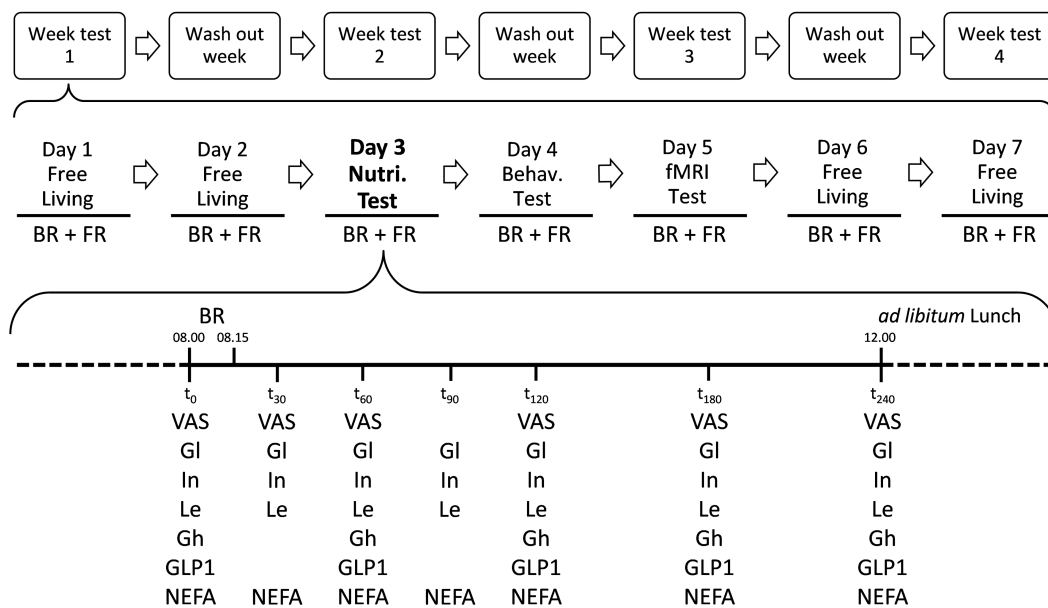


Figure 24 - Study design and schedule of experimental procedures.

Nutri Test: Nutritional test; Behav. Test: Behavioural test; fMRI: functional magnetic resonance imaging.

BR: breakfast; FR: food record; VAS: visual analogue scales for appetite and satiety; Gl: Glucose; In:

Insulin; Le: Leptin; Gh: Ghrelin; GLP1: Glucagon-like peptide-1; NEFA: Non esterified fatty acids.

The study was conducted in accordance with the Declaration of Helsinki, after Ethics Committee of Parma (Italy) approved the protocol. The trial was registered at the US National Institute of Health on clinicaltrials.gov as BRNN-014 NCT02516956.

3.2.2.3. Breakfast meals

Breakfasts were three experimental meals (BR1, BR2, and BR3) composed of foods usually consumed in Italy and differing just for one single food, and one control, non-caloric meal (BR4). BR1, BR2, and BR3 were iso-caloric and similar for protein and dietary fibre contents. BR1 and BR2 were similar for total carbohydrate, sugar and lipid profiles, while BR3 had a lower lipid and higher carbohydrate and sugar content compared to BR1 and BR2. The meal composition and nutritional profile are shown in Table 4 (Chapter 3.1.2.2).

Moreover, the three experimental meals were designed to be matched for GI. In fact, BR1 and BR2 had similar and lower GI (<55) then BR3 (GI>55). Finally, the four breakfast meals presented some differences in their perceived characteristics (healthy, palatable, satiating, energizing, and caloric attribute), as assessed in the previous Chapter and reported in Table 5.

Table 5 - Perceptual characteristics of the four breakfast meals.

Perceptual attribute	Breakfast 1	Breakfast 2	Breakfast 3	Breakfast 4
Healthy	4.5 ± 0.2 ^{ab}	5.4 ± 0.2 ^a	5.1 ± 0.2 ^{ab}	3.3 ± 0.5 ^b
Satiating	5.5 ± 0.2 ^a	4.9 ± 0.3 ^a	5.3 ± 0.2 ^a	1.2 ± 0.1 ^b
Palatable	5.5 ± 0.2 ^a	5.1 ± 0.2 ^a	5.1 ± 0.3 ^a	1.9 ± 0.3 ^b
Energizing	5.8 ± 0.2 ^a	4.7 ± 0.3 ^b	4.8 ± 0.3 ^b	1.2 ± 0.1 ^c
Caloric	5.1 ± 0.2 ^a	3.8 ± 0.3 ^b	3.9 ± 0.2 ^b	1.1 ± 0.1 ^c

Values are means (n=15) ± SEM. Evaluation was done before breakfast consumption through a 7-point LS (0=not at all; 7=extremely). Different letters in the same row indicate statistically significant differences ($p < 0.05$) among breakfast meals.

3.2.2.4. Appetite and satiety ratings

A self-reported appetite questionnaire was completed at baseline (t_0) and every 30 minutes up to 4 hours after breakfast consumption (t_{30} , t_{60} , t_{120} , t_{180} , t_{240}). The questionnaire had two components: hunger and fullness. Subjective appetite and satiety profiles were rated by answering to question “How hungry do you feel right now?” and to question “How full do you feel right now?” through 100-mm

Visual Analogue Scales (VAS) (Flint *et al.*, 2000). VAS were anchored by “not at all” on the left (0 mm) and “extremely” on the right (100 mm). At each time point, participants were instructed to mark a “X” on the 100-mm line at the point that better represented their degree of hunger and fullness respectively. Ratings were scored by measuring the distance to the closest millimetre from the left anchor to the point where the “X” mark intersected the line.

3.2.2.5. Blood sampling and laboratory analyses

Blood was sampled to evaluate glucose, insulin, leptin, ghrelin, GLP-1, and non-esterified fatty acids (NEFA), at baseline (t_0) and up to 4 hours after consuming breakfast (t_{15} , t_{30} , t_{45} , t_{60} , t_{90} , t_{120} , t_{150} , t_{180} , t_{210} , t_{240}). Scheduled sample timing for each marker is reported in Figure 24. Blood samples were collected through a 3-way tap intravenous cannula in the antecubital region of the arm. At each collection time, the first mL was discarded to avoid likely contamination with the saline solution used to keep the cannula’s patency. Blood was drawn into different tubes (Trust BD Vacutainer®) depending on metabolic parameter to be analysed: BD Vacutainer® Fluoride/EDTA Vol. 2.0 mL (cod. 368920) tubes were used for glucose and NEFA; BD Vacutainer® SST™ II Advance Vol. 5.0 mL (cod. 367955) tubes were used for insulin and leptin; BD Vacutainer® K2 (EDTA) Vol. 3.0 mL (cod. 364664) tubes were used for ghrelin; BD Vacutainer® K2 (EDTA) Vol. 3.0 mL (cod. 364664) tubes, added of 30µL Diprotin A (6.25 mg/mL - Diprotin A Enzo® Life Sciences ALX-260-036-M005), were used for GLP-1. Collected samples were centrifuged (2000x g, 10 min, 20 °C) and stored as serum or plasma (-80 °C) for subsequent analysis. Glucose concentration was determined by an automated analyser (YSI 2900 Biochemistry Analyzer, YSI Incorporated, Yellow Springs, OH, USA), while the remaining markers were analysed through commercially available high sensitivity ELISA kits (Insulin: Insulin ELISA Human kit, D.B.A. Italia s.r.l., Segrate, Italy; Leptin: Human Leptin ELISA kit, Merck Millipore S.p.A., Darmstadt, Germany; Ghrelin: Ghrelin (human) EIA Kit, Tema Ricerca, Castenaso, Italy; GLP-1: Glucagon-like Peptide I (active) ELISA KIT, Tema Ricerca; NEFA: NEFA-HR - R1 e NEFA-HR – R2, Kardia s.r.l., Milan, Italy).

3.2.2.6. *Ad libitum* lunch

Four hours after breakfast consumption, following the last blood sample, participants were given the opportunity to consume food *ad libitum*. Meals were prepared on the same day and served identical in all intervention conditions as a buffet. The *ad libitum* lunch consisted of four different dishes, with a 2x2 design based on nutritional and health-perceived characteristics of meals. Courses were classified as: i) protein-based (salad with ham and cheese, and chicken nuggets) or carbohydrate-based (pasta with vegetables and pizza with fries), related to the nutritional composition, and ii) healthy (pasta with vegetables and salad with ham and cheese) or unhealthy (pizza with fries and chicken nuggets), based on an evaluation of healthiness perception (Chapter 3.1.). Energy content, macronutrient values, and experimental categories of the test dishes are presented in Table 5. Each course was offered in excess of the estimated intake, and presented in a single tray or bowl. Pasta and salad were served in bowls, while trays were used for pizza and nuggets. Participants were instructed to eat what and how much they wanted until feeling “comfortably full and satisfied”. Double weighing of food (prior to and following consumption) was set up to evaluate food choices, and energy and nutrient intakes of lunch.

Table 6 - Description, nutritional composition, nutritional category, and healthiness perception of the four dishes offered during the *ad libitum* lunch.

Nutritional information	<i>Pasta</i> with mixed vegetables	<i>Pizza</i> with French fries	<i>Green salad</i> with ham and cheese	Chicken <i>nuggets</i>
Energy (kcal/100g)	195.1	212.6	178.4	246.0
Protein (g/100g)	5.5	6.9	12.6	18.0
Fat (g/100g)	4.4	7.9	12.1	14.0
Carbohydrates (g/100g)	31.9	27.4	1.7	13.0
Nutritional category	carbs-based	carbs-based	protein-based	protein-based
Healthiness perception	healthy	unhealthy	healthy	unhealthy
Energy (kcal/100g)	195.1	212.6	178.4	246.0

3.2.2.7. Dietary assessment

Eating habits of participants were assessed for the whole week during each experimental week. A 7-day weighed food diary was used to collect data on all foods and beverages consumed. Participants were trained in the use of the food diary by a nutritionist immediately after enrolment. Participants were asked to provide a complete description of all foods and beverages consumed during the day, describing recipes and methods of preparation, and/or noting the brand of manufactured products. Participants were also requested to record the weight of each food/beverage consumed by weighing the product or, if not possible, by evaluating the portion size through standard household measures and a food atlas (Turconi & Roggi, 2007). Time and place of consumption were also specified for all meals. The accuracy of the information reported in the 7-day food diary was checked by a nutritionist with the participant on the third and the last day of each experimental week. Daily intake of energy and macronutrients was calculated by linking food and beverage consumption with the food database of the European Institute of Oncology (Gnagnarella *et al.*, 2008) through a Microsoft Access application.

3.2.2.8. Statistical analysis

Data were presented as means \pm SEM of fifteen independent measurements in response to each breakfast for i) satiety and appetite profile; ii) biomarkers of appetite regulation; iii) intakes of foods, energy, and macronutrients at lunch; and iv) daily intake of energy and macronutrients. Incremental areas under the curves (iAUC) were determined for the VAS score profile and the metabolic parameters by using the trapezoidal method for the post-breakfast period (0-120 min, and 0-240 min). The effects of breakfast meals on all variables were analysed by repeated measurement GLM (breakfast x time) using Greenhouse–Geisser or Huynh–Feldt corrections whether epsilon was lesser or greater than 0.75, respectively. Bonferroni *post-hoc* tests were used for multiple comparisons. The statistical

analysis was performed with the Statistical Package for Social Sciences software (IBM SPSS® Statistics, Version 22.0. IBM Corp., Chicago, IL). A difference was considered significant at $p < 0.05$.

3.2.3. Results

3.2.3.1. Self-reported appetite and satiety ratings

The response curves for perceived appetite and satiety after breakfast consumption are shown in Figure 25. There were main effects of breakfast, time, and the interaction of breakfast x time for satiety ($p < 0.001$ for all) and appetite ratings ($p < 0.01$ for breakfast, and $p < 0.001$ for both time and breakfast x time). Independently of the type of breakfast, a suppression of appetite ratings was detected after breakfast consumption, showing the lowest peak at 30 minutes. Appetite responses were gradually restored to fasting values at 120 minutes for BR4, while at 180 minutes for BR1, BR2, and BR3 (Figure 25A). Conversely, ratings of satiety increased significantly after breakfast consumption under the three test BRs, showing the highest peak at 30 minutes after breakfast and returning to baseline levels between 180 and 240 minutes (Figure 25B). As expected, satiety values fluctuated around baseline for the whole 4h period for BR4, which represented the control breakfast and basically mimicked fasting condition. After BR4, participants reported the highest appetite and the lowest satiety responses compared to the other breakfasts. iAUC were also used to analyse VAS scores for self-reported appetite and satiety in both the 0-120 min and 0-240 min interval periods (Figure 25). Statistically significant differences between BR4 and the other three test breakfasts were shown for both appetite and satiety ratings, without noting significant differences among BR1, BR2, and BR3.

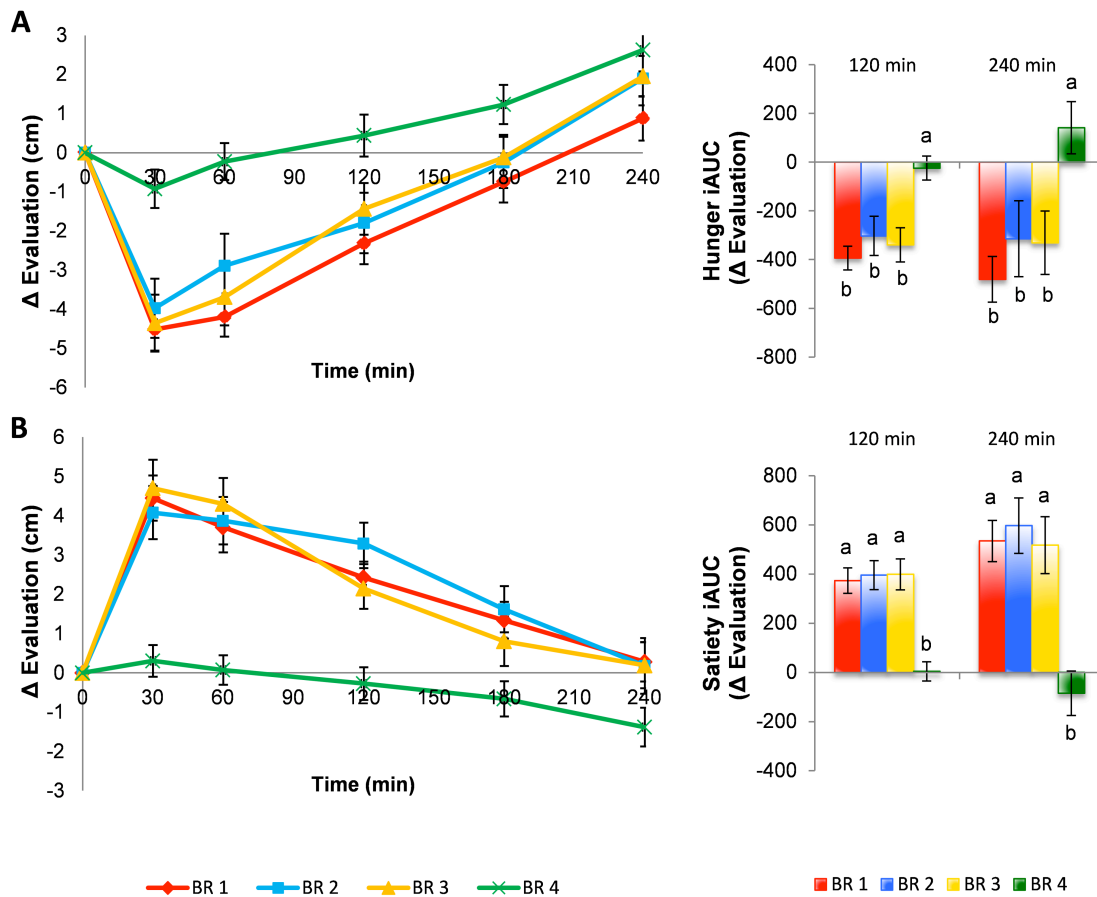


Figure 25 - Postprandial appetite (A) and satiety (B) sensations over time (incremental curves and corresponding iAUC), as measured by VAS score answering to question A: “How hungry do you feel right now?” and to question B: “How full do you feel right now?”. Values are means ($n=15$) \pm SEM. Different letters in the same grouped bars indicate statistically significant differences ($p<0.05$) among breakfast meals. BR: breakfast.

3.2.3.2. Metabolic parameters related to food intake regulation

Blood glucose responses to breakfast consumption were conditioned by the kind of breakfast, the time, and the breakfast \times time interaction ($p<0.001$ for all). Blood glucose concentrations peaked at 30 min post-breakfast, turning back to the basal levels 120 min after breakfast (Figure 26A). Variations in glucose concentrations were significantly higher following consumption of BR3 than for the other two test breakfasts (BR1 and BR2) in terms of blood concentration (Figure 26A). Moreover, BR3 was the only test breakfast that showed a hypoglycaemic profile at 120 min. Glucose values did not change as a consequence of BR4 consumption. iAUC at 120 min indicated significant differences between the

glycaemic response of BR1 and BR3, respectively the breakfast with the lowest and the highest GI (Figure 26A).

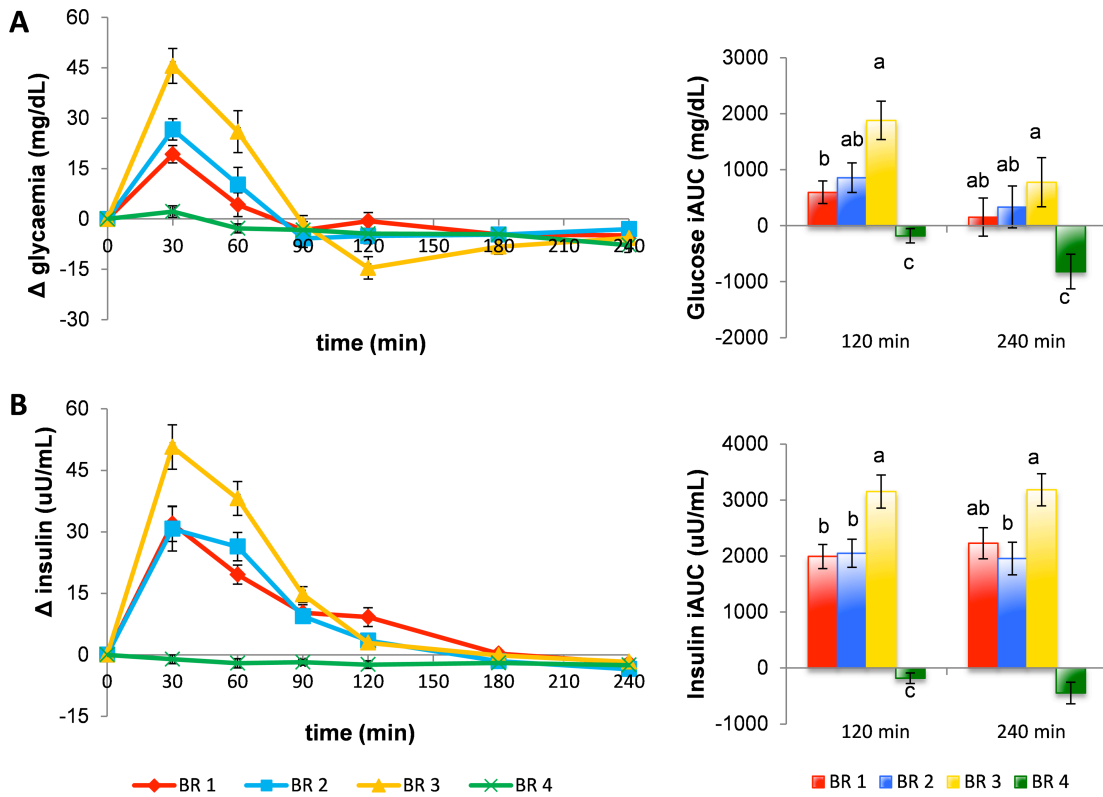


Figure 26 - Glycaemia and insulinaemia responses to breakfast. Postprandial incremental concentration and relating iAUC for Glucose (A), Insulin (B). Values are means ($n=15$) \pm SEM. Different letters in the same grouped bars indicate statistically significant differences ($p<0.05$) among breakfast meals. BR: breakfast.

In the case of postprandial insulin responses, significant effects of breakfast, time, and breakfast x time interaction were observed (all $p<0.001$), and followed the same trend of blood glucose: BR3 elicited the highest increase in serum insulin concentrations, with mild increases for BR1 and BR2 (Figure 26B). BR4 did not cause modifications in insulin levels. These differences among breakfasts were also evident when iAUC values were taken into account (Figure 26B).

Decreases in leptin values following breakfast consumption were not different after the different breakfasts ($p>0.05$) and were kept constantly under baseline values (Figure 27A).

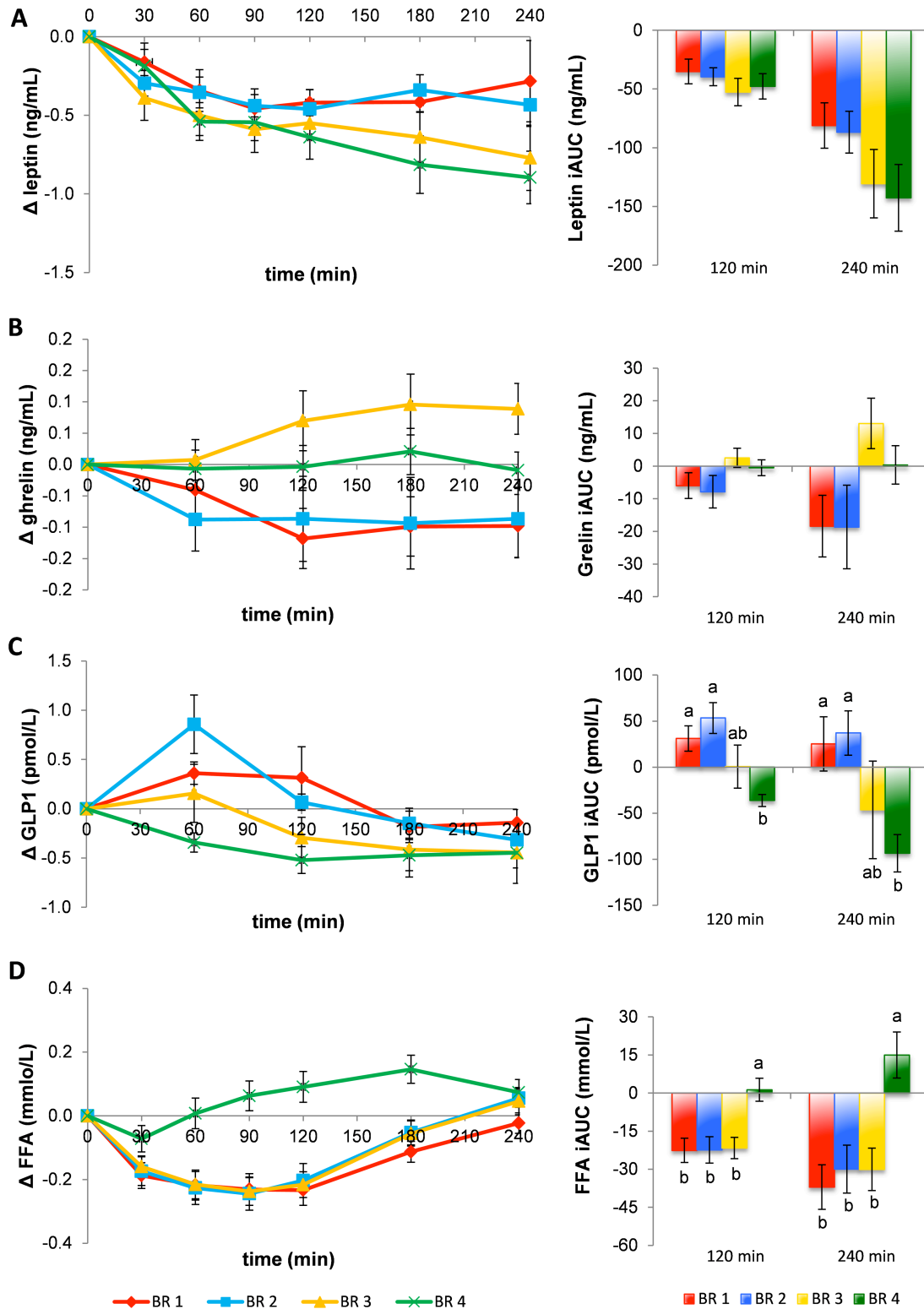


Figure 27 - Metabolic parameters related to food intake regulation. Postprandial incremental concentration and relating iAUC for leptin (A), ghrelin (B), GLP-1 (C), and NEFA (D). Values are means (n=15) ±SEM. Different letters in the same grouped bars indicate statistically significant differences (p<0.05) among breakfast meals. BR: breakfast.

In the case of the other hormone involved in metabolic control, ghrelin, the high inter-individual variability in its circulating levels did not allow to spot statistically significant differences among breakfasts ($p>0.05$). Nevertheless, a trend in ghrelin levels was observed, as while BR1 and BR2 decreased postprandial ghrelin concentration, this was visibly raised following BR3 (Figure 27B).

Postprandial plasma GLP-1 concentrations were not significantly affected by the type of breakfast ($p>0.05$), but there was a main effect of time ($p<0.05$). GLP-1 values were higher for BR1 and BR2 with respect to BR4 only at 60 min. This increased production of GLP-1 after consumption of BR1 and BR2 became more evident when iAUC data were taken into account for the periods 0-120 min and 0-240 min (Figure 27C).

Both time and type of breakfast affected NEFA responses ($p<0.001$ for both), as well as a breakfast x time interaction ($p<0.001$). BR1, BR2, and BR3 similarly decreased post-prandial NEFA concentrations, while the response to BR4 was characterised by an increase in NEFA levels (Figure 27D). This significant reduction in NEFA levels as a consequence of BR1, BR2, and BR3 was also displayed in terms of iAUC (Figure 27D).

3.2.3.3. Food choices at subsequent lunch meal

Food choices at lunch were not significantly modulated by the different breakfasts. Regardless of the kind of breakfast, even the one mimicking fasting conditions (BR4), subjects consumed the same amount of individual food items ($p>0.05$, Figure 28A). No differences among breakfasts were registered when foodstuffs were grouped neither by nutritional categories (protein- vs. carbohydrate-based courses) nor by healthiness perception (healthy vs. unhealthy) with the intent of evaluating these specific groupings instead of single items (Figure 28B and 28C). In agreement with these results, energy and macronutrient intake at lunch did not change significantly following different breakfasts (Table 7).

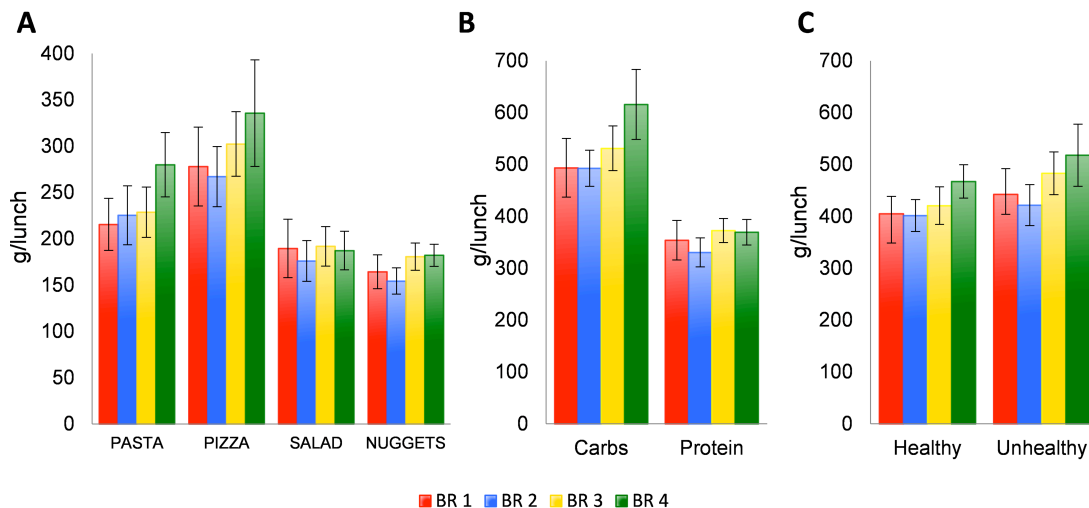


Figure 28 - Food choices at lunchtime. Amount (g) of food consumed during the ad libitum lunch for each single dish (A), nutritional category (B), and healthiness perception (C). Carbs: carbohydrate-based (pasta with vegetables and pizza with fries); Protein: protein-based (salad with ham and cheese, and chicken nuggets); Healthy: food perceived as healthy (pasta with vegetables and salad with ham and cheese); Unhealthy: food perceived as unhealthy (pizza with fries and chicken nuggets). Values are means ($n=15$) \pm SEM.

3.2.3.4. Dietary habits throughout the week

Dietary records were kept for each experimental week to evaluate the impact of each breakfast on participants' food choices. Daily energy intake did not vary among the different breakfasts ($p>0.05$) and was about 2700-2800 kcal/day (Table 7). This fact, considering that BR1, BR2, and BR3 provided about 330 kcal/day (~12% of the total daily energy intake) while BR4 was deprived of caloric content, accounted for an increase in energy intake throughout the day following BR4 consumption. Actually, when energy intake due to breakfast was not included, a significant higher energy intake during the day was observed for BR4 with respect to BR2 and BR3 (Table 7). Regarding macronutrients, daily intakes of proteins and fats were not affected by the kind of breakfast (Table 7). Daily carbohydrate intake was lower for BR4 than for BR1 and BR3 (Table 7). However, when nutrient intake during the day was adjusted for the nutrient value of breakfast, subjects tended to consume more proteins and fats as a result of morning fasting (BR4) in comparison with BR2 and BR3, respectively (Table 7).

Table 7 - Description, nutritional composition, nutritional category, and healthiness perception of the four dishes offered during the ad libitum lunch.

Intakes	Breakfast 1	Breakfast 2	Breakfast 3	Breakfast 4
Lunch:				
Energy (kcal)	1761.5 ± 149.0	1704.5 ± 85.0	1878.7 ± 112.6	2042.7 ± 156.3
Protein (g)	76.3 ± 6.7	73.1 ± 3.8	81.1 ± 4.6	85.8 ± 5.9
Fat (g)	78.2 ± 6.7	74.0 ± 4.1	82.5 ± 4.7	87.0 ± 6.3
Carbohydrates (g)	177.7 ± 17.5	176.6 ± 11.3	192.2 ± 13.6	217.2 ± 19.8
Total day:				
Energy (kcal)	2805.7 ± 130.0	2706.6 ± 155.6	2706.1 ± 157.4	2711.9 ± 198.8
Protein (g)	106.7 ± 5.0	99.3 ± 5.3	100.7 ± 5.0	104.4 ± 6.6
Fat (g)	114.6 ± 5.5	114.1 ± 8.1	104.6 ± 6.9	120.9 ± 10.2
Carbohydrates (g)	321.6 ± 19.5 ^a	300.3 ± 18.4 ^{ab}	324.0 ± 20.2 ^a	279.9 ± 20.9 ^b
Rest of the day*:				
Energy (kcal)	2472.8 ± 130.0 ^{ab}	2378.9 ± 155.6 ^b	2376.1 ± 157.4 ^b	2711.9 ± 198.8 ^a
Protein (g)	97.2 ± 5.0 ^{ab}	90.0 ± 5.3 ^b	92.6 ± 5.0 ^{ab}	104.2 ± 6.6 ^a
Fat (g)	103.4 ± 19.5 ^{ab}	103.0 ± 18.4 ^{ab}	100.6 ± 20.2 ^b	120.9 ± 20.9 ^a
Carbohydrates (g)	273.0 ± 5.5	251.9 ± 8.1	257.5 ± 6.9	279.9 ± 10.2

Values are means (n=15) ± SEM. Different letters in the same row indicate statistically significant differences ($p < 0.05$) among breakfast meals. *Rest of the day values were calculated as total day intakes minus breakfast intakes.

3.2.4. Discussion

The present study investigated the effect of breakfast consumption on self-reported appetite and satiety perception, metabolic parameters relating to food intake regulation, food choices at lunch, and 7-day dietary habits in healthy subjects. The four breakfast meals (three iso-caloric and one non-caloric) were composed of foods that are commonly consumed at breakfast in Italy (Marangoni *et al.*, 2009), and were different in terms of nutrient composition, GI, and perceptual characteristics.

The effects of different breakfasts on similar physiological targets have been already approached. For instance, it has been reported that protein-based breakfasts have a greater effect on satiety than carbohydrate-based ones (Bonnema *et al.*, 2016; Fallaize *et al.*, 2013). For this reason, we attempted to investigate the metabolic and behavioural effect of balanced breakfasts differing just for one single food item (Marangoni *et al.*, 2009).

In the present investigation, as expected, breakfast consumption increased satiety and reduced appetite in the early postprandial phase, while ratings for non-caloric breakfast fluctuated around baseline (Figure 25A and 25B). However, no differences in satiety and appetite among the three iso-caloric breakfasts were detected, contrary to the expectations that specific breakfast characteristics (i.e., physical form, GI, and subjective perception) could influence these parameters in a specific way. A thorough comparison with the literature was difficult because test breakfast meals in previous investigations often contained foods that are not commonly consumed at breakfast in Italy. Moreover, when foods similar to the ones used in the current study were evaluated, conflicting results on satiety-sensations were observed, with heterogeneity partially ascribable to the different target populations (Berti *et al.*, 2015; Geliebter *et al.*, 2015).

Taking into account the perceived characteristics of these breakfasts before consumption (Table 5), postprandial ratings herein obtained matched well the perceived features given for the different breakfasts. For instance, the estimations for satiety perception of the three iso-caloric breakfasts, perceived as more satiating than the non-caloric one (Table 5), corresponded with better satiety postprandial profiles (Figure 25B). Regarding perceived palatability and caloric content, diverging results have been reported when using measures of subjective appetite sensations (Sorensen *et al.*, 2003). In this work, a greater perceived palatability and energy content (Table 5) was associated with higher satiety and lower appetite postprandial profiles following breakfast (Figure 25A and 25B).

In terms of objective attributes of breakfast items and their relation with self-reported appetite and satiety perception, differences among the experimental

breakfasts were expected. Satiety is generally affected by the physical form of foodstuffs, with more solid food usually resulting in greater satiety (Samra & Anderson, 2007). Similarly, carbohydrates in liquid food have been reported to produce lower satiety than carbohydrates provided in solid form (Pan & Hu, 2011). Cereals included in BR2 and BR3 presented a semi-solid consistency since they were consumed with a spoon and, thus, it was hypothesised that they might elicit a lower satiety postprandial profile than BR1, which contained solid food items. However, this fact was not observed and differences among treatments due to the consistency of the breakfast items were not observed. A likely explanation can be found in the different composition of our breakfast meals with respect to those reported by Samra and Anderson (2007), since apples were not present in the latter study.

With regard to macronutrients, carbohydrate composition of breakfasts has previously shown to differently influence eating behaviour. Consumption of low GI breakfasts resulted in a higher control of hunger and a lower subsequent food intake with respect to high GI breakfasts (Liljeberg *et al.*, 1999). Regarding GI, no differences were observed in terms of modulation of appetite and satiety sensations in this study (Figure 25A and 25B), and this could be attributed to the relatively low difference in breakfast GIs. Nevertheless, the GI of breakfast affected markers of glucose metabolism, with postprandial glucose and insulin peak values that were significantly lower after consumption of low GI breakfast meals (BR1 and BR2, Figure 26A and Figure 26B), in agreement with previous findings in normal weight subjects (Geliebter *et al.*, 2015; Samra & Anderson, 2007). Therefore, these findings seem to encourage the consumption of low GI foods at breakfast, considering the potential role of glycaemic control in the prevention of chronic diseases (Barkoukis *et al.*, 2007; Dong *et al.*, 2011; Jenkins *et al.*, 2014; Livesey *et al.*, 2013; Ma *et al.*, 2012).

Concerning food intake, low GI foods or meals have been proved to elicit higher satietogenic effects than high GI ones during the subsequent lunch (Bornet *et al.*, 2007). Nonetheless, low GI breakfasts did not significantly impact food intake at lunch after direct assessment of *ad libitum* intake by double-weighting of foods

(Figure 28). Actually, no significant differences were observed among the breakfast meals at the subsequent lunch for both food quantity and nutrient intake (Figure 28A and Table 7). However, food and nutrient intake tended to be reduced after caloric breakfasts if compared with a non-caloric one (Figure 28 and Table 7). These findings could be partially affected by the high inter-individual variability registered, but might also suggest a potential compensation at lunchtime for omitted energy intake through breakfast skipping. Similar results were registered by Geliebter *et al.* (2015) for normal weight subjects, while this is partially contrasting with Chowdhury *et al.* (2016), who did not observe compensatory intake during an *ad libitum* lunch following extended morning fasting in obese adults. The different target population (obese vs. normal weight subjects) may explain such differences. In addition, in spite of different appetite and satiety ratings and metabolic profiles in the early postprandial phase, it should be noted that values tended to return to baseline levels 4 hours after breakfast (Figure 25, Figure 26, and Figure 27). In fact, subjects reported almost the same satiety-sensations before consuming the lunch independently from the type of breakfast (Figure 25). In this context, previous studies showed that the impact of a test meal in reducing subsequent energy intake is decreased as the period between two meals increases, suggesting that periods of time between breakfast and lunch greater than 4 h may limit the effects of breakfast on satiety (Astbury *et al.*, 2011; Fallaize *et al.*, 2013). With respect to the food choices, volunteers showed a preference for carbohydrate-rich foods (i.e. pizza and pasta) independently on the type of breakfast (Figure 28B). No differences were observed between choices for healthy or unhealthy foods, suggesting no association between the healthiness perceptions of foods consumed at breakfast and the intake of healthy/unhealthy foodstuffs at lunch (Figure 28C).

It is worth mentioning that food intake at lunch was very high, confirming that the *ad libitum* feeding is generally associated with excessive food intake, as previously reported by other authors (Fallaize *et al.*, 2013; Livingstone *et al.*, 2000). This was further demonstrated by analysing the 7-day dietary records, that showed a mean energy intake during the *ad libitum* lunch similar to two third of the daily mean energy intake. The analysis of 7-day dietary behaviours revealed that energy

was compensated during the day in the case of BR4, suggesting that calories not consumed at breakfast were integrated during the remaining part of the day (Table 7). It has already been proposed that omitting breakfast can lead to a positive daily energy balance due to an increased consumption of foods later in the day, even if a recent review of the literature identified breakfast skipping as an effective strategy to reduce total energy intake (Clayton & James, 2016). The mean energy intake was 2700-2800 kcal/die with no differences among the type of breakfast (Table 7). Therefore, the daily energy intake was compliant to the recommendation (SINU, 2014), considering anthropometric characteristics and lifestyle of volunteers. However, macronutrient contribution to total daily energy intake diverged from the recommended values, being about 41-48%, 35-40%, and 15% for carbohydrates, lipids, and proteins, respectively. Except for the latter, these were found to be quite different from recommended values of 55-75% and 15-30%, respectively (SINU, 2014; WHO & Joint Consultation, 2003). The low carbohydrate intake was particularly emphasized after the non-caloric breakfast meal, probably ascribable to the missing consumption of carbohydrates at breakfast that were not compensated during the day (Table 7).

In light of all these results, according also to Giovannini *et al.* (2008), recommendation for the best breakfast composition for Italian-like contexts cannot be provided, although evidence seems to suggest that an ideal breakfast should include milk and milk-derived products, whole grain and low GI cereals, and fruits.

The present study was performed on a small group of healthy volunteers. This can represent a possible limitation of the work, despite the crossover design requires a smaller sample size to reliably estimate the magnitude of the treatment effect and the homogeneity of the sample strengthens the results. Future studies with larger and more heterogeneous subjects (i.e., overweight and obese, both gender, different age) are needed to better investigate the effects of breakfast consumption in different target populations.

3.2.5. Conclusions

In summary, findings from the present study suggest that the consumption of breakfast positively affects postprandial satiety if compared with non-caloric breakfast, but no differences were observed among breakfasts with slight diverse nutritional or perceived characteristics. However, the type of breakfast could affect the metabolic and endocrine responses, with low GI breakfasts composed of an apple and semi-skimmed milk, and white bread with chocolate hazelnut spread, or muesli with chocolate and nuts, resulting in a better postprandial metabolic profile. In addition, although total daily energy intake did not differ significantly between breakfast and breakfast skipping, as a compensating mechanism was observed, consumption of breakfast seems to help better matching the nutrient intake recommendation. In conclusion, our findings support the hypothesis that subjects should be encouraged to keep eating breakfast, preferably lower GI ones, composed of palatable and varied foods.

3.3. STUDY 3

Effects of four different breakfast meals on cognitive performance at lunchtime

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3.3.1. Introduction

Health-related consequences of fasting have been largely addressed, both from a metabolic and a cognitive perspective. In this context, breakfast emerges as a daily eating occasion able to interrupt the overnight fasting, providing energy and nutrients for the morning. Breakfast consumption is generally associated with healthier lifestyles, better dietary habits, and improved cognitive performance (Marangoni *et al.*, 2009).

The beneficial impact of breakfast on brain activity is mainly known in children and adolescents, as cognitive and school performance of students have been largely assessed (Edefonti *et al.*, 2014). Breakfast consumption induces positive cognitive effects in comparison to breakfast omission, as pointed out in a recent systematic review of the literature. In particular, breakfast seems to positively affect attention and memory tasks across the morning. In the different studies analysed by Adolphus *et al.* (2016), positive consequences of having breakfast have been observed at different postprandial times, from immediately after breakfast ingestion until late morning, but the greatest difference between breakfast consumption and breakfast omission emerges especially at around 3 hours after eating. Moreover, the positive consequences on brain function could be differently modulated on the basis of the specific nutritional characteristics of breakfast meals. When cognitive performance was evaluated by comparing different types of breakfast meals, a better result was observed after having low GI relative to high GI breakfasts (Adolphus *et al.*, 2016). In particular, lower-GI breakfasts were found to be the most effective in preserving attention, specifically by reducing the natural decrease in accuracy of sustained attention over time in schoolchildren (Edefonti *et al.*, 2014).

The cognitive advantages of having breakfast, and the effects of different breakfast meals have also been revealed in adults. However, while breakfast has been proved to produce small but consistent memory gain in healthy adults, the advantages for executive function and attention are less clear and no effects have been found on language function (Galioto & Spitznagel, 2016).

All this evidence has been linked to differences in blood glucose regulation, which may elicit different patterns of cognitive performance in healthy subjects, both adult and young. Since the brain is particularly responsive to variation in energy and glucose homeostasis, and energy deprivation could lead to negative effects on its function (Roh *et al.*, 2016), cognitive performance might be facilitated by adequate and less oscillating blood glucose concentrations. In this sense, breakfast consumption could help in sustaining blood glucose and having better postprandial glycaemic responses compared to breakfast skipping, with low-GI breakfast meals emerging as the better option (Jenkins *et al.*, 2014).

Despite these insights, general conclusions cannot be drawn, as the literature reveals a lack of consistency among the small number of available studies. According to these considerations, the present study was conducted to evaluate the impact of breakfast on cognitive performance at lunchtime. To achieve this purpose, sustained and selective attention was tested 4-h after having breakfasts differing in nutritional and perceptual characteristics.

3.3.2. Material and Methods

3.3.2.1. Study design and experimental procedures

The same 15 volunteers that took part in the study "*Effects of four different breakfast meals on satiety-related sensations, metabolic responses and food choices and intake later in the day*" (Chapter 3.2.) were also subjected to the present set of experiments. Besides the inclusion criteria considered for the assessment of satiety-related sensations, metabolic responses, and food choices, volunteers matched some additional inclusion criteria. In particular, they were Italian native speakers, right-handed, had a normal or corrected to normal vision, and had a normal colour perception. Handedness, visual acuity, near vision, and colour perception were assessed by using the Edinburgh Handedness Inventory (Oldfield, 1971), the Snellen Test (Snellen, 1862), the Italian version of the Radner Test (Calossi *et al.*, 2014;

Radner *et al.*, 1998), and the Ishihara Test (Ishihara, 1917), respectively. The characteristics of each volunteer are reported in Table 8.

Table 8 – *Handedness, visual acuity, near vision, and colour perception of the 15 volunteers.*

Volunteer	Edinburgh Test	Snellen Test	Radner Test	Ishihara Test
1	0.89	left/right: 7/10	OK	OK
2	0.26	left/right: 10/10	OK	OK
3	0.31	left/right: 10/10	OK	OK
4	0.84	left/right: 9/10	OK	OK
5	0.63	left/right: 10/10	OK	OK
6	0.97	left/right: 9/10	OK	OK
7	0.63	left/right: 10/10	OK	OK
8	0.79	left/right: 10/10	OK	OK
9	0.52	left/right: 10/10	OK	OK
10	0.65	left/right: 10/10	OK	OK
11	0.89	left/right: 10/10	OK	OK
12	0.42	left/right: 10/10	OK	OK
13	0.79	left/right: 10/10	OK	OK
14	0.42	left/right: 10/10	OK	OK
15	0.60	left/right: 8/10	OK	OK

Each Thursday of the four experimental weeks, the day after the “Nutritional test”, participants performed the cognitive performance tests at lunchtime (as represented in Figure 29). Volunteers arrived at the laboratory at 12.00 a.m. after having one of the four breakfasts at around 8.00 a.m., without eating any additional meal/snack during this 4-hour period. Study design and experimental procedures are reported in Figure 29.

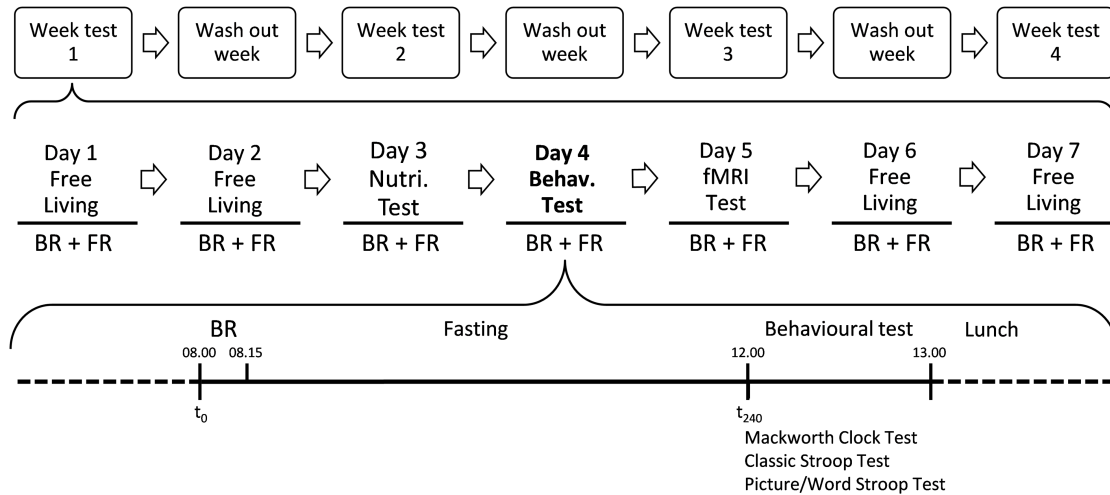


Figure 29 - Study design and schedule of experimental procedures.

Nutri Test: Nutritional test; Behav. Test: Behavioural test; fMRI: functional magnetic resonance imaging; BR: breakfast; FR: food record.

Volunteers were individually conducted to the same experimental room set up with the identical environmental conditions used for the rating tasks described in the Chapter “*Food perception at lunchtime does not depend on the nutritional and perceived characteristics of breakfast*” (Section 3.1.2.1, Food stimuli definition and evaluation). During each test day, volunteers were evaluated for their sustained and selected attention by using a cognitive task battery: i) the Mackworth Clock Test, ii) the Stroop Colour Word Test, and iii) the Stroop Food Picture Word Test, a modified version of the Stroop Test in which food image stimuli are used instead of the classic colour/name stimuli. Tests were administered in a random and counterbalanced order for the four experimental days and across subjects, and they were run by using the same apparatus described in the Chapter “*Food perception at lunchtime does not depend on the nutritional and perceived characteristics of breakfast*” (Section 3.1.2.1, Food stimuli definition and evaluation). There was a short break between two tests, depending on volunteer’s needs.

3.3.2.2. Mackworth Clock Test

During the Mackworth Clock Test (Mackworth, 1948), subjects were shown a circular arrangement of 24 grey dots simulating a clock on a computer screen (an example is shown in Figure 30).

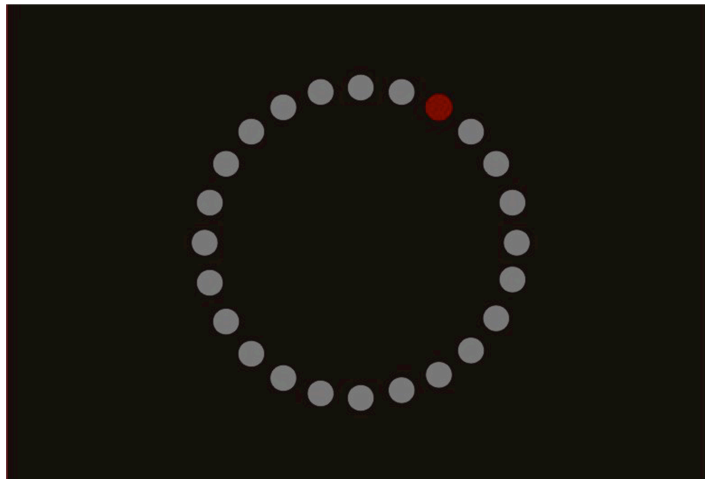


Figure 30 - Mackworth Test. Example of the experimental display.

Dots were briefly illuminated in red in clockwise rotation (rate: one per 500 ms) with a 15°-jump. Participants were instructed that occasionally the red dot would proceed with a 30°-jump by skipping one of the dots in the regular sequence. When the red circle skips a position, participants were required to respond as fast as possible by pressing the space bar using a computer keyboard with the right hand. A correct detection was registered if the response occurred within 2 s after the signal. A total of 240 signals (12 signal per minutes) randomly occurred during the 20 minutes of the task. In particular, 60 signals were presented in 4 successive 5-min periods. For each one of the four time periods, behavioural outcome measures were mean reaction time of correct detections, number of omissions, number of false detections, and total number of errors (omissions + false detections).

3.3.2.3. Stroop Colour Word Test

The second test used was the Stroop Colour Word Test (Stroop, 1935). Volunteers were instructed to name, as fast as possible, the colours of the ink in which colour names were written (Figure 31).

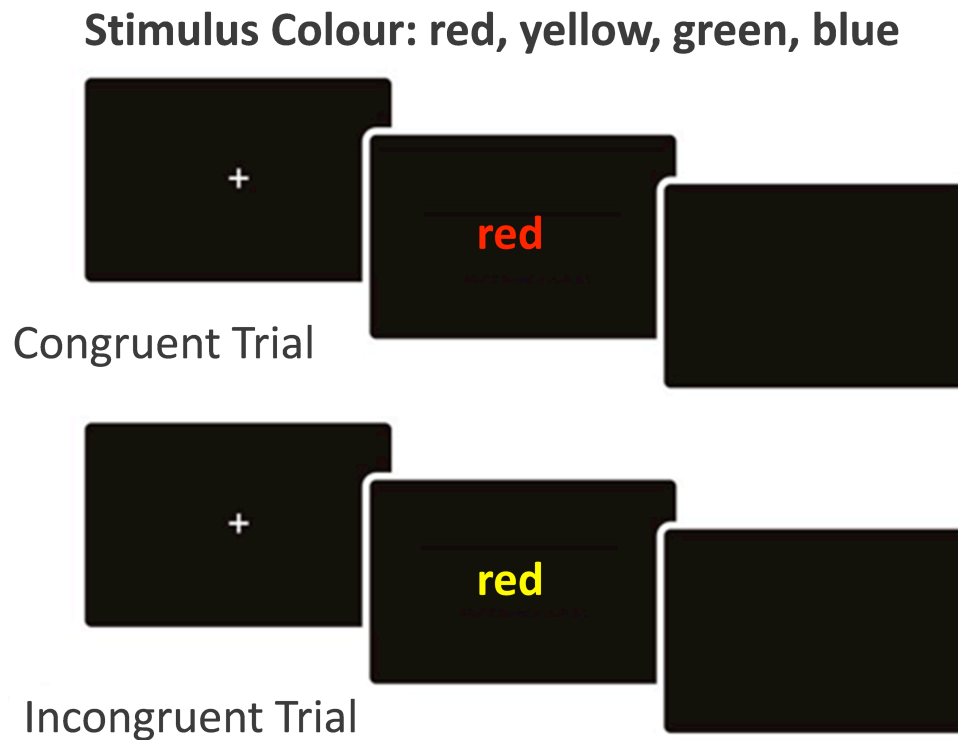


Figure 31 - Stroop Colour Word Test. Example of the experimental display.

Ink colour should be named without focusing on the written word itself. Four colours were used in the test: red, yellow, green, and blue. Colour names were singularly presented on the screen and could be written in congruent or incongruent ink. Congruent stimuli were names of colours written in the same colour of the written word (e.g. the word "red" written in red letters), while incongruent stimuli were written in dissonant colour (e.g. the word "red" written in yellow letters). After 800 ms of fixation on a cross at the centre of the screen, a colour word stimulus was shown for 1500 ms, following by another fixation cross which introduced to the next trial. A total of 48 trials for each test session were presented, consisting of 24 congruent and 24 incongruent trials (6 trial for each colour in both congruent and incongruent conditions). Stimuli and conditions were

randomised. Participants' vocal response time (RT) in milliseconds and the number of correct, incorrect, and no responses were registered for each trial. If a mistake was made, participants were allowed to correct themselves. In such a way, the RT was increased by the correction itself and the number of error trials was very low (0.98%), as expected in healthy subjects. Since the number was very low, incorrect responses were excluded. Further, outlier responses (vocal RT < 80 ms and vocal RT > 1150 ms) were excluded from the analysis. The mean vocal RT by congruent and incongruent conditions and breakfast groups were obtained.

3.3.2.4. Stroop Food Picture Word Test

Results from the Stroop Colour Word Test were used as control data for the Stroop Food Picture Word Test. This modified version of the Stroop Colour Word Test was used to study food-related selective attention (attentional bias). Food images and food-related names were used (Figure 32), instead of the classic Stroop.

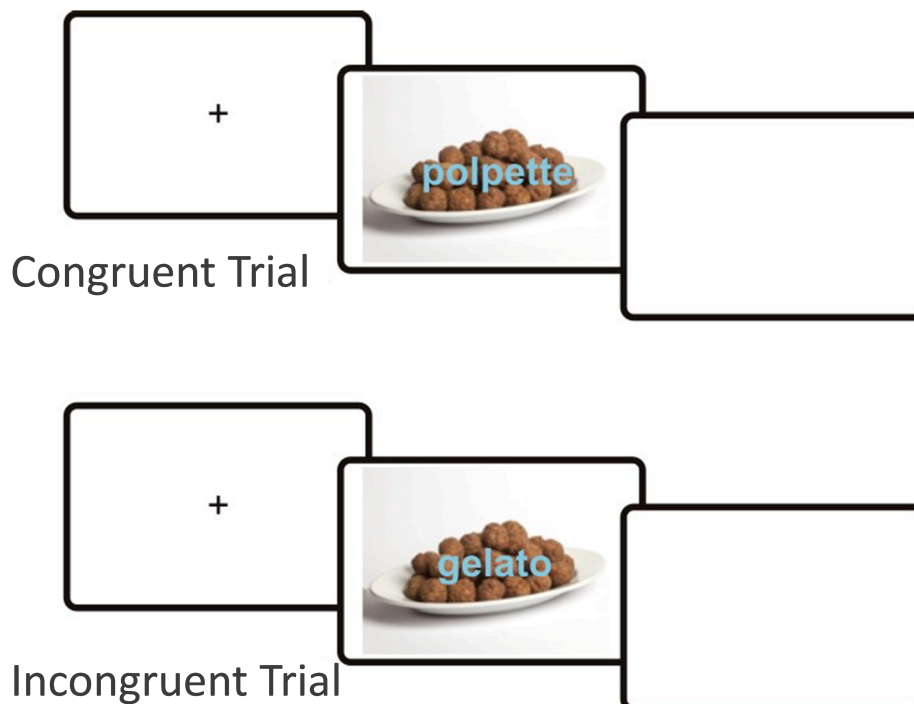


Figure 32 - Stroop Food Picture Word Test. Example of the experimental display.
“polpette” means meat balls; “gelato”, ice cream.

Twenty food-images (Figure 33), the same used in the rating task on food stimuli evaluation at lunchtime (presented at Section 3.1.2.4.), were easily recognisable typical Italian foods, matched for lexical frequency, word length, and syllable number. In addition, they were homogeneous with respect to the four nutritional category previously identified: 5 HEC foods, 5 HEP foods, 5 LEC foods, and 5 LEP foods (Section 3.1.2.4.).



Figure 33 - Food images selected as stimuli for the Stroop Food Picture Word Test. HEC, High Energy Carbohydrate; HEP, High Energy Protein; LEC, Low Energy Carbohydrate; LEP, Low Energy Protein. Potato dumplings; lasagne, tray; chips; pizza; ice cream; sandwich; fish coated in crumbs; meat balls; chocolate pudding; hamburger; biscuits; bread wholegrain; pasta with vegetables; polenta (porridge made with corn flour and salted water); rice; yogurt; salad; Parma ham; bresaola, arugula and parmesan cheese; boiled egg.

Each food image was presented with a food name that could be congruent or incongruent with respect to the picture. In the congruent condition, the word was the name of the food represented in the picture (e.g. the word “pizza” written on a picture representing a tray of pizza). In the incongruent condition, the word described another food that could be referred to the *same* nutritional category (e.g. the word “pizza” written on a picture representing a tray of lasagne, both HEC foods), or to a *different* nutritional category (e.g. the word “pizza” written on a

picture representing a boiled egg, a HEC and a LEP food, respectively). The test included two experimental tasks. In Task A, participants were instructed to name, as fast as they could, the food shown in the image, without focusing on the written word. Contrarily, in Task B, they were asked to name the written word without paying attention to the food shown in the figure. Three hundred and twenty experimental trials were run for each task, for a total of 640 trials. For both Task A and Task B, there were 4 experimental sessions, each of 80 trials. Every experimental session was composed of 40 congruent trials (10 trials for each nutritional category), and of 40 incongruent trials (20 trials with picture and word referred to the *same* nutritional category and 20 trials with picture and word referred to *different* nutritional category). As in the Stroop Colour Word Test, participants were allowed to correct themselves, leading to a low number of errors (1.23% and 0.31% in Task A and Task B, respectively), so the incorrect responses were excluded from the analysis. In addition, outlier responses (vocal RT < 80 ms and vocal RT > 1150 ms) were omitted. For each task, the mean vocal RT by congruent and incongruent conditions, nutritional categories, and breakfast groups were obtained.

3.3.2.5. Statistical analysis

Data were presented as mean \pm SD. The statistical analysis was performed with the Statistical Package for Social Sciences software (IBM SPSS® Statistics, Version 22.0. IBM Corp., Chicago, IL). A difference was considered significant at $p < 0.05$. For all tests, data were analysed by repeated measurement GLM, using Greenhouse–Geisser correction if epsilon was lesser than 0.75 or Huynh–Feldt correction if epsilon was greater than 0.75, and Bonferroni post-hoc tests for multiple comparisons, considering as factors:

i) time (0-5 min, 5-10 min, 10-15 min, 15-20 min) X breakfast type (BR1, BR2, BR3, BR4), in the Mackworth Clock Test;

ii) congruency (congruent/incongruent) X breakfast type (BR1, BR2, BR3, BR4), in the Stroop Colour Word Test;

iii) congruency (congruent/incongruent) X nutritional category (HEC, HEP, LEC, LEP) X breakfast type (BR1, BR2, BR3, BR4), in the Stroop Food Picture Word Test. In addition, nutritional categories were split in 2 X 2 compositional factors: energy content (high/low energy) X nutrient composition (carbohydrate-based/protein-based). In Task A, an additional analysis was the performance related only to incongruent conditions, taking into account incongruence type (*same* nutritional category/*different* nutritional category) X energy content (high/low energy) X nutrient composition (carbohydrate-based/protein-based) X breakfast type (BR1, BR2, BR3, BR4).

3.3.3. Results and Discussion

3.3.3.1. Mackworth Clock Test

The Mackworth Clock Test was used to study the impact of breakfast consumption on sustained attention performance at lunchtime. This monotonous monitoring task addressing sustained attention in a low-stimulus observation situation was carried out at different time intervals for 20 min. Considering that vigilance is impaired after the first 20-30 min (Teichner, 1974), a decreased sustained attention might be expected for the last intervals of time (10-15 and 15-20 min). Since breakfast consumption may have positive cognitive effects with respect to breakfast skipping (Adolphus *et al.*, 2016), an improvement in sustained attention due to iso-caloric breakfasts consumption (BR1, BR2, and BR3) compared with the breakfast mimicking fasting conditions (BR4) was hypothesized.

Performance in the sustained attention task was not significantly modified on the basis of different breakfasts, which refused the study hypothesis. In particular, the type of breakfast, regardless of its nutritional composition, did not change the reaction time for correct responses, the number of lapses, the number

of false alarms, and the total number of erroneous responses ($p>0.05$ for all the parameters). The lack of differences on vigilance might be due to the excellent performance of the volunteers in the test. In fact, the rate of total errors was low (<1%), which accounted for the ease of the test for these healthy young adults. At the same time, the homogeneity of response for the outcomes measured may be associated with the physiological conditions of participants. Since sustained attention performance after consumption of BR4 did not change with respect to that recorded after consumption of BR1, BR2, and BR3, a fasting-like effect would have happened at lunchtime. This fasting-like condition did not prompt differences on readily available energy sources and the benefits of consuming breakfast on sustained attention were likely nullified after 4 hours (Adolphus *et al.*, 2016). This fact was supported by the similar metabolic status of volunteers at lunchtime, as already indicated at Chapter 3.2.

With respect to the effect of time during the execution of the Mackworth Clock Test, reaction time was significantly impaired after 5 minutes ($F_{3,42}=18.75$, $p<0.001$, $\eta_p^2=0.57$; Figure 34A). The number of total errors showed a trend towards an increased number of incorrect responses at the second time interval ($F_{3,42}=2.74$, $p=0.055$, $\eta_p^2=0.16$; Figure 34B). These factors may also account for the fasting-like behaviour of participants since data did not account for an active attention performance (Galioto & Spitznagel, 2016).

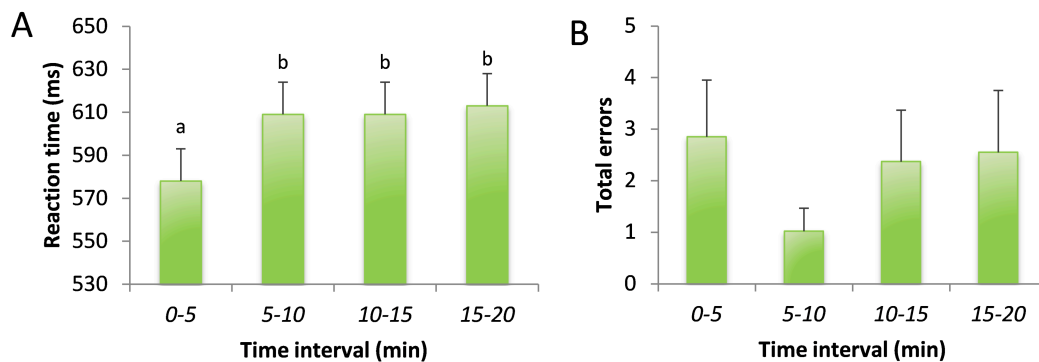


Figure 34 - Mackworth Test results. A) Reaction time for the different time intervals, regardless the type of breakfast; B) total errors, regardless the type of breakfast, for the different time intervals. Different letters indicate statistically significant differences ($p<0.05$).

3.3.3.2. Stroop Colour Word Test

The classic version of the Stroop Test was used to study attentional bias and as a control for the Stroop Food Picture Word Test. This test was based on stimuli not related to foods to check attention control. Attending to the nature of this test, relations between the type of breakfast and task performance were not expected. Actually, data indicated that there were no main effects for both the breakfast and the congruency between word and ink X breakfast interaction (all $p > 0.05$).

As expected, a longer reaction time was registered when ink colours of colour names were incongruent with the written colour name (Stroop effect) ($F_{1,14} = 32.94$, $p < 0.001$, $\eta_p^2 = 0.70$). The reaction time for the congruent responses was 736 ms, while the time for incongruent ones was 817 ms. Therefore, the interference or congruity effect, the time between average congruent and incongruent responses, was 81 ms.

3.3.3.3. Stroop Food Picture Word Test

Cognitive bias may condition dietary habits (Roefs *et al.*, 2015). For instance, it may promote excessive calorie intake in an environment rich in food-related stimuli or makes obese and overweight individuals hard to resist their craving for food (Roefs *et al.*, 2015; Werthmann *et al.*, 2014). Selective attention is a cognitive component involved in food intake and, although evidence for food-related attentional biases is inconsistent, it seems that attentional bias may condition the response of subjects to food stimuli (Bazzaz *et al.*, 2017). In the present study, food-related attentional bias was studied by using a modified Stroop test which calculates the respondents' reaction times to congruent/incongruent picture-word food-related stimuli. It was hypothesised that food consumption determines an attentional bias towards foods with nutritional characteristics similar to the products consumed at breakfast, encouraging the consumption of foodstuffs with different nutritional characteristics (compensatory effect). Therefore, a better performance was expected for foodstuffs with nutritional categories diverse to

those previously consumed at breakfast, since they would be harder to be omitted. According to this, protein-based foods should be preferred compared to carbohydrate-based ones after having BR1, BR2 and BR3, and no preferences were expected after BR4 consumption. The interference/congruency effect, due to the longer time required to answer to incongruent conditions, may be modulated by the nutritional categories of the food pictures.

As expected, results for the Task A of the food-related Stroop Test accounted for a congruency effect ($F_{1,14}=97.50$, $p<0.001$, $\eta_p^2=0.87$; Figure 35), having slower responses for the picture-word conditions describing different foods than to picture-word conditions describing the same food. In particular, average reaction time for congruent conditions was 775 ms, while it was 853 ms for incongruent conditions. This interference effect of 78 ms was similar to the interference effect recorded for the classic version of the Stroop test (81 ms).

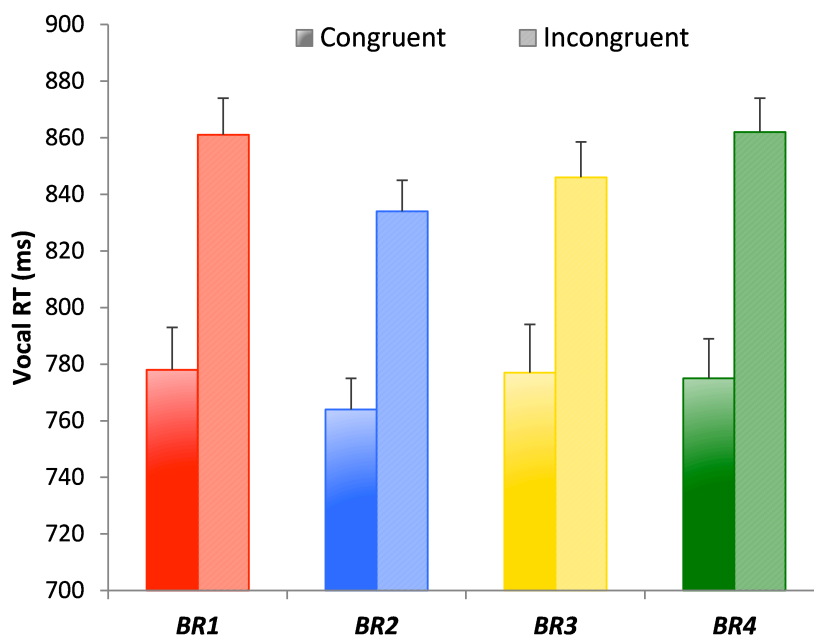


Figure 35 - Stroop Food Picture Word Test. Vocal response time on the basis of different breakfasts for the congruent and incongruent conditions.

There were not statistically significant main effects for the type of breakfast ($F_{3,42}=0.38$, $p=0.77$, $\eta_p^2=0.03$), nor for the breakfast X congruency interaction ($F_{3,42}=0.68$, $p=0.57$, $\eta_p^2=0.05$) (Figure 35). Overall, the different GI of the tree test breakfasts (BR1, BR2, and BR3) did not condition the cognitive performance in

terms of attentional bias of volunteers, although it was hypothesised (Adolphus *et al.*, 2016). This may be related to the fasting-like metabolic conditions of volunteers at lunchtime, as stated in the previous Chapter (Section 3.2.4.). However, the breakfast X nutritional category interaction was statistically significant ($F_{3,42}=3.43$, $p<0.03$, $\eta_p^2=0.20$; Figure 36). This interaction pointed out quicker responses for protein-based foods after consumption of BR2, suggesting an attentional bias towards those foodstuffs.

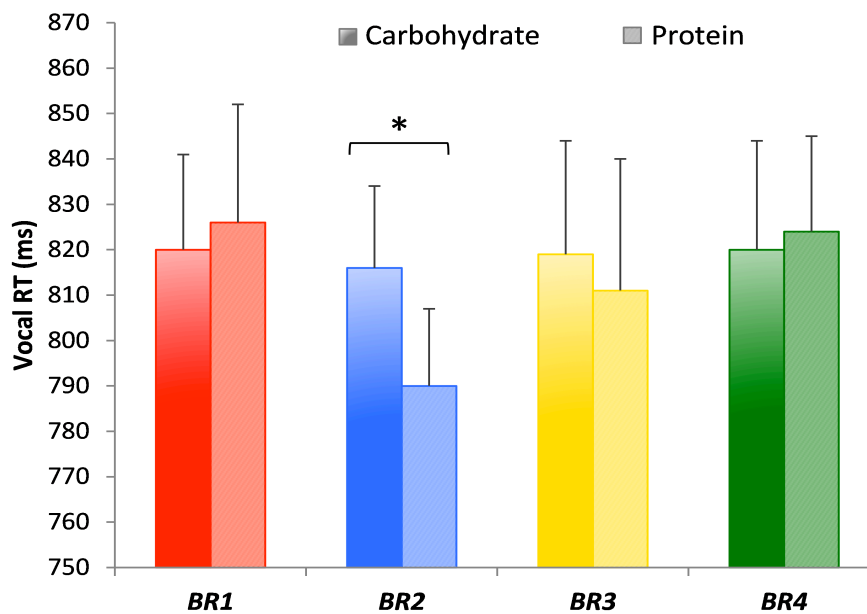


Figure 36 - Stroop Food Picture Word Test. Vocal response time for breakfast and nutritional category. The asterisk accounts for a significant difference ($p<0.05$) in the RT between nutritional categories within the same breakfast.

Average reaction time to food stimuli was affected by the energetic content (high/low) of the presented foods, but not for the nutritional category to which the foods belonged to (carbohydrate-based/protein-based) ($F_{1,14}=14.43$, $p<0.002$, $\eta_p^2=0.51$ for energy; Figure 37A and $F_{1,14}=1.41$, $p=0.26$, $\eta_p^2=0.09$ for nutritional category; Figure 37B). The attentional bias for highly energetic foodstuffs may be related to the fasting-like physiological conditions of the volunteers 4 hours after breakfast consumption.

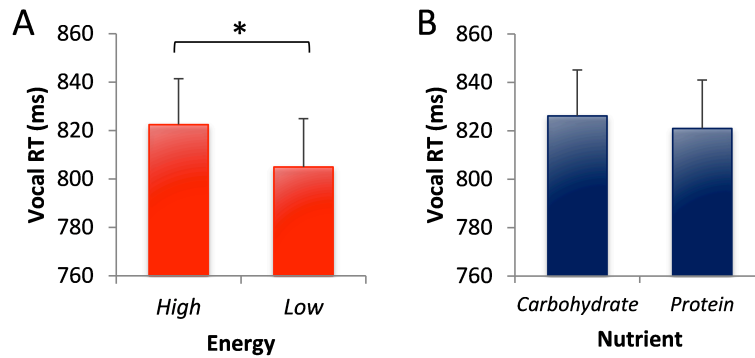


Figure 37 - Stroop Food Picture Word Test. A) Vocal response time for energy content, and B) vocal response time for nutrient composition, regardless the breakfast type. The asterisk accounts for a significant difference ($p < 0.05$).

When incongruent trials were decomposed in those representing words and pictures of the *same* nutritional category and in those showing words and pictures of *different* nutritional categories, main effects were observed for the incongruence X breakfast interaction ($F_{3,42}=3.19$, $p < 0.04$, $\eta_p^2=0.19$; Figure 38A). Only BR1 showed a different trend to that recorded for the rest of breakfasts, having longer vocal reaction times for the *different* conditions than for the *same* ones. The magnitude of effect for BR1 was of -24 ms (*same* RT – *different* RT), while the attentional bias for the *same* conditions after BR2, BR3, and BR4 was of 8, 18, and 20 ms, respectively (Figure 38B). The difference between BR1 and the rest of the breakfasts was statistically significant ($p = 0.02$).

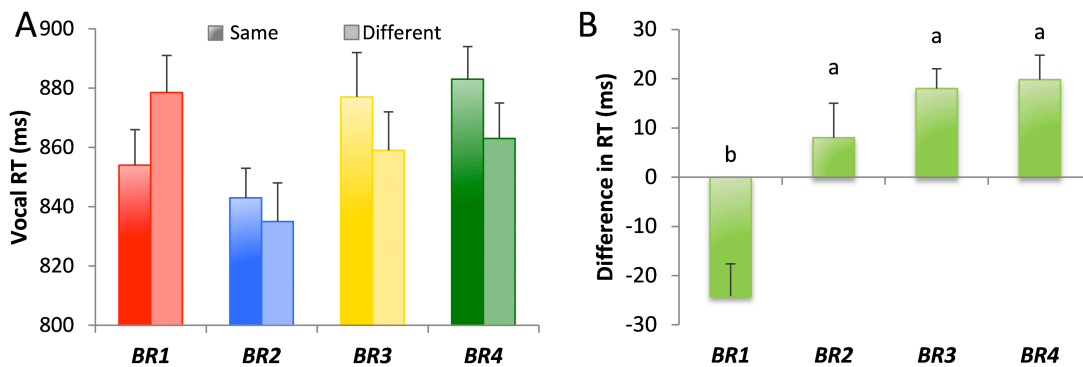


Figure 38 - Stroop Food Picture Word Test. A) Vocal RT for incongruent conditions belonging or not to the same nutritional category; B) Variation in the RT when naming incongruent conditions belonging to the same nutritional category minus the RT for incongruent conditions belonging to different nutritional categories. Different letters indicate significant differences ($p < 0.05$).

Task B of the food-related Stroop Test was based on reading as quickly as possible the word, omitting the picture. There were no main effects for the type of

breakfast and the congruence effect was not present ($F_{1,14}=0.03$, $p=0.87$, $\eta_p^2=0.002$). In particular, reaction time for congruent and incongruent responses was 542 and 545 ms, respectively.

The Stroop Food Picture Word Test carried out by using food-related stimuli confirmed an interference/congruency effect when picture and word were referred to different foods (incongruence) and subjects named the picture neglecting the word (Task A). The energetic perception of the pictured food conditioned participants' reaction time (Figure 37A). Longer times were required to properly name high energy foods, which may account for the need of a deeper analysis of this class of foods, or the fact that it might be considered a more demanding processing. Another interesting result was the inverse trend evidenced for BR1 with respect to the reaction times required to name food pictures belonging or not to the same nutritional category (Figure 38B). A higher interference effect could be expected when foods belong to the same nutritional category, since it may be more difficult to disregard the word when it belongs to the same semantic area of the picture. This hypothesis was confirmed for BR2, BR3, and BR4 (Figure 38A). The outputs obtained for BR1 may rely on a lower selective attention for the food represented in the picture, linked to an attentional bias for words reporting foods with nutritional characteristics different to those of the pictures. This might provoke, hence, a higher interference effect and a lower flexibility in food-related attention. However, these effects were not observed in terms of food choices, as it was already reported at Chapter 3.2.

No major effects of the type of breakfast on cognitive attention were observed at lunchtime, due likely to the physiological status of the subjects. Nevertheless, a series of insights on the effect of foods belonging to different nutritional categories on attention control were identified. Attention performance, both vigilance and attentional bias, may be influenced and may condition food intake on the basis of different compositional characteristics and the metabolic state of the subjects, in line with previous reports addressing the impact of food on cognitive performance (Adolphus *et al.*, 2016; Galioto & Spitznagel, 2016).

3.4. STUDY 4

Effects of four different breakfast meals on brain activation at lunchtime

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3.4.1. Introduction

Mechanisms underlying food intake regulation have been largely investigated. In humans, food intake is regulated by two parallel systems: the physiological/homeostatic and the hedonic systems (Lutter & Nestler, 2009; Saper *et al.*, 2002). The homeostatic system includes hunger, satiety, blood glucose and adiposity regulations, and affects food intake but, at the same time, it could also be affected by eating habits (conditioning) (Berthoud, 2004). Moreover, in healthy individuals, food intake is controlled by the brain mechanism of the rewarding value of food, suggesting an interchange between metabolic regulators and hedonic system in the brain (Berridge, 2007). The cross-talk between metabolic and emotional-cognitive regulatory systems determines nutritional requirements, as well as the desire to eat. Food-related sensory information is integrated together with physiological satiety signals to set up a mental representation of the food in the CNS. These signals allow the evaluation of the food reward value, the regulation of the feeding behaviour, and the generation of affective responses (Li *et al.*, 2012). In addition, this complex mechanism is affected by the mood, since appetite-related regions of the brain can be differentially activated by the affective state, and the positive affective state may influence on early stages of sensory processing (Killgore & Yurgelun-Todd, 2007).

To better understand how appetite and food intake control are affected by the interaction among homeostatic, reward, and cognitive systems, the functional magnetic resonance imaging (fMRI) combined with behavioural test, has been proposed as a promising technic (Farr *et al.*). In addition, neural responses to food have been investigated in fMRI by using food cue reactivity paradigms showing food and non-food stimuli, such as images and smells, that vary in their desirability (Kahathuduwa *et al.*, 2016). Succeeding approaches should be not only focused on specific functional paradigms but must be oriented towards complex models to understand how various brain regions of interest (ROIs) interact to regulate food intake (Kahathuduwa *et al.*, 2016). In this context, brain region stated to be involved in food intake regulation include: the hypothalamus (the homeostatic brain system),

the parietal and visual cortices (attention system), the amygdala and hippocampus (emotion and memory systems), the prefrontal cortex (cognitive control system), and the ventral tegmental area and striatum (reward system) (Farr *et al.*).

For all these reasons, there is an increasing interest on motivational and decisional aspects of food choices, eating behaviours, and how they are influenced by both metabolic status and food characteristics. The present study aimed to investigate the impact of breakfast on brain processes implicated in food choice at lunchtime. To this purpose, fMRI sessions were performed to assess brain responses of healthy adults 4 hours after having breakfast.

3.4.2. Material and Methods

3.4.2.1. Study design and experimental procedures

The same 15 volunteers that took part in the previous investigations “*Effects of four different breakfast meals on satiety-related sensations, metabolic responses and food choices and intake later in the day*” (Chapter 3.2.) and “*Effects of four different breakfast meals on cognitive performance at lunchtime*” (Chapter 3.3.) were also subjected to the present test. Inclusion and exclusion criteria and participants characteristics are presented in sections 3.2.2.1 and 3.3.2.1.

Each Friday of the four experimental weeks, the day after the “Behavioural test”, participants were involved in a fMRI session at lunchtime. Volunteers reached the fMRI laboratory at 12.00 a.m., in a 4-hour starving condition after having one of the four breakfasts at around 8.00 a.m. Study design and experimental procedures are reported in Figure 39.

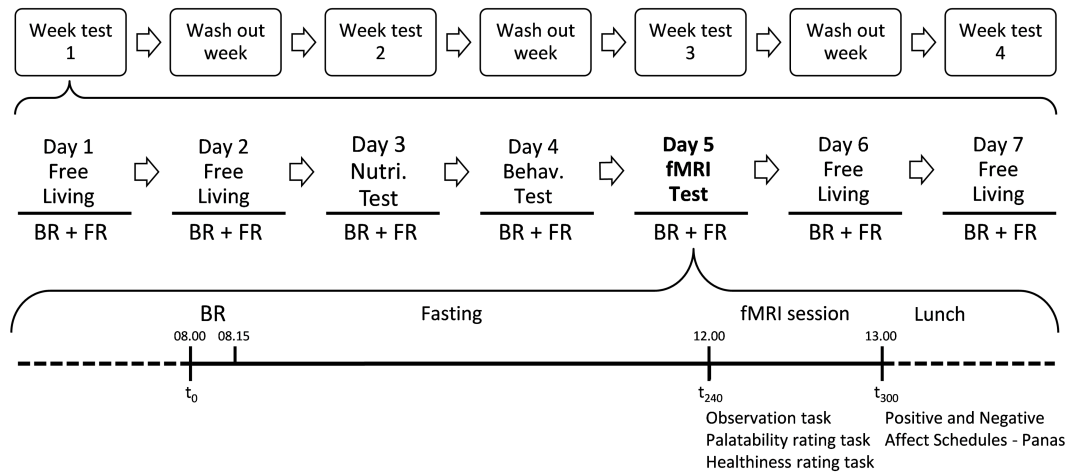


Figure 39 - Study design and schedule of experimental procedures. *Nutri Test*: Nutritional test; *Behav. Test*: Behavioural test; *fMRI*: functional magnetic resonance imaging; *BR*: breakfast; *FR*: food record.

Participants were trained on the test and were prepared for the fMRI scansion. They were laid down in the scanner in a dimly lit environment. Three different tasks were performed by each volunteer: an observational task, an explicit desire-to-eat judgment, and an explicit healthiness perception judgment. The condition order was kept fixed for the observation task, while it was randomised between the two rating tasks. In such a way, the observation condition was always run as the first task and was used to measure unbiased (spontaneous) brain responses to the stimuli, representing a control for the other conditions. The same images were used as stimuli in all the three experimental condition (as described at the *Stimuli* section below). During the observational task, volunteers were instructed to image themselves in a restaurant and to simply observe the image. They were also asked to indicate that they paid attention to the picture by using the index or middle or the ring finger of the right hand when a red circle appeared on the screen. During the desire-to-eat and the healthiness judgment, participants were instructed to image themselves in a restaurant and to observe the image. When a red question mark appeared on the screen, they were asked to rate their appetitive perception in the desire-to-eat judgment task and their health perception in the healthiness judgment task. Images were rating through a 3-point LS using the index or middle or the ring finger of the right hand to indicate “not at all”, “enough”, and “extremely”, respectively (Figure 40).

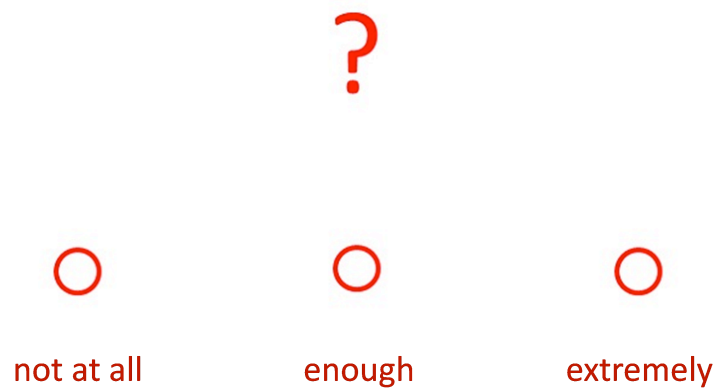


Figure 40 - 3-point Likert Scale used in the desire-to-eat and the healthiness perception judgment.

At the end of each scanning session, participants were asked to complete the Positive and Negative Affect Schedule (PANAS) (Watson *et al.*, 1988). The PANAS was used to explore 20 affects (10 positive and 10 negative) correlated with mood and brain activation: interested, distressed, excited, upset, strong, guilty, scared, hostile, enthusiastic, proud, irritable, alert, ashamed, inspired, nervous, determined, attentive, jittery, active, and afraid.

3.4.2.2. Stimuli and Stimulation Paradigms

Twenty-four images of various food (Figure 41), 24 scrambled images, and 6 non-food images (Figure 42) were selected for the present study.

Food stimuli were chosen out of an initial pool of 65 images representative of an omnivorous diet and comprising food items that may be consumed throughout the day (as previously described in Section 3.2.2.4.). The 24 selected picture were easily recognisable typical Italian food. Food stimuli were presented in a 2 x 2 design, with two levels of stimulus energy content (high and low energy) and two levels of stimulus nutrient composition (carbohydrate-based and protein-based). Those 24 food images were scrambled by using the Matrix Laboratory software (MATLAB®, the MathWorks® Inc., Natick, Massachusetts, USA). In such a way, scrambled images were presented using the same 2 x 2 design used for food

stimuli. Finally, 6 non-food images were selected from a picture database containing coloured images of common objects.

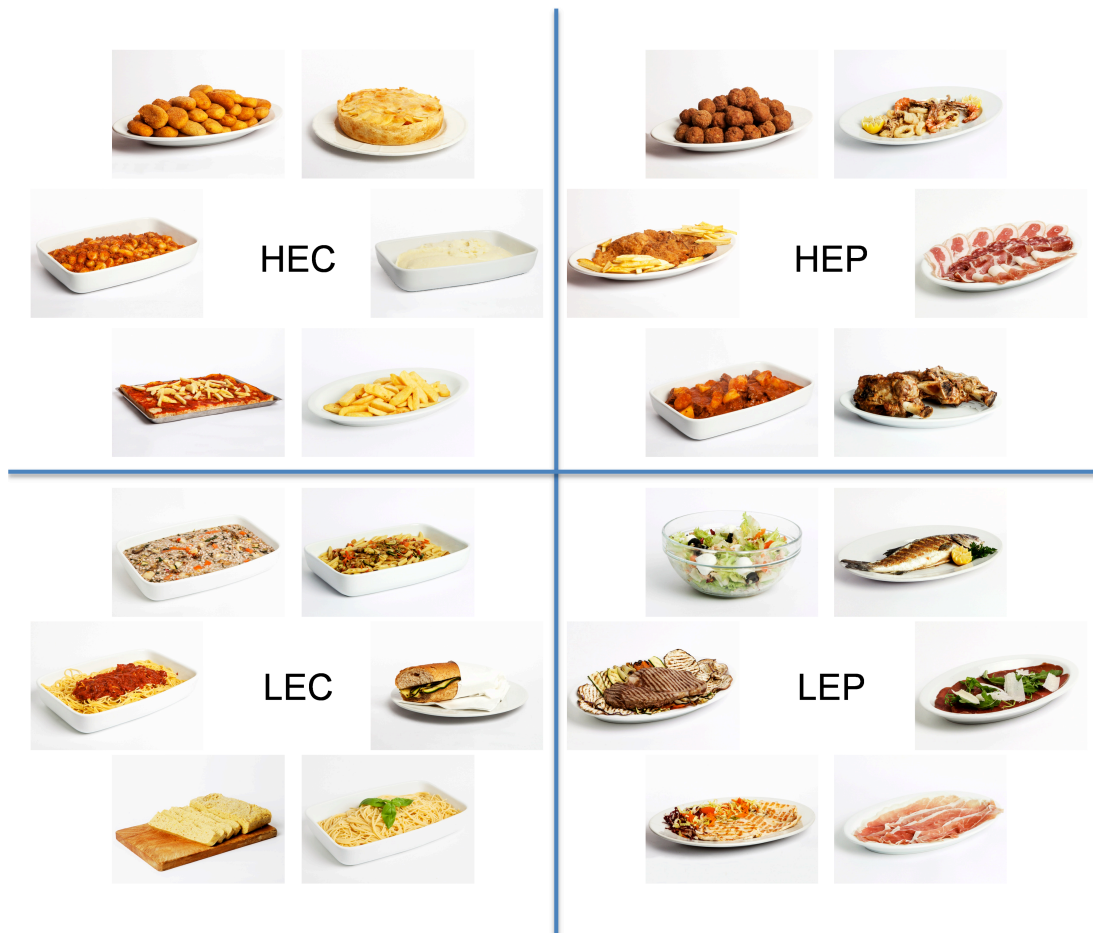


Figure 41 - Food images selected as stimuli for the observational task and the desire-to-eat and healthiness perception judgments, by nutritional categories. HEC, High Energy Carbohydrate; HEP, High Energy Protein; LEC, Low Energy Carbohydrate; LEP, Low Energy Protein.

Stimuli were displayed for 2500 ms and presented in blocks of 18 sec for the three experimental conditions (observation, desire-to-eat judgment and healthiness judgment), corresponding to three separate fMRI runs of 17 min. During each run, 6 different stimuli belonging to the same category were shown within each block, for a total of 6 blocks X 5 stimulus category (HEC, HEP, LEC, LEP, non-food) in the observational tasks, and a total of 4 block X 5 stimulus category in both judgment tasks. After each block of food images, an identical block of scrambled images was run, using as baseline explicit condition. Stimuli order was randomised and counterbalanced across subjects.

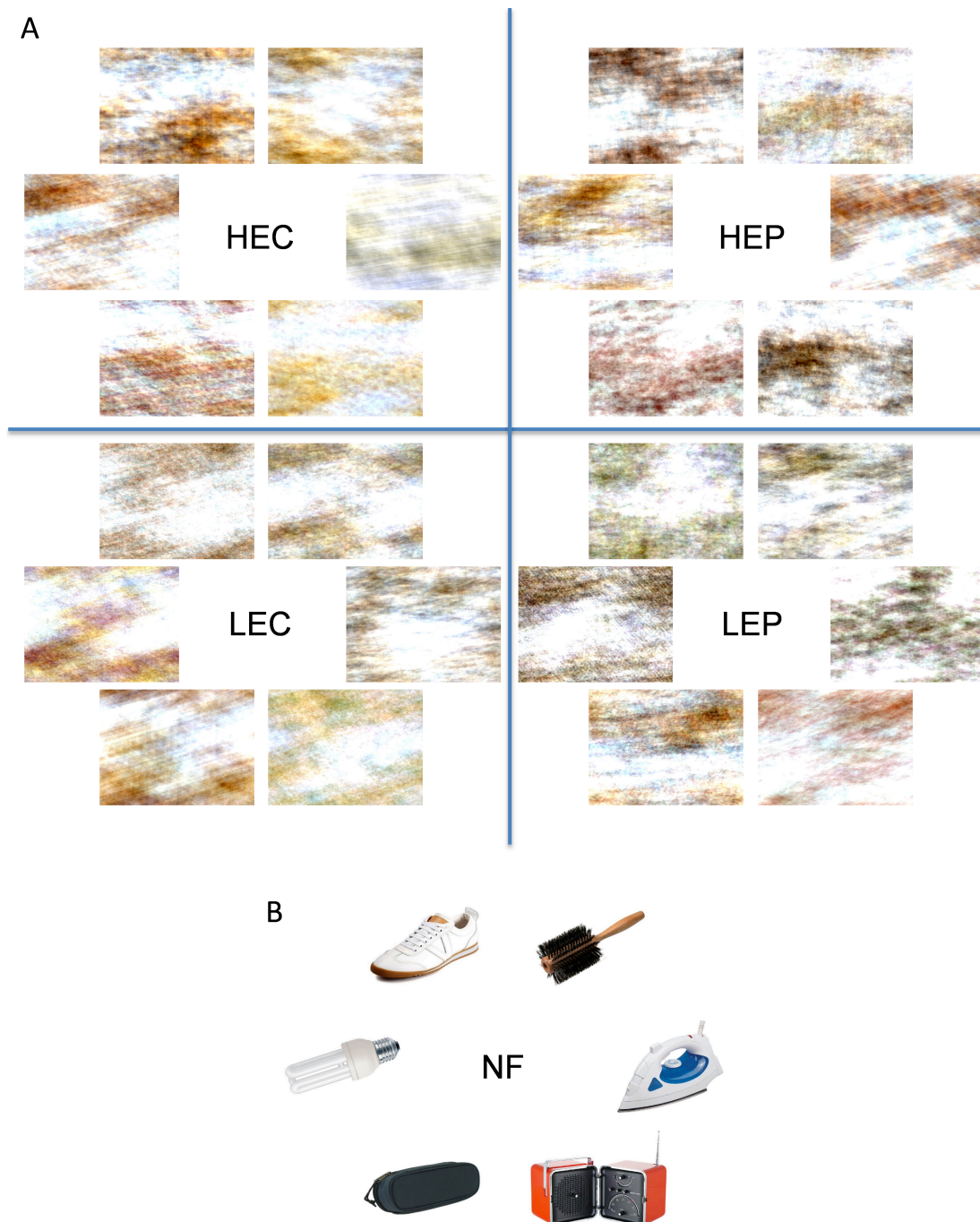


Figure 42 - Non-food images selected as stimuli for the observational task and the desire-to-eat and healthiness perception judgments. A) Scrambled images by nutritional categories; B) Common objects. HEC, High Energy Carbohydrate; HEP, High Energy Protein; LEC, Low Energy Carbohydrate; LEP, Low Energy Protein.

Stimuli were shown using digital visors (VisuaSTIM) with a resolution of 500,000 pixel x 0.25 square inch and horizontal eye field of 30, applied directly on volunteer's face. Signal was digitally transmitted to the scanner via optic fibre. Stimuli were presented through the software E-Prime 2 Professional (Psychology

Software Tools, Inc., Pittsburgh, PA, USA). The same software was also used to record answers of participants during the different tasks.

3.4.2.3. fMRI data acquisition

Anatomical T1-weighted and functional T2*-weighted magnetic resonance images were acquired with a 3 Tesla General Electric's scanner equipped with an eight-channel receiver head-coil. Functional images were acquired using a T2*-weighted gradient-echo, echo-planar pulse sequence (acceleration factor 2, 37 interleaved transverse slices covering the whole brain, TR=2100 ms, TE=30 ms, flip angle=90°, FOV=205x205 mm², inter-slice gap=0.5 mm, slice thickness=3 mm, in-plane resolution 2.5x2.5x2.5 mm³). Each scanning sequence comprised 306 sequential volumes. Immediately after the functional scanning, a high-resolution inversion recovery prepared T1-weighted anatomical scan (acceleration factor 2, 156 sagittal slices, matrix 256x256, isotropic resolution 1x1x1 mm³, TI=450 ms, TR=8100 ms, TE=3.2 ms, flip angle 12°) was acquired for each participants in each experimental session.

3.4.2.4. Statistical analysis

Image pre-processing and statistical analysis were performed using the Statistical Parametric Mapping software (SPM8, Wellcome Department of Imaging Neuroscience, London, UK), implemented in the Matrix Laboratory software (MATLAB®, the MathWorks® Inc., Natick, Massachusetts, USA). Significance was set at $p < 0.05$.

Data on desire-to-eat and healthiness perception was analysed by using a repeated measurement GLM (Greenhouse–Geisser correction if epsilon was lesser than 0.75 or Huynh–Feldt correction if epsilon was greater than 0.75, and Bonferroni post-hoc tests for multiple comparisons) considering as factors nutritional category (HEC, HEP, LEC, LEP) X breakfast type (BR1, BR2, BR3, BR4). In

particular, nutritional categories were split in 2 X 2 compositional factors: energy content (high/low energy) X nutrient composition (carbohydrate-based/protein-based).

A repeated measurement GLM was used to analysed fMRI imaging data by stimulus type (food picture/non-food picture). Based on this analysis, brain ROIs were defined. ROIs were examined by using a repeated measurement GLM considering as factors experimental task (observation, desire-to-eat rating and healthiness perception rating) X energy content (high/low energy) X nutrient composition (carbohydrate-based/protein-based) X breakfast type (BR1, BR2, BR3, BR4).

Data on the level of each one of the 20 positive and negative affects (PANAS test) was analysed by using repeated measure GLM by breakfast type (BR1, BR2, BR3, BR4).

3.4.3. Results and Discussion

3.4.3.1. Evaluation of the desire-to-eat and healthiness perception during the fMRI scanning session

Participants were asked to report their desire-to-eat and healthy perception of a series of visual stimuli belonging to different nutritional categories. No statically significant differences were observed among the different breakfasts for both attributes (all $p > 0.05$). In terms of nutritional categories, low energy carbohydrate-based foods (LEC; e.g., spaghetti with oil and basil) were slightly less desirable than the other three categories (Figure 43A). Low energy and protein-based foods were rated as the healthiest products ($p < 0.05$) (Figure 43B). Moreover, low energy protein-based foods (LEP) were perceived as healthier than low energy carbohydrate-based foods (LEC) (i.e., iron steak > spaghetti with oil and basil; $p < 0.05$; Figure 43B).

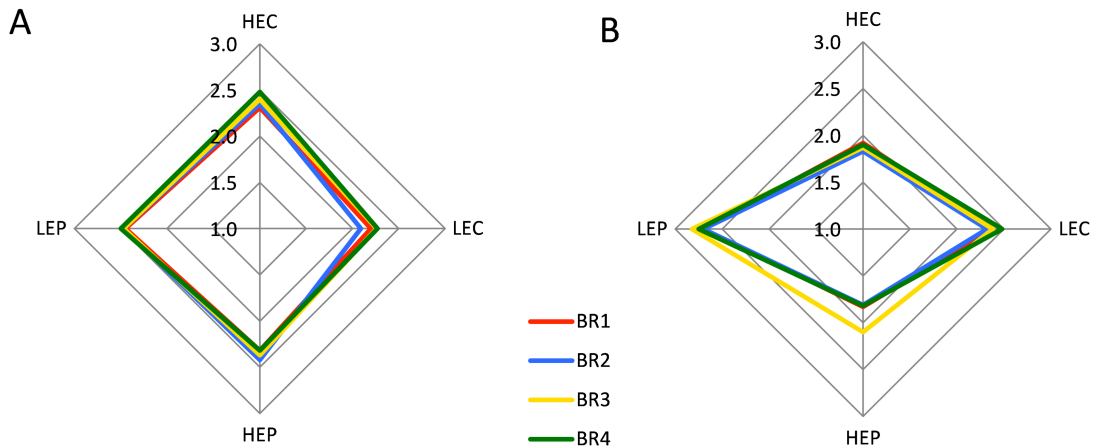


Figure 43 - A) *Desire-to-eat* and B) *healthiness perception rating at lunchtime of foods belonging to different nutritional categories*. A) *Scrambled images by nutritional categories*; B) *Common objects*. HEC, High Energy Carbohydrate; HEP, High Energy Protein; LEC, Low Energy Carbohydrate; LEP, Low Energy Protein.

3.4.3.2. Assessment of brain activation by fMRI

Initially, brain activation linked to the observation of *scrambled* pictures was used as baseline for the evaluation of the neural correlates associated with food choices. It might have served as reference signal for the comparison of brain activation due to different food categories. However, imaging data showed a strong brain activation for this category of stimuli. Therefore, with the aim of avoiding false negatives -lack of real existing activation-, activation due to *scrambled* stimuli was neglected and the activation due to non-food pictures was used as baseline. This did not preclude the study of the neural activation related to nutritional processes. When the brain areas involved in the nutritional processes were identified, some of them were selected as ROIs. Neural signals for these ROIs were analysed on the basis of the four type of breakfasts (BR1, BR2, BR3, and BR4), nutritional categories (HEC, LEC, HEP, and LEP), and experimental tasks (observation, desire-to eat judgment, and healthiness judgment).

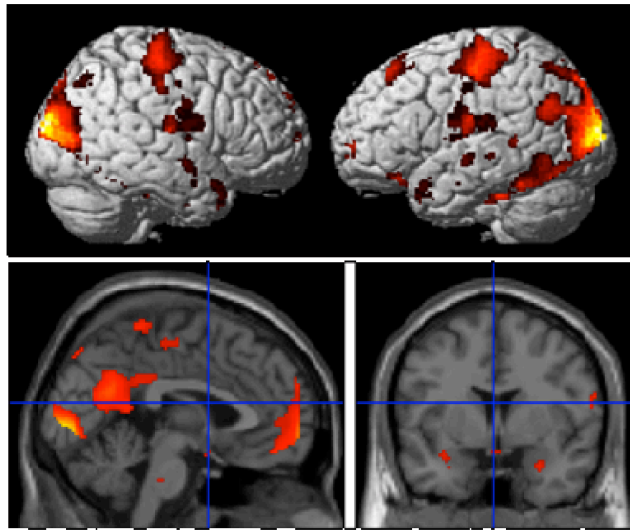
3.4.3.2.1. Global activation

Brain activation associated with food picture viewing in the observation, desire-to eat rating, and healthiness rating tasks is illustrated in Figure 44. They

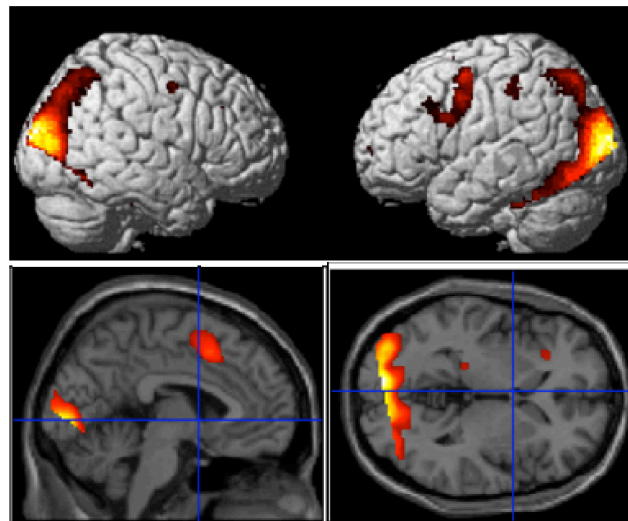
were obtained by subtracting the average activity of participants registered for each task in response to food picture viewing to the activity registered for non-food picture viewing (food – non-food stimuli activation). They served to highlight the specific brain response related to food viewing for each experimental task (Figure 44).

Imaging data showed the vast activation of the same cortical areas for the three experimental tasks, being most extended for the observational task (Figure 44). Brain activation included: the occipito-temporal cortex visual areas, for the primary and complex analysis of the stimulus; the parietal, pre-motor, and prefrontal areas, mainly involved in visual-motor processing; and, during the observational task, the medial frontal cortex and somatic area. Specific activations were also found for the observational task in comparison to the two rating tasks. These activations involved the posterior insular cortex, the orbitofrontal cortex, and the hypothalamus. On the contrary, both rating tasks showed a higher reactivity for the prefrontal-parietal circuitry, including the activation of the posterior parietal and pre-motor cortices, and the supplementary motor cortex. The healthiness judgment also activated the bilateral middle frontal gyrus, linked to cognitive monitoring and selection of the stimulus. This activation fitted with the experimental question of this rating task, focused on assessing whether the observed food picture was less or more healthy. Regarding the desire-to-eat rating, a higher reactivity in comparison with the other tasks was shown for the left anterior insula. The activation of this part of the insula was coherent with the activation of the pre-motor cortex, which was highly active for both rating tasks. These two cortices are actually closely connected to one another. Functional classification of the anterior insular indicates a more emotional function of the ventral area, while a more cognitive and motor-related function of its dorsal part. This last function agrees with the expression of an explicit judgment in both rating tasks and, in particular, in the desire-to-eat rating, where the “emotional” value of food was higher than for the healthiness rating.

A) Observational Task (-NF, FWE .05)



B) Desire-to-eat Rating (-NF, FWE .05)



C) Healthiness Rating (-NF, FWE .05)

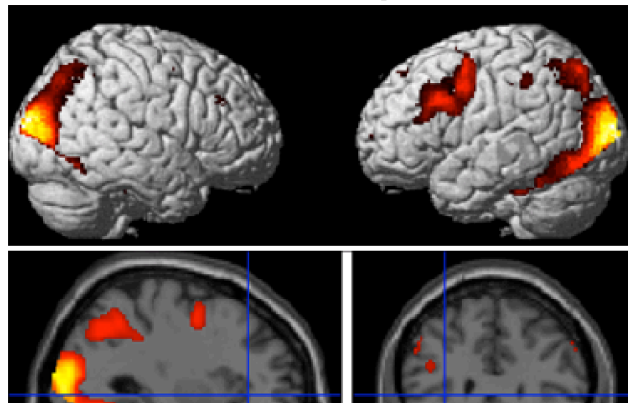


Figure 44 - Functional activity mediated by food picture stimuli in A) the observational task, B) the desire-to eat rating task, and C) the healthiness rating task. Upper images show cortical activation, while lower images are related to subcortical activation.

All these brain activations triggered by food stimuli viewing differed from those activations not related to food stimuli. Non-food pictures provoked mainly visual activations. These data suggested that the observation of stimuli not related to food was not interesting for the participants or it was not relevant for the aims of the task, but they served to highlight the effectiveness of the experimental stimuli chosen to promote brain reactivity to food pictures.

Based on the specific activations registered for each task in response to food pictures (Figure 44), some anatomical structures associated with food-related processes were identified to create ROIs. They were thoroughly studied to better understand the differences on brain activation as affected by the type of breakfast, the nutritional category of the food stimuli, and the experimental task.

3.4.3.2.2. Regions of Interest

ROIs were created for the following anatomical structures:

- Hypothalamus
- Middle orbitofrontal cortex
- Anterior cingulate cortex
- Anterior insula

Hypothalamus

The hypothalamus has been long recognised as the master clock of food intake/metabolic cycles, playing a central role in body energy balance and, thus, in feeding behaviours and body weight regulation (Challet, 2013; Morton *et al.*, 2006; Schwartz & Porte, 2005). It is composed of different nuclei: the ventromedial nucleus is most commonly associated with satiety, while the lateral hypothalamus acts like a hunger/appetite centre. Moreover, the paraventricular nucleus participates in the regulation of appetite, controlling neural and hormonal signals involved in food intake (King & Frohman, 1985). In healthy subjects, the inhibition of fMRI signals within the areas corresponding to paraventricular and ventromedial

nuclei was found after glucose oral intake (Matsuda *et al.*, 1999), with a maximum inhibitory response occurring 2 hours after glucose intake and correlating well with blood glucose and insulin levels. This sustained decrease in hypothalamic activation after glucose intake was related to the concomitance of two factors: the taste (sweet) and the energy content (Smeets *et al.*, 2005). The hypothalamus serves as a key homeostatic site receiving and integrating neural, nutrient and hormonal signals (*i.e.*, leptin, PYY, and ghrelin) and orchestrating appropriate efferent responses impacting on our physical (endocrine) and mental (mood, behaviour) systems. It also modulates the pleasure of eating and the reward value of food (Batterham *et al.*, 2007).

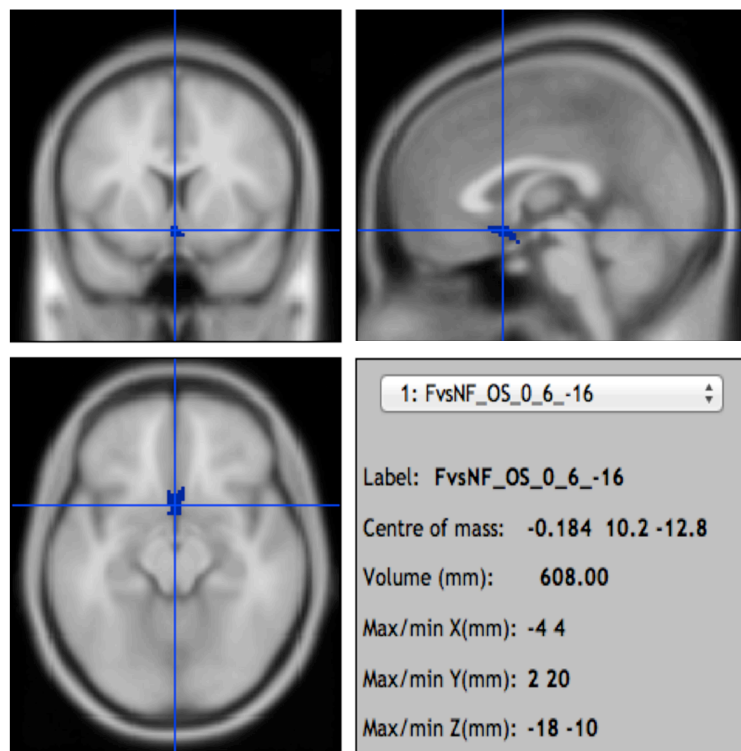


Figure 45 - Render of the ROI created for the hypothalamic region (0 6 -16).

As expected based on the global activation imaging data, statistical analysis evidenced a main effect of the task ($F_{2,28}=17.36$, $p<0.001$, $\eta_p^2=0.55$, $\delta=1$), with the observational test activating the hypothalamus to a greater extent. A further exploration of hypothalamic activation within the observational task did not reveal statistically significant differences ($p>0.05$), despite a clear trend in the activation of hypothalamic areas was observed after BR4 consumption in comparison to the other breakfast types (Figure 46). This trend may be explained considering the

master clock function of metabolic cycles by hypothalamus. Actually, the hypothalamic activation registered after consumption of BR4 may be likely associated with the meal skipping-like condition of this breakfast, in comparison with the rest of the experimental breakfasts.

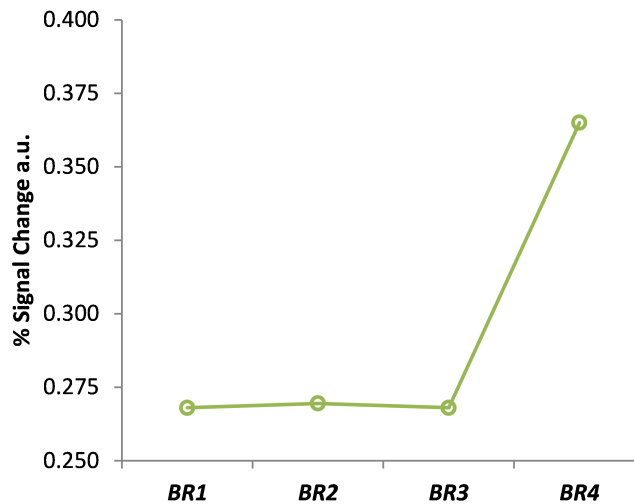


Figure 46 - Hypothalamic activation profile for the 4 breakfast types during the observational task, regardless of the nutritional category of the food pictures.

Middle orbitofrontal cortex

Eating behaviour is deeply conditioned by non-homeostatic factors such as cognition, emotion, motivation and decision making, which are mainly processed in corticolimbic brain areas (Berthoud, 2004). Changes in neural activity within the caudolateral orbitofrontal cortex have been observed under conditions of high PYY plasmatic concentrations (Batterham *et al.*, 2007). Reactivity increases in this area under fullness/satiety conditions (high PYY) predicted subsequent feeding behaviour independently of meal-related sensory experiences. On the contrary, under low plasmatic levels of PYY (appetite/hunger conditions), the hypothalamus was the area predicting eating behaviour at the subsequent meal (Batterham *et al.*, 2007). Therefore, the presence of a postprandial satiety factor (high/low plasmatic PYY levels) switches food intake regulation from a homeostatic need to a hedonic experience taking place in the corticolimbic area. Moreover, the activation of corticolimbic brain areas, also comprising the orbitofrontal cortex, increases in hungry subjects when they observe high-caloric food pictures compared with low-

caloric ones (Goldstone *et al.*, 2009). These results also demonstrated the interaction between homeostatic and hedonic aspects of feeding behaviour.

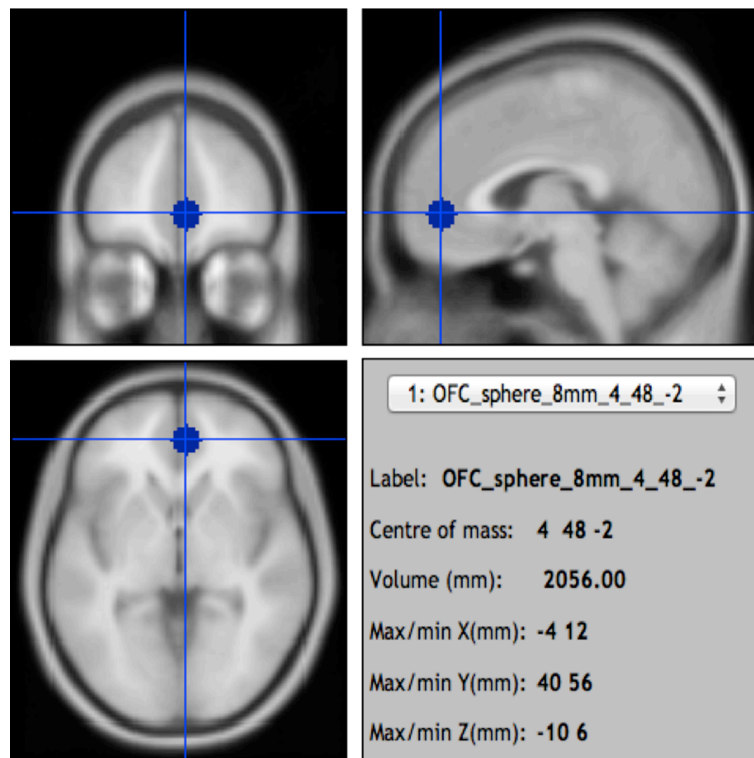


Figure 47 - Render of the ROI created for the orbitofrontal region (4 48 -2).

In the present study, activation of the orbitofrontal cortex was specifically registered at the observational task, when participants observed food pictures with respect to non-food pictures while they imaged themselves in a restaurant without perception rating. Under these conditions, the response to the experimental stimuli can be regarded as “spontaneous” (non-related to cognitive tasks). There was a main effect of the task ($F_{2,28}=25.7$, $p<0.001$, $\eta_p^2=0.65$, $\delta=1$) but not of the type of breakfast nor the nutritional category of the food stimuli. In particular, a higher activation of the orbitofrontal cortex was recorded during the observational task in comparison with both perceptual rating tests, in line with the imaging results. To further explore the main effect of the observational task on brain reactivity at the orbitofrontal cortex area, a second GLM model focused on the observational test was created. In this case, although statistical differences were not found ($p>0.05$), there was a clear trend pointing at a lower activation of this brain area at lunchtime following BR1 (Figure 48).

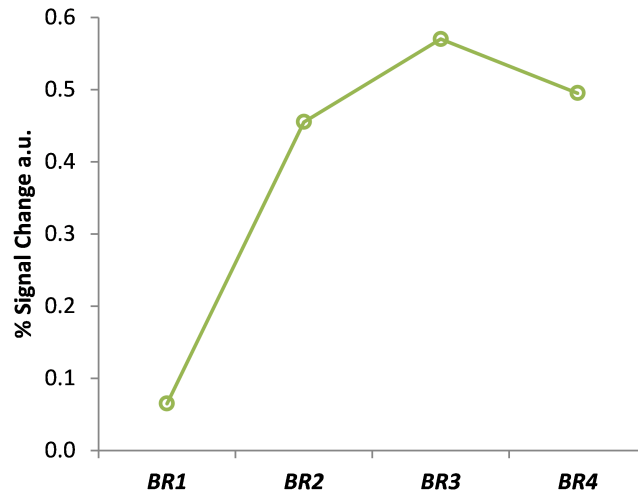


Figure 48 - Orbitofrontal cortex activation profile for the 4 breakfast types during the observational task, regardless of the nutritional category of the food pictures.

This trend in the activation of the orbitofrontal cortex region indicated a higher saliency of the food picture stimuli after consuming BR2, BR3, and BR4 than after consuming BR1. The lower saliency of food images following BR1 might account for a higher satiety state with respect to the rest of the breakfast meals tested. However, this effect was not observed when subjects rated their perceived satiety during the postprandial period (stated at Chapter 3.2.).

Anterior cingulate cortex

The anterior cingulate cortex establishes neural connections with different cortical areas, such as the orbitofrontal and the insular, and is part of the reward circuit. It is involved in the elaboration of stimuli and responses having emotional saliency, and its activation is usually associated with learning processes. This brain area participates from cognitive processes of appetite control together with other brain regions such as the superior frontal areas, insula, and superior parietal areas (Tuulari *et al.*, 2015). The functional role of anterior cingulate cortex in voluntary control of feeding behaviour has been widely emphasised for eating disorders (Uher & Treasure, 2005).

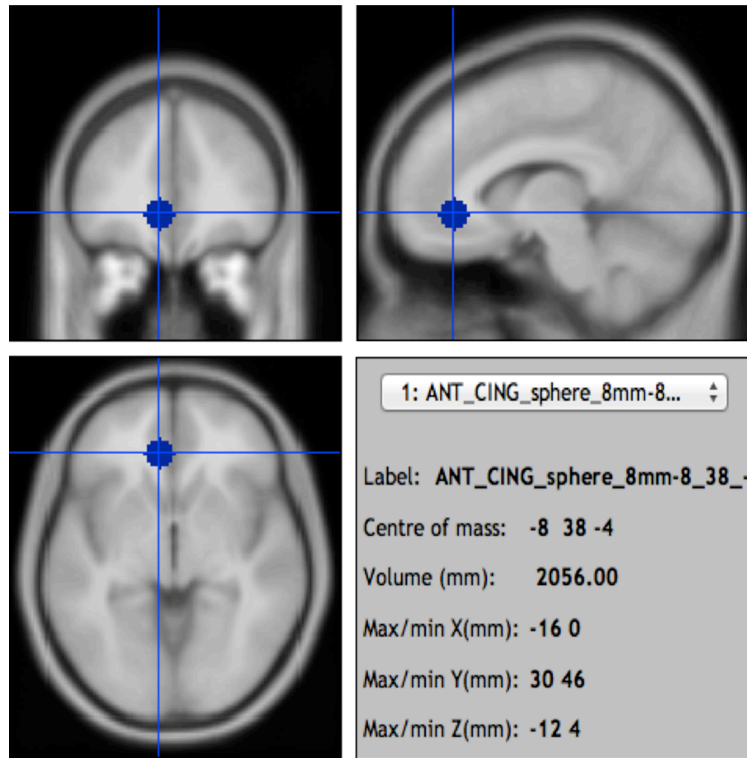


Figure 49 - Render of the ROI created for the anterior cingulate cortex (-8 38 -4).

Neuroimaging data from the present study showed a high activation of the cingulate gyrus during the observational task for all the breakfast types. Although statistically significant differences were not found ($p > 0.05$), a trend in the activation of the anterior cingulate cortex was evidenced after consumption of breakfast meal BR1 (Figure 50).

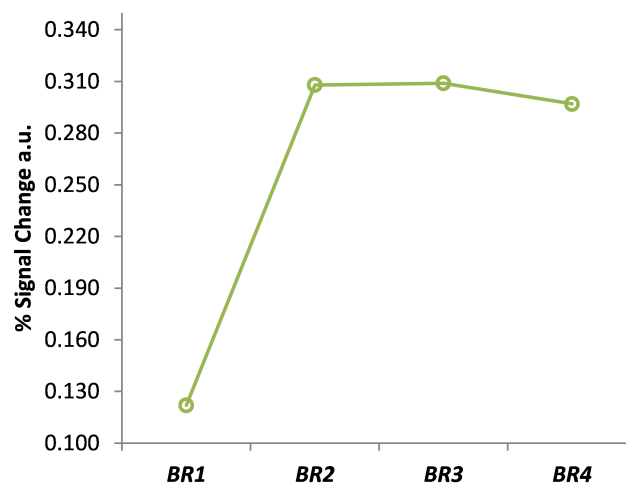


Figure 50 - Anterior cingulate cortex activation profile for the 4 breakfast types during the observational task, regardless of the nutritional category of the food pictures.

This result agreed with the activation observed in the middle orbitofrontal region following BR1 consumption (previously described at Section 3.4.3.2.2.2.). The lower reactivity of this brain region during the observation of food pictures after having BR1 might account for a lower effort to inhibit feeding needs, while BR2, BR3, and BR4 would have stressed more this brain area by provoking higher efforts to counteract feeding needs.

Anterior insula

The anterior insula participates from the primary taste cortex and it is interconnected with other brain areas such as the amygdala and the cingulate and orbitofrontal cortices, which are also activated in tasks related to visual processing of food stimuli. The anterior insula processes information associated with food taste and depicts the subjective value of the food (Kim *et al.*, 2012). It is involved in food choice processing and integrates the information linked to food taste, aspect, odour, and consistency with internal homeostatic markers and feeding needs.

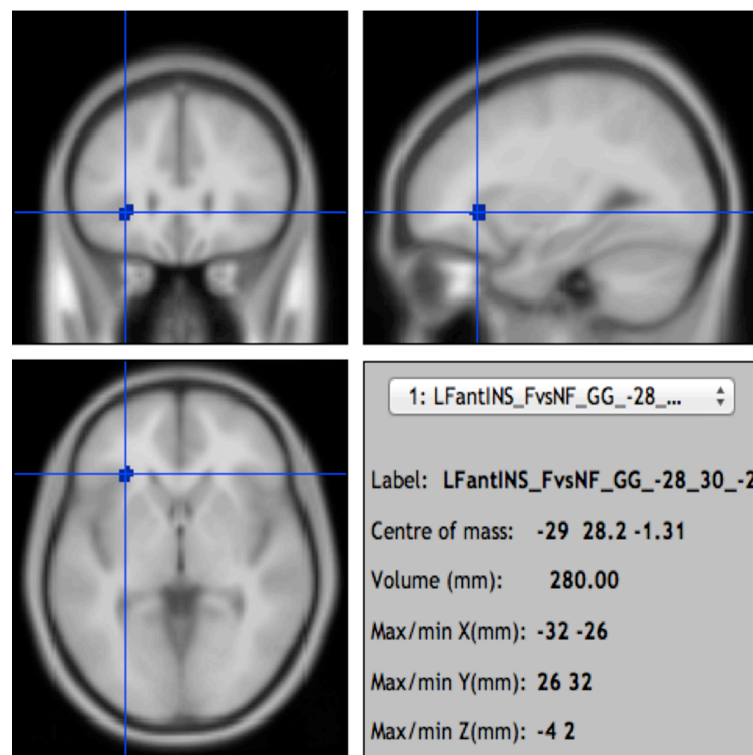


Figure 51 - Render of the ROI created for the anterior insula (-28 30 -2).

Imaging data analysis did not display differences in the activation of the left anterior insula among the three experimental tasks ($p>0.05$), although rating tasks caused a slightly higher activation of this area in comparison to the observational task. On the other hand, the activation of this brain region did not show statistically significant differences ($p>0.05$) among the diverse breakfast meals. Nevertheless, BR1 and BR2 presented a lower activation of the anterior insula with respect to BR3 and BR4 (Figure 52).

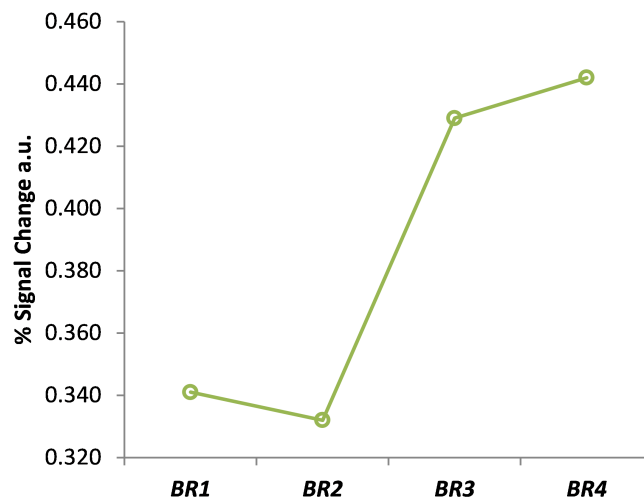


Figure 52 - Average activation profile for the three experimental tasks of the anterior insula, for the 4 breakfast types regardless of the nutritional category of the food pictures.

Taking into account the imaging data recorded for the three experimental tasks and the effect of breakfasts differing for their perceptual and compositional characteristics on specific brain areas, it can be concluded that no major effects on brain activation at lunchtime were observed. However, clear trends in the reactivity of the selected ROIs were observed as a consequence of having different types of breakfast. These aspects might be further explored at different time points (for instance, between breakfast and lunch times) to better understand how the cognitive responses to food stimuli are affected by the type of breakfast, since these are not relevant under fasting-like scenarios. On the other hand, attending to the lack of differences in brain activation when observing or rating foods belonging to the HEC, HEP, LEC, and LEP nutritional categories, it can be pointed out that food itself is enough to activate cognitive brain circuits at lunchtime, regardless of the energy content and nutritional composition.

3.4.3.3. Positive and negative affective schedule

Participants' mood and emotional status was evaluated by using the PANAS test after the fMRI evaluation, where a series of affects are correlated with mood and brain activation. A total of 20 affects were assessed: interested, distressed, excited, upset, strong, guilty, scared, hostile, enthusiastic, proud, irritable, alert, ashamed, inspired, nervous, determined, attentive, jittery, active, and afraid.

Comparison among the different types of breakfasts did not evidenced statistically significant differences ($p>0.05$) for any affect. These results were in agreement with the lack of major effects due to breakfast composition recorded for the fMRI scanning session in the three different tasks.

3.5. STUDY 5

***How to improve food choices through
vending machine:
the importance of healthy food availability
and consumers' awareness***

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3.5.1. Introduction

Globally, about 2 billion adults are obese or overweight regardless of gender and age (WHO, 2016). Excessive body weight is a critical risk factor for different pathologies, such as cardiovascular disease, diabetes, and some types of cancer, accounting for more than 3 million deaths every year (NCD, 2016; Smith & Smith, 2016). For this reason, obesity represents, at the same time, a major public health concern and possibly the most important challenge of the 21st century. Among the many factors involved in the process of body weight gain, the positive energy balance resulting from excessive energy intake and reduced physical activity is probably one of the most relevant (Hill *et al.*, 2000; Schmitz & Jeffery, 2000). With regard to eating habits, numerous environmental factors have been pinpointed to contribute to the obesity epidemic by changing eating patterns towards unhealthy behaviours. Among them, the growing trend to eat outside, the continuously increased size of food portions, the greater availability of high-fat, energy-dense foods and sugar-sweetened drinks, and the increased frequency of snacking occasions (Bray *et al.*, 2004; Bray & Champagne, 2005; Piernas & Popkin, 2010; Wilson *et al.*, 2016; Young & Nestle, 2007) are the most representative.

In this context, vending machines (VMs) could be considered among the environment elements contributing to increase the availability of nutrient-poor and energy-dense foods products, being healthy options scarcely offered or totally absent (Grech & Allman-Farinelli, 2015; Matthews & Horacek, 2015). VMs are widely used and are available in a multitude of locations, such as schools, healthcare facilities, workplace, and many public spaces (Matthews & Horacek, 2015). Contextually, it was demonstrated that VMs are the main and readily available source of foods for people working long hours (Escoto *et al.*, 2010), when VMs are used to buy main meals (to save time) and snacks. The problem is that the most popular, nutrient-poor snacks may negatively impact weight management (Hess *et al.*, 2016).

Interventions on VMs emerge therefore as an opportunity to improve product quality and to assist users in food choices and body weight control, since

environmental interventions have been proved to be effective in influencing consumers behaviour and eating habits (Cohen, 2008; Matthews & Horacek, 2015; Story *et al.*, 2008; Swinburn *et al.*, 1999). Among different strategies, pricing or promotion interventions have been widely demonstrated to be effective approaches to address and improve consumer choices among VM products (French *et al.*, 2001; French, 2003; Voss *et al.*, 2012). Although product price has been described as a relevant variable that influences consumer purchasing behaviour and represents a potential barrier to buying healthy products (Callaghan *et al.*, 2010), a long-term reduction of the selling price of healthy products does not appear as a viable strategy for VM companies. In keeping with this consideration, other interventional approaches such as point-of-choice nutrition information and healthy food alternatives enrichment have been tested, but the effectiveness of these actions in encouraging healthy food choices is not completely clear yet (Grech & Allman-Farinelli, 2015).

Given the wide presence of VMs in schools, and the critical period of childhood and adolescence for eating habits acquisition, several behavioural and educational intervention have been conducted in school settings (Adachi-Mejia *et al.*, 2013; Alaimo *et al.*, 2013; Bucher *et al.*, 2016; Han-Markey *et al.*, 2012; Kocken *et al.*, 2012). Nonetheless, the transition between adolescence and adulthood should be addressed, since young adults very often face severe lifestyle changes associated with moving away from home, gaining independence while facing the development of their own dietary habits and, thus, increasing the risk of weight gain (Arnett, 2000; Sparling, 2007). In particular, college students could have problems in healthy body weight maintenance, as they usually have busy schedules, less time available for physical activity, academic stress, and unhealthy eating patterns (low in fruits and vegetables and rich in high-fat and high-sugar foods and drinks) (Crombie *et al.*, 2009; Kicklighter *et al.*, 2010; Lake *et al.*, 2009; Lien *et al.*, 2001). Although the need for easily accessible healthy foods and for nutrition education in university campuses, linked to VMs, has been previously highlighted, a limited research has been conducted to date on the topic (Ali *et al.*, 2015).

For all these reasons, the present study tried to enrich the existing literature, testing a simultaneous multiple-action intervention on food and beverage VMs in a University setting. In particular, this work aimed at raising purchases and intakes of healthy foods/beverages in VMs by applying two different strategies focused on: i) nutritional improvement of the product portfolio, and ii) nutritional communication to promote the healthier options.

3.5.2. Material and Methods

The present study was conducted at the University of Parma (North of Italy), in collaboration with the university's vending services. The study was carried out between April 2014 and December 2015, during standard university terms, with no holiday and with regular opening time, to avoid uncontrolled events that might impact vending sales.

Three VMs out of the total set of dispensers of the university, located in three university's sites, were chosen for the study. Selection criteria for VMs were: i) to be located in a site that was easily accessible, with no restrictions; ii) to be located in a site that was not in any of the university food courts; iii) to be located in a site with no other VM except for water and hot-drink dispensers; iv) to have minimum weekly sales variation; v) to be refrigerated; and vi) to have an equal size.

All potential customers of the selected VMs were students, researchers, professors, and university administrative staff members. Choices were measured retrospectively by the amount of sales and not by real-time single purchase. Therefore, no specific information was collected for the VM customers, corresponding to a heterogeneous group of adults of both genders.

University Review Board's approval was not necessary for this study since only data regarding food item sales in the university VMs, but not personal data, were collected.

3.5.2.1. Intervention 1 – Increasing the availability of healthy food options

One out of the three VMs was randomly selected for the first intervention. The intervention was conducted in two time periods: an initial baseline phase and an experimental phase. During the baseline phase, each purchase of a food or drink item was assessed over a 24-week period in 2014. Water and hot beverages were excluded from the test. The VM was in its usual configuration during the baseline period, keeping the same products and prices occurring before the intervention. Purchase data were collected for the same VM during the equivalent 24 weeks in 2015, once unhealthy foods and drinks were partially replaced by healthier ones to test the effect of increasing the availability of healthy food options on sales (experimental phase). During this time frame, products and VM settings remained constant.

3.5.2.1.1. Food items evaluation and selection

Before the beginning of the study, each food item in the vendor's portfolio was categorised as snack, ready-to-eat meal, and drink. The ingredient list and nutritional values of a total of 280 products were obtained by using the nutritional label. Complete data for ingredients, calories, and selected nutrients (total carbohydrate, sugars, total fat, saturated fat, cholesterol, sodium, and fibre) per serving size were collected. Each item was evaluated for its nutritional quality on the basis of criteria derived from i) local guidelines for the availability of healthy food and beverage options in schools (Giunta della Regione Emilia Romagna, 2012), ii) the Italian recommended levels for energy and nutrients (SINU, 2014), and iii) the Italian guidelines for healthy eating (Cialfa *et al.*, 2007). Specific criteria for each food group are provided in Table 9. Depending on its nutritional profile, each product was then classified into one of four possible nutritional categories: "healthy +", "healthy -", "unhealthy -", and "unhealthy +". A total of 32 products (20 snacks, 4 ready-to-eat meals, and 8 drinks), equally distributed in the four nutritional categories (25% "healthy +", 25% "healthy -", 25% "unhealthy -", and 25% "unhealthy +"), were selected for the intervention.

Table 9 - Criteria to classify every food item in a nutritional category, for each food group.

Nutritional information	Nutritional quality indicator	Positive if...
Snacks	Energy (kcal/serving)	< 150
	Fat (g/serving)	< 5
	Sugars (g/serving)	< 5
	Sodium (g/serving)	< 0,12
	Fibre (g/serving)	> 3
	Trans fat acids	NO
	Hydrogenated fat acids	NO
	Whole grain	YES
	With fruit/vegetables	YES
	Nuts with no oil or salt	YES
	Fermented Milk	YES
	With no salt on the surface	YES
	From organic farms	YES
	100% fruits	YES
Ready-to-eat Meals	Energy (kcal/serving)	< 500
	Fat (g/serving)	< 30
	Sugars (g/serving)	< 30
	Sodium (g/serving)	<0,75
	Fibre (g/serving)	> 3
	Trans fat acids	NO
	Hydrogenated fat acids	NO
	Whole grain	YES
	With fish	YES
	With chicken	YES
	With fruit/vegetables	YES
	DOP	YES
	IGP	YES
	STG	YES
	From local farms	YES
	From organic farms	YES
	Nuts with no oil or salt	YES
	Fermented Milk	YES
With no salt on the surface	YES	
Drink	Energy (kcal/serving)	< 150
	Fat (g/serving)	< 5
	Sugars (g/serving)	< 4
	Sodium (g/serving)	< 0,12
	Alcohol	NO
	Fruit (%)	> 70
	Stimulating substance (e.g. caffeine)	< 0,15
	With added sugars	NO
	100% fruits	NO

3.5.2.1.2. Food items arrangement within the VM

To better promote healthy options, the selected products were arranged within the VM in a 2 (food group) x 2 (nutritional category) display layout (Figure 53).

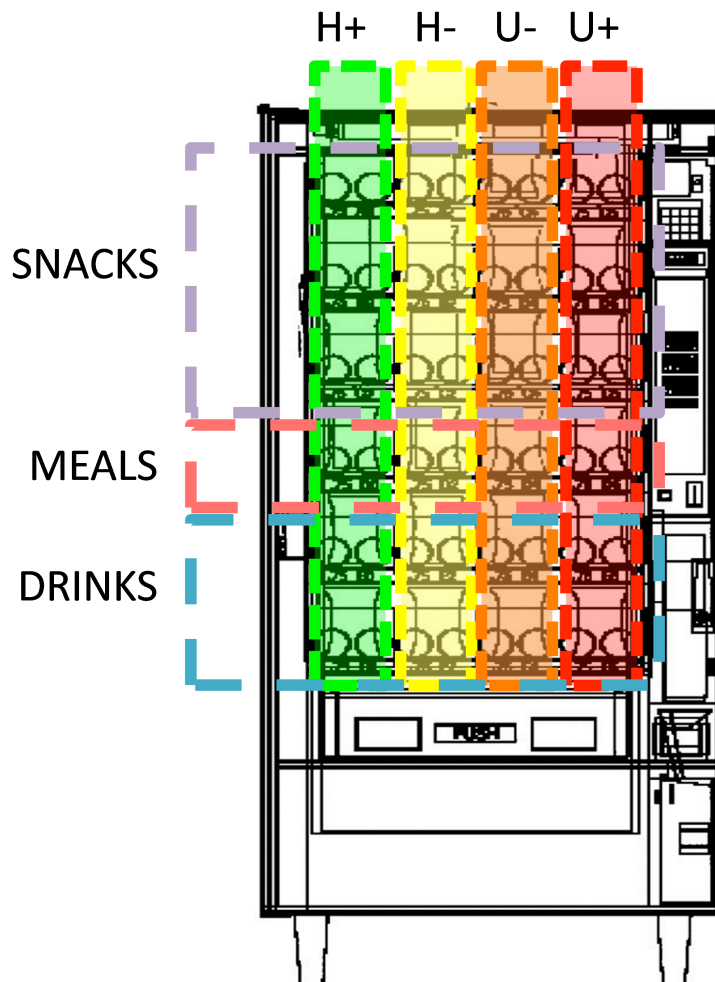


Figure 53 - Vending machine layout by food group (snacks, meals, and drinks) and nutritional category (H+, H-, U-, and U+). Nutritional categories: H+, “healthy +”; H-, “healthy -”; U-, “unhealthy -”; and U+, “unhealthy +”.

Food groups were used to organise products horizontally in the VM, with items belonging to the snack, ready-to-eat meal, and drink groups allocated respectively in the upper, central, and lower shelves. Through this arrangement, the lightest items -like snacks- were placed at the top of the machine, whereas the heaviest -beverages- at the bottom to avoid damaging the pack after the vertical drop. Within each food group, products were organised vertically by nutritional

categories, with each shelf divided into 4 sections -from left to right- corresponding to the four defined nutritional categories (“healthy +”, “healthy -”, “unhealthy -”, and “unhealthy +”). As stimuli have been reported to be perceived faster when positioned on the left rather than on the right of the observer (Porcheddu *et al.*, 2011), placing the “healthy +” items in the first vertical section on VMs (on the left of the observer) should promote their sales.

3.5.2.2. Intervention 2 – Nutrition information through VMs

A group-randomized, crossover, controlled nutrition intervention was conducted in the three selected VMs for 24 weeks in 2015. Each VM was equipped with the same food products, organised in an equal layout (as previously described in the Section 3.5.2.1.2, *Food items arrangement within the VMs*). No changes were made in the VM used in the first intervention after increasing the availability of healthier food options. This VM represented the “control” condition, since no nutrition information was disclosed. The other two VMs were randomly assigned to one of two experimental conditions, and crossed-over (12 weeks + 12 weeks). The two experimental conditions are described in the following paragraph. Communication materials were shown in these two VMs to provide information about the nutritional quality of each product.

3.5.2.2.1. Nutrition information of food items

Based on its nutritional quality, each of the 32 selected products was characterised by two types of information: “stars” and “claims”. The “star” information system was a graphical summary of a star rating scale of zero to three stars used to classify products (Figure 54A and 54B). The number of stars for each item was determined according to its nutritional category, with more stars describing healthier products. Food items belonging to the “healthy +”, “healthy -”, “unhealthy -”, and “unhealthy +” categories were branded with three, two, one, and zero stars, respectively. The “claim” information system was a textual description of the nutritional proprieties of foodstuffs (Figure 54C and 54D). A

nutritional claim was identified for each product, trying to describe in a few words its key nutritional features (e.g. “100% fruit”; “high fibre”; “high energy”). The claim was combined with information values for energy (kcal), total fat (g), sugars (g), and sodium (g) per portion, with a related green or red icon underling value adequacy.



Figure 54 - Examples of nutrition information provided by the two information systems tested, “stars” and “claims”. A) “stars” rating scale; B) example (almonds) of a “stars” information card; C) “claims” rating; D) example (almonds) of a “claims” information card. Translation: A) Nutritional value of product: *** Excellent, ** Good, * Sufficient, ⁰ Inadequate. B) Press B button to confirm or select another product. C) Nutritional value: V, adequate nutritional value; X, inadequate nutritional value. D) Claim: 100% fruit; Per portion: Energy, Fat, Sugars, Sodium. Press B button to confirm or select another product.

3.5.2.2.2. Nutrition information layout and arrangement in the VMs

Nutrition information was displayed in two different sites in the intervention VMs: the lateral display and the banner above products. The graphic LCD 240x128 pixel display was placed at the VM’s side in the users’ visual line and the displayed contents were adapted for the study. During the VM standby mode, a descriptive legend of the “stars” or the “claims” information was presented (Figure 54A and 54C) to help users understand the nutritional concepts to which they were exposed. During the VM usage, the complete “stars” or “claims” information card (Figure 54B

and 54D) was displayed, after the selection of an item and before its purchase confirmation or rejection. Moreover, the number of stars or the nutritional claim were continuously posted in rotation with the price in the electronic banner inside the VM, above each item.

3.5.2.3. Data Collection and Analysis

The primary outcome of the study referred to sales data. The frequency of purchase for each item was recorded using standardised procedures to avoid record bias. The number of sold items was collected continuously throughout the 24-week period in each of the two phases. Sale data were registered by vending employees during routine service visits and VM restock, by means of a manual daily inventory count through a handheld computerized device. Number of purchased items was entered into the vendor database and then exported into a spreadsheet file. For each time phase and intervention condition, total sale data were gathered summing purchased items, and daily sale data for each item were grouped into the 3 food groups (snacks, ready-to-eat meals, and drinks). For each food group, daily sales were pooled across days to create a total for each of the 24 weeks. Weekly sales data were pooled across weeks to generate a grand total for the whole 24-week period. Variables were expressed as total sales and as mean \pm SEM of 24 independent measurements, corresponding to the 24 weeks analysed for both interventions. For intervention 1, paired-sample Student's t-tests between baseline (2014) and intervention (2015) were performed to compare i) total weekly mean sales, ii) total weekly mean sales by nutritional category, and iii) weekly mean sales by nutritional category for each food group. For intervention 2, one-way analyses of variance with Tukey's *post hoc* tests were performed to determine differences among the three experimental groups (control, stars, and claims) in terms of i) total weekly mean sales, ii) total weekly mean sales by nutritional category, and iii) weekly mean sales by nutritional category for each food group. All data analyses were conducted using the Statistical Package for Social Sciences software (IBM SPSS® Statistics, Version 22.0. IBM Corp., Chicago, IL). Significance was accepted for $p < 0.05$.

3.5.3. Results

3.5.3.1. Effect of increasing the availability of healthy food options on VM sales

The replacement of unhealthy food products by healthier items in 2015 did not modify the VM weekly sales with respect to 2014 baseline sales (Table 10). Mean product sales reached about 10100 units/year and were led mainly by snack items, which represented the 73.0% of the total VM sales. Drinks were about the 21.4% of the total sales, while ready-to-eat meals accounted just for the remaining 5.6%.

Table 10 - Total sale, mean weekly sale, and percentage of items sold by nutritional category before and after increasing the availability of healthy options.

Intervention 1	Baseline "2014"	Intervention "2015"
Total sale (24 weeks)	10199	10005
Weekly mean \pm SEM	425.0 \pm 21.8	416.9 \pm 20.7
% "healthy +" items	0%	7%
% "healthy -" items	3%	29%
% "unhealthy -" items	52%	37%
% "unhealthy +" items	45%	28%

Sale distribution by healthy category was analysed to check whether increasing healthy food availability was associated with an increased purchase of healthier products (Figure 55A). The change in the point-of-sale settings towards healthier foodstuffs improved the nutritional quality of purchases, leading to increases in sales of healthy foods while decreasing those of unhealthy items (Figure 55A). Product substitution allowed the purchase of "healthy +" foods, increased 8-fold the purchases of "healthy -" products, and reduced the sales of "unhealthy -" and "unhealthy +" foods by 29.7 and 39.3% with respect to the baseline conditions. The impact of increasing the availability of healthier food products on the sales of snack items followed the same pattern (Figure 55B), and

this was expected considering the large snack contribution to the total product sale. A similar trend was observed for ready-to-eat meals and drink items (Figure 55C and 55D, respectively), except for the sales of “unhealthy +” ready-to-eat meals, where an increase in sales was observed with respect to the baseline setting.

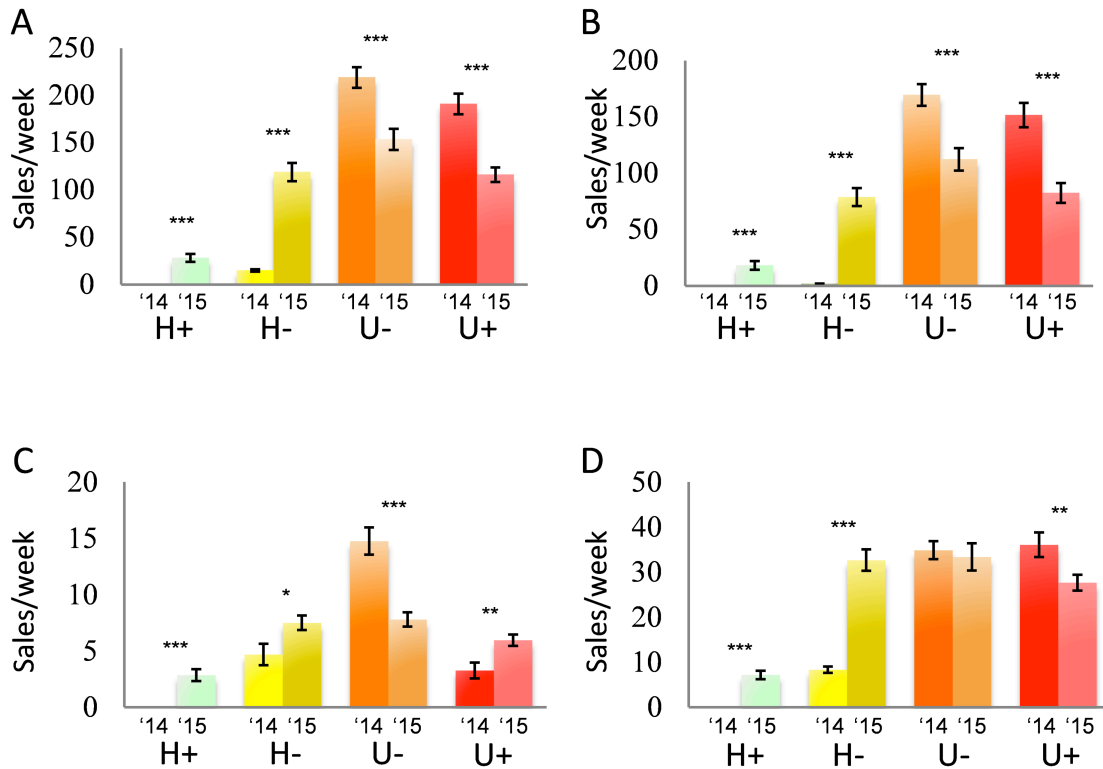


Figure 55 - Weekly sales by nutritional category for each intervention group before ('14) and after ('15) increasing the availability of healthy options for: A) total items, B) snacks, C) ready-to-eat meals, and D) drinks. Mean ($n=24$) \pm SEM. Nutritional categories: H+, “healthy +”; H-, “healthy -”; U-, “unhealthy -”; and U+, “unhealthy +”. Significant at $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) vs. baseline.

3.5.3.2. Effect of nutrition information on VM sales

Once the effectiveness of product replacement on increasing healthy food purchasing was asserted, the adoption of healthier food purchase habits through nutrition information was evaluated. VM weekly sales did not change among the three different experimental groups, highlighting the lack of effect of introducing nutrition information on VM total sales (Table 11). Mean sales distribution by food groups for the three experimental groups was 70.7% snack items, 4.1% ready-to-eat meals, and 25.2% drink items.

Table 11 - Total sales, mean weekly sales, and percentage of items sold by nutritional category in each intervention group with or without nutritional information.

Intervention 2	Control "2015"	Intervention "stars"	Intervention "claims"
Total sale (24 weeks)	10005	9332	10294
Weekly mean \pm SEM	416.9 \pm 20.7	388.8 \pm 19.8	428.9 \pm 38.6
% "healthy +" items	7%	7%	7%
% "healthy -" items	29%	33%	30%
% "unhealthy -" items	37%	41%	41%
% "unhealthy +" items	28%	19%	22%

The effect of nutrition information on sales by healthy category was negligible for three categories, namely "healthy +", "healthy -", and "unhealthy -", while it was statistically significant in the case of the "unhealthy +" products (Figure 56A). In particular, the purchase of "unhealthy +" items was drastically reduced when nutrition information was provided using stars labels (about 37.6% in comparison with control group). A moderate but not statistically significant decrease (by 19.5%) in sales of "unhealthy +" items was observed when using nutritional claims (Figure 56A). In terms of food groups, the positive contribution of nutrition information on total sales by healthy category was also registered for snack items, where decreases in "unhealthy +" product purchases were observed (Figure 56B). This reduction in the number of sales of "unhealthy +" snacks was linked to a shift towards a healthier profile, as the sales percentages of "unhealthy +" snack items changed from 28.3% in the control group to 19.5% and 22.6% in the stars and claims groups, respectively (Figure 57A).

Nutrition information did not modify the number of "healthy +" ready-to-eat meals (Figure 56C), while it decreased significantly the sales of ready-to-eat meals belonging to the other three nutritional categories, regardless of the kind of information provided. However, despite weekly sales of ready-to-eat meals fell in the "stars" and "claims" groups, the contribution of healthy products (as the sum of "healthy +" and "healthy -") augmented in percentage, from 43.0% for the control

group to 49.4% and 48.6% for the stars and claims groups, respectively (Figure 57B). In the case of drink items (Figure 56D), the effect of nutrition information was specific for certain nutritional categories: while information provided by stars increased the purchases of “healthy -” and “unhealthy -” foodstuffs without modulating purchases in the other two categories, the claims approach just reduced the sales of “unhealthy +” drinks with respect to the control group. When considering drink sales percentages (Figure 57C), it should be noted that nutrition information did not modify the purchase of healthier foods, as the sum of “healthy +” and “healthy -” categories accounted for about 39.6% of the drink sales for all the experimental groups. However, it favoured a purchasing shift from “unhealthy +” to “unhealthy -” drinks (Figure 57C).

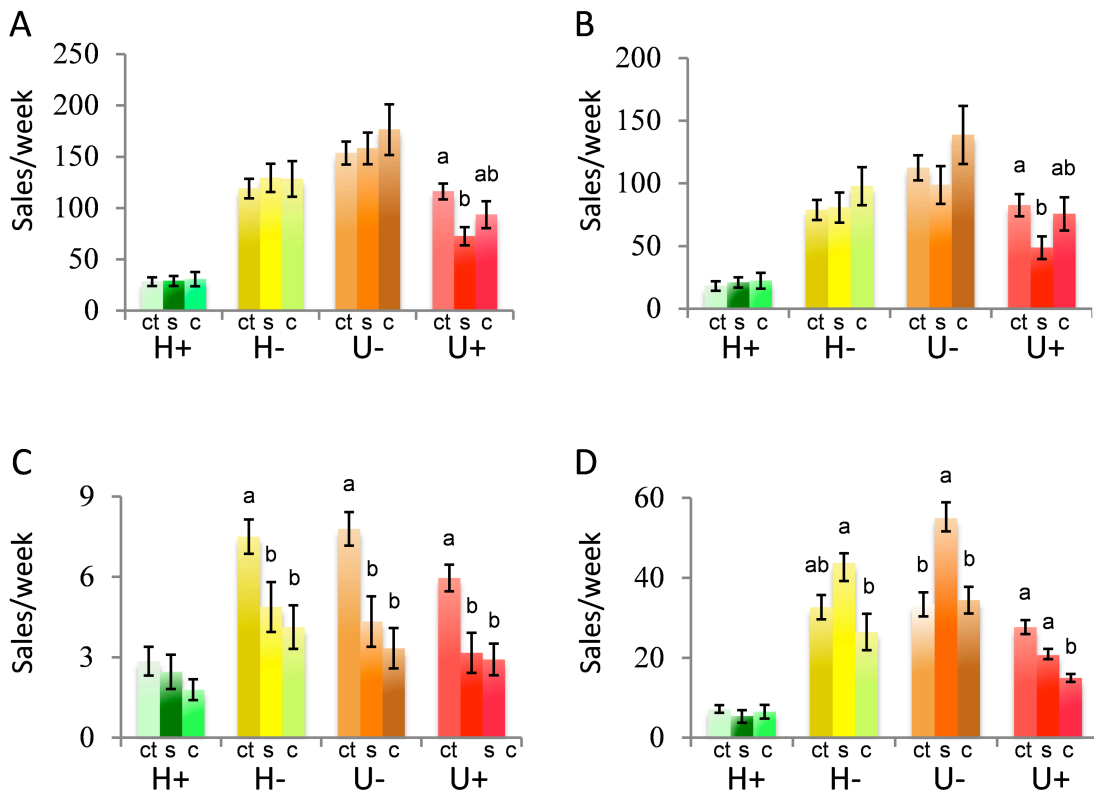


Figure 56 - Weekly sales by nutritional category for each intervention group without (ct, control) or with nutritional information (s, “stars”, and c, “claims”) for: A) total items, B) snacks, C) ready-to-eat meals, and D) drinks. Mean (n=24) ± SEM. Nutritional categories: H+, “healthy +”; H-, “healthy -”; U-, “unhealthy -”; and U+, “unhealthy +”. Different letters in the same grouped bars indicate statistically significant differences (p<0.05) among intervention groups.

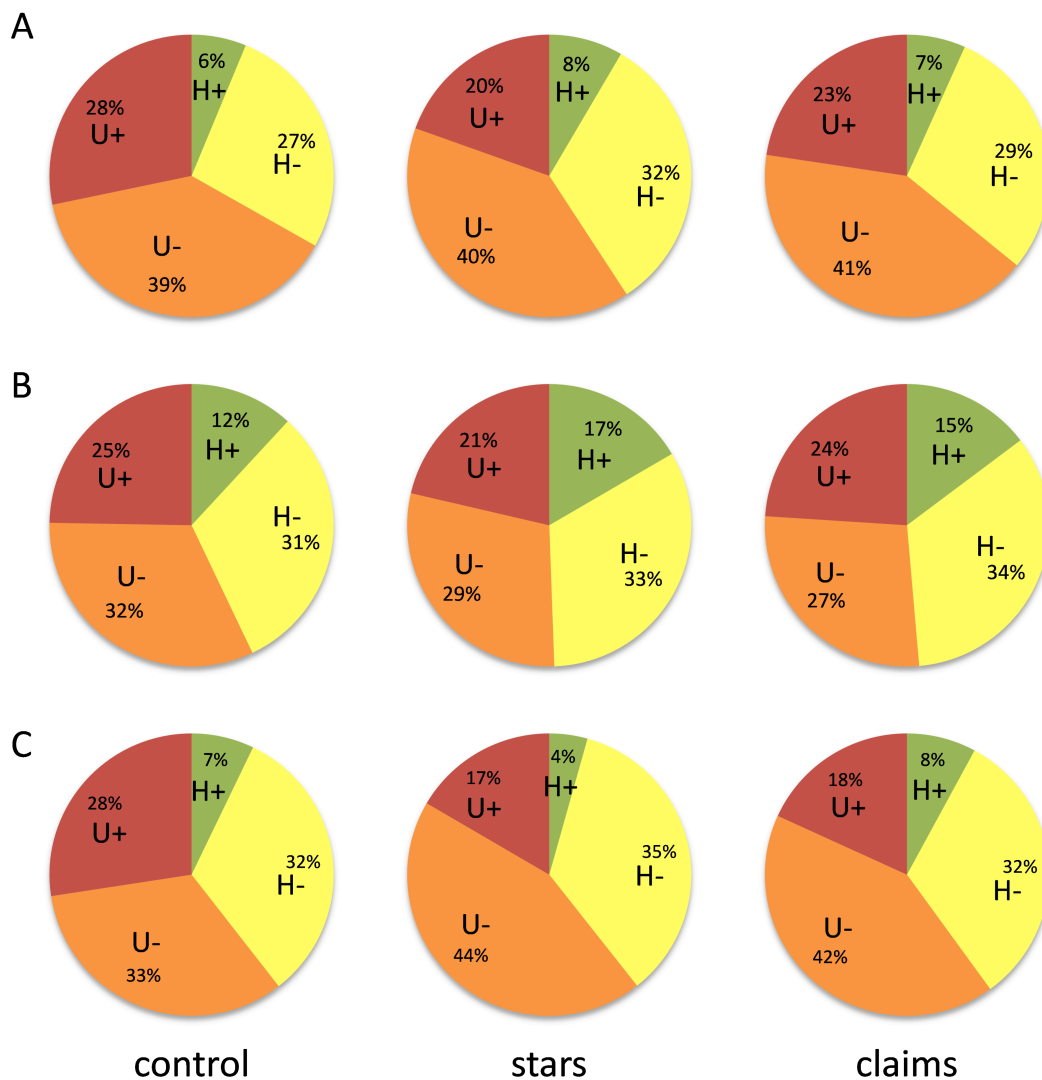


Figure 57 - Sales percentages by nutritional category for each intervention group without (“control”) or with nutritional information (“stars” and “claims”) for: A) snacks, B) ready-to-eat meals, and C) drinks. Nutritional categories: H+, “healthy +”; H-, “healthy -”; U-, “unhealthy -”; and U+, “unhealthy +”.

3.5.4. Discussion

The present research attempted to increase purchases of healthy foods in VMs and indirectly improve consumers food choices and intakes. VM users increasingly purchase foods throughout the day and, at the same time, they are becoming more careful about their diets, searching for diverse and high quality products (Vicini *et al.*, 2013). To satisfy consumers’ needs, nutritious food options should be proposed in VMs. In this study, products offered at baseline in the

university VMs were found to be of low nutritional quality, with only a small amount of the total baseline sales evaluated as moderately healthy (3% of “healthy -” items in 2014, Table 10). This nutritional analysis was in line with the few previous studies that quantitatively evaluated the nutritional quality of food products usually available in university VMs (Byrd-Bredbenner *et al.*, 2012; Raposo *et al.*, 2016). According to these studies, most snacks and beverages sold in VMs were of poor quality, being high in total calories, fat, sugars, and sodium, while low in fibre (Byrd-Bredbenner *et al.*, 2012; Raposo *et al.*, 2016).

The poor variety and nutritional quality of the available products in VMs favours the purchase of unhealthy snacks, and VM users are prone to unfavourable daily eating behaviours with respect to non-users (Park & Papadaki, 2016). Therefore, given the potential association among this “obesogenic” environment, unhealthy dietary habits, and body weight gain (Matthews *et al.*, 2015), a revolution in the VM sector towards healthier scenarios is desirable. Attending to the heterogeneity of point-of-purchase research studies on VMs and their still not clear efficacy to raise healthy choices (Grech & Allman-Farinelli, 2015), good quality interventions are required to fill the gap in the actual effectiveness of nutrition environmental interventions encouraging healthy choices (Liberato *et al.*, 2014). At this point, our study tried to reduce this lack of knowledge by implementing two different strategies focused on: i) the enhancement of healthy product availability, improving the nutritional quality of VM stock, and ii) the promotion of healthier food options through nutrition communication. To reach these goals, the study was performed in experimental sites, not within the food court of the University of Parma, and with no other VM areas, as nutrition environmental interventions appear to be more successful in enhancing food choices in places with no or only few other points of food purchase (Seymour *et al.*, 2004). The primary outcome was the impact of different experimental conditions on VM sales, a helpful indicator able to reveal the effectiveness of this study in improving consumers’ behaviours.

The total number of purchased products was not affected by the replacement of unhealthy by healthier items during 24 weeks of intervention (Table 10). However, product replacement was successful in increasing “healthy +” and

“healthy -” product sales while decreasing unhealthy options (Figure 55). These results, suggesting a switch of consumers’ purchases toward healthier choices, were in agreement with other works where an enhanced availability of healthy food options in VMs did not impact sales volume, but led to higher sales of favourable products (Fiske & Cullen, 2004; Grech & Allman-Farinelli, 2015; Kocken *et al.*, 2012). Similarly, the sales of less desirable foods may increase when the availability of these products is higher, as it occurred for the ready-to-eat meals falling into the “unhealthy +” category (Figure 55C). These facts pointed out the need for a reduction in the supply of unhealthy products while enhancing the availability of healthy ones to address VM user choices toward a more healthful pattern.

As consumers are increasingly becoming more tech-skilled, expecting VMs to supply food as well as services (Vicini *et al.*, 2013), we exploited VM functionalities to promote nutritious products through LCD screens. To our knowledge, this is the first study assessing how nutrition communication can impact sales in a university scenario using VMs able to communicate nutrition information rather than printed labels and/or posters.

Once healthy food options were added into VMs, offering point-of-purchase nutritional information did not affect volume sales during the educational intervention (Table 11). This result was in accordance with previous researches, in which total sales were not associated with promotion conditions (Bergen & Yeh, 2006; Fiske & Cullen, 2004; French *et al.*, 2001). Studies promoting healthier options by providing nutrition information at VMs also revealed significant increases in purchases of healthy food products, ranging from 1 to 5% (Brown *et al.*, 2014; Dingman *et al.*, 2015; Larson-Brown, 1978). Conversely, we did not measure important changes in the mean sales by nutritional category of both total products and snacks after adding nutrition information in VMs (Figure 56A and 56B). Only the sales of products considered to be the worst choice decreased when “stars” labels were used (“unhealthy +” products, Figure 56A and 56B). These results suggested that iconographic labels in VMs could change only partially consumer choices, reducing the purchase of less recommendable products. Similar results were achieved by Brown and colleagues (2014), who reported a decrease in unhealthy

item sales when colour labels were used to inform university students. On the other hand, the nutritional claim information system produced a significant reduction in the purchase of “unhealthy +” drinks (Figure 56D). A previous study on the impact of point-of-purchase information to increase university student awareness through claim labels and nutrient information also revealed small but significant effects only when additional educational and motivational materials (as posters) were employed (Bergen & Yeh, 2006). Regardless of nutrition information typology, we found a significant reduction of purchase of the three less favourable categories (“healthy -”, “unhealthy -”, and “unhealthy +”) of ready-to-eat items (Figure 56C). According to these results, the efficacy of point-of purchase information may be limited, as, due to the heterogeneity and limited number of researches using this intervention approach, it is difficult to draw clear conclusions on the effect of nutrition information on improving VM users choices (Grech & Allman-Farinelli, 2015).

Studies conducted in the VM framework to date are insufficient to confirm a strong evidence of the impact of both increased availability of healthy food products and nutrition education through communication at the point-of-sale (Liberato *et al.*, 2014). The present study shed light on the effectiveness of these interventional strategies. Nevertheless, other approaches can be put in effect in order to stimulate healthy food purchasing, and, in this sense, price reductions of healthier food options have been described as effective strategies to increase their sale (Grech & Allman-Farinelli, 2015). When prices of low-fat snacks were reduced between 10% and 50% in VMs, purchases changed significantly toward the discounted items, with sale growth ranging from 9 to 93% (French *et al.*, 2001). However, the effectiveness of price reduction approaches may be compromised by declines in revenues (French *et al.*, 1997), and with loss of profits representing a key concern for vending contractors, we chose not to implement a price-discount strategy. This point should be considered as a strength of the study design, since it accounts for the feasibility of this kind of environmental interventions in real-life VM scenarios (Kocken *et al.*, 2012). Moreover, price should not be considered as a confounding factor as comparisons between (intervention 1) and among (intervention 2) groups were done for mean sales within the same nutritional

category, where the price was kept constant for each intervention group. Another point worth mentioning is that longer periods of study, with a bigger number of VMs and university sites, could have obviously strengthened the present findings (Dingman *et al.*, 2015). However, the fact that VM positions were geographically dislocated within the city, to avoid mutual influences among experimental conditions, reduced the chance of using a larger set of dispensers. Future environmental studies should address the implementation of new, multi-approach strategies to promote healthier choices in VM use and establish the contribution of sold foods to total diet quality and eating behaviour.

3.5.5. Conclusions

The findings of the present study indicated that enhancing the availability of better nutritional quality items in VMs increases the purchase of healthy foods. Differently, addition of point-of-sales nutrition information only discouraged choices of less favourable foods, without significantly impacting on consumer behaviour. These interventions did not limit the freedom of choice of consumers and did not force them to choose healthy products, as consumer decisions remained free, even though oriented towards healthy options when the nutritional quality of the product assortment was increased and nutrition communication was introduced. Overall, this study yielded useful insights for the implementation of nutrition interventions through VMs, addressing food choices and promoting healthy dietary patterns in everyday life. Since a strong relationship between diet quality and population health is now a solid paradigm, this kind of environmental interventions could lead to advantages in body weight management and health outcomes, entailing eating behaviour improvement. In keeping with these considerations, further research is needed to understand whether point-of-purchase nutritional interventions in VMs can really play an active role in improving consumer health.

Chapter 4 - General Remarks

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In the present Doctoral Thesis, two different interventional approaches were carried out to tackle issues about food choices and eating behaviour. The first investigation, a randomised controlled trial, was oriented to better understand the physiological, cognitive, and emotional mechanisms driving food intake (Chapters 3.1., 3.2., 3.3., and 3.4.). Its results were used to define and implement a second nutritional intervention addressing individual practical needs, preferences, and habits in a real-life setting (Chapter 3.5.).

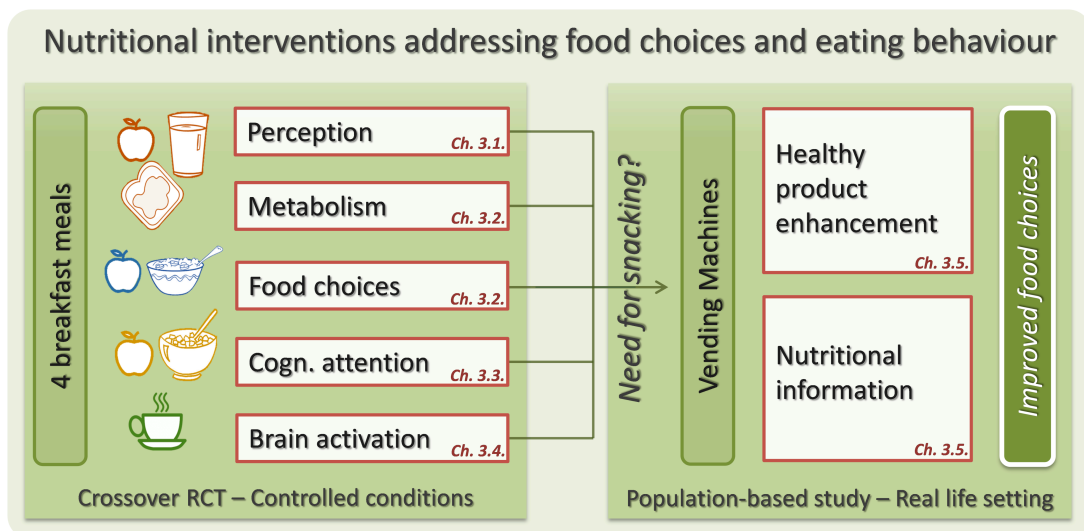


Figure 58 - Doctoral Thesis outline.

During the first part of the present Doctoral research, the impact of breakfast on several factors linked to food intake was assessed through a series of nutritional, behavioural, and neurological studies. In particular, breakfast was chosen because epidemiological evidence has shown favourable effects of regular breakfast consumption, based on its nutrient composition and on the overall quality of the diet, in entailing health and nutritional advantages (Marangoni *et al.*, 2009; O'Neil *et al.*, 2014; Rosato *et al.*, 2016).

Since food perception and hedonic value of food seem to affect food choices (Buckland *et al.*, 2015; Farr *et al.*; McCrickerd & Forde, 2016), a preliminary study

was performed to investigate the effect of minor changes in the composition of breakfast meals on the perceived attributes of foods, both at breakfast and at lunchtime (Chapter 3.1.). The efficacy of the food picture viewing approach to categorise foods on the basis of some cognitive and hedonic attributes (healthy, palatable, satiating, energizing, and caloric) was evaluated. This assessment was used to select the visual stimuli to be used in the remaining studies (Chapters 3.2., 3.3., and 3.4.). Moreover, the same perceptual attributes were used to characterise four different breakfast meals. While the energy/caloric content of the breakfast was rated on the basis of single food items, breakfast healthiness was judged on the basis of the whole meal. This accounted for how the association of different ingredients can modify the perceived health value of foods.

After breakfast definition and stimuli selection, a randomised, crossover, controlled trial was carried out, with four experimental conditions consisting in the four different breakfast meals. The appetite/satiety perception and the metabolic mechanisms underneath breakfast consumption (glucose, insulin, leptin, ghrelin, GLP-1, and NEFA), as well as food choices at subsequent meal and food intakes during the whole day, were assessed (Chapter 3.2.). In addition, the impact of the four breakfasts, differing in nutritional and perceptual characteristics, on brain processes implicated in food choice was explored at lunchtime, through behavioural-neurocognitive tests (Chapter 3.3.) and fMRI (Chapter 3.4.).

Overall findings from Chapter 3.2. indicated that the consumption of breakfast positively affected postprandial satiety, if compared with a non-caloric breakfast. However, no differences were observed among the breakfasts characterised by slightly different nutritional and perceived characteristics. On the other hand, the type and nutritional composition of breakfast modified the metabolic and endocrine responses, with a time-related effect. Actually, glycaemia and hormonal levels returned to their homeostatic values 2-3 hours after breakfast consumption. Thus, no differences were observed at lunchtime for both the satiety-related perception and the satiety-related metabolic parameters in comparison with the control breakfast, which was designed to mimic a fasting scenario. This fasting-like metabolic condition occurred 4 hours after breakfast, which likely

influenced food choices and food intake and may account for the perceptual and neurocognitive responses at lunchtime.

To study the cerebral factors involved in food intake control, real life experiences were simplified into behavioural-neurocognitive tests by using the presentation of food images (Chapter 3.3.). The similar fasting-like conditions observed for all the experimental groups at lunchtime may have contributed to nullify the benefits of consuming breakfast on sustained and selective attention to food stimuli. An attention bias for highly energetic foods regardless of breakfast type may be associated with this fasting-like physiological condition. Moreover, the metabolic status may have affected the perceptual evaluation of lunch-related food images at lunchtime, which was the same independently of the energy and nutrient contents of the different experimental breakfasts (Chapter 3.3.). In addition, positive and negative affects, evaluated to define the individual mood/emotion at lunchtime, were not distressed by having breakfasts differing slightly for nutritional composition and perceived characteristics (Chapter 3.4.).

Since behavioural-neurocognitive tests alone are useful to explore cognitive performance but not to describe the potential neural correlates associated with food choices, these tests were combined with fMRI scans, one of the best tools currently available for the detection of the brain reactivity in response to food stimuli (Farr *et al.*). Although no significant differences were detected, well-defined trends in the activation of specific ROIs were observed after having different breakfast meals (Chapter 3.4.). These findings suggested that breakfast may alter the saliency of food stimuli at lunchtime. In particular, brain areas linked to the cognitive and the affective control of food behaviour were activated, suggesting that the energetic/caloric perceived content of food may play a key role in the saliency of food stimuli and in the consequent food choices and intakes.

Despite changes in metabolic condition and brain activation could be related to real life decisions in eating behaviour (Farr *et al.*), no differences were registered for the food choices and nutrient intake during an *ad libitum* lunch (Chapter 3.2.). This result was imputable to the compensatory mechanisms of the homeostatic

system, being the metabolic status at lunchtime the same independently of the breakfast type. In this sense, the expected influence of the hedonic-cognitive system, based on the different trend in brain activation related to food stimuli saliency at lunchtime, was not confirmed in real-life. It should be noted that the *ad libitum* lunch, generally associated with excessive food intake (Livingstone *et al.*, 2000), could have overridden the brain circuitry that controls eating behaviour. This complex interaction among physiological, cerebral, and behavioural factors acting to define food intake could be partially confirmed when dietary habits throughout the week were observed. Although breakfast skipping could be compensated later in the day by increasing the consumption of foods, breakfast consumption seemed to help better matching the nutrient intake recommendations.

These findings supported the hypothesis that healthy subjects should be encouraged to keep eating breakfast, but the impact of breakfasts differing in their nutritional and perceptual characteristics should be further explored in a shorter time period like, for instance, at the morning snacking occasion. This could avoid reaching fasting-like status, while still retaining an effect on the aforementioned factors involved in food intake regulation.

Snacking is generally defined as any consumption of food or drink between main meals. Nonetheless, snacking is also linked to specific periods of time, amounts of foods, and nutritional profile of foods (Hess *et al.*, 2016). Snacking behaviour is often characterised by an unhealthy pattern, ascribed to energy unbalance and body weight gain. In this regard, vending machines have been pointed out as an obesogenic factor contributing to increase the availability of unhealthy, nutrient-poor, and energy-dense food products throughout the day (Chapelot, 2011). However, snacking by choosing healthy products may be considered as an optimal way to avoid fasting conditions. To investigate deeper the impact of perceptual characteristics and nutritional awareness on food choices during snacking occasions, an environmental and educational nudging intervention was performed (Chapter 3.5.).

To overcome the intrinsic limits of experimental settings, the study was conducted in an every-day life environment, without limiting or conditioning the individual freedom of choice. Consumer food choices were nudged through the enhancement of healthy product availability and through the promotion of healthier food options by nutrition communication. Improving the nutritional quality of products caused an increase in the purchases of healthy foods. Product replacement was successful in increasing healthy product sales while decreasing unhealthy options, suggesting a switch of consumers' purchases toward healthier choices. Differently, when healthiness and energetic/caloric content of foods were explained by adding nutrition information at the point-of-decision, these factors scarcely affected food choices. The communication of product attributes only discouraged choices of less favourable foods, without impacting notably on consumer choices. Overall, this study yielded useful insights for the implementation of nutrition interventions aimed to promote healthy food choices. In general, this kind of environmental interventions could lead to advantages in body weight management and health outcomes, entailing eating behaviour improvement.

In conclusion, the findings obtained within this Doctoral Thesis contributed to shed light on the complex interaction of several drivers of food choices and eating behaviour. Breakfast could be included among the factors contributing to food intake, positively affecting appetite control in healthy adults. Nutritionally balanced breakfast meals may influence the homeostatic system signals and could efficiently modulate the postprandial satiety cascade. The positive influence of breakfast on body homeostasis did not persist 4 hours after consumption. Thus, the impact of breakfast on behavioural, sensory, attention, cognitive, emotional, and hedonic factors was not confirmed at lunchtime. Further investigations are needed at a closer time after breakfast consumption to better understand the interconnection between homeostatic and cerebral systems and their modulation by the presence of single breakfast product. A strategic approach in appetite control may be snacking between meals, to prolong the satiety sensation until the subsequent traditional meal and to avoid fasting-like metabolic conditions that could result in overeating. In such a way, the sensory, saliency, and reward-related

attributes of food that can stimulate food intake might be reduced in favour of physiological and cognitive factors. Indeed, adoption of healthy eating behaviour could be facilitated by nudging food choices through cognitive aspects and educational approaches.

Chapter 5 - Conclusions



Chapter 5 – Conclusions

1. Breakfast healthiness is perceived considering breakfast meal as a whole, while its energy/caloric content is evaluated on the basis of single food components. In this sense, the association of different ingredients/foods could modify the perception of the health value of breakfast meals.
2. Heuristic judgment of food stimuli at lunchtime does not change following consumption of breakfasts differing for their perceptual and compositional characteristics.
3. The consumption of breakfast positively affects postprandial satiety-related perceptions and physiological factors when compared with a non-caloric breakfast, while minor differences in nutritional or perceived characteristics of breakfast meals do not have a major impact on postprandial satiety response.
4. Consumption of a non-caloric breakfast does not entail nutritional advantages, since it leads to a compensatory mechanism in total daily energy intake and hinders the achievement of nutrient intake recommendations.
5. Breakfasts differing for their perceptual and compositional characteristics do not affect sustained and selective attention, or neuronal activation at lunchtime, although they may account for specific reactivity of some brain regions in response to food stimuli.
6. Mood status at lunchtime, considering both positive and negative affects, is not influenced by breakfast, or by the perceptual or compositional characteristics of different breakfast meals.
7. Enhancing the availability of healthy foods at the point-of-choice, in real life settings, increases their purchase, thus the exposure to healthy foods nudges towards healthy food choices.
8. The addition of nutrition information highlighting the health attributes of foods at the point-of-choice only discourages choices of less favourable foods, without significantly affecting the purchase of products having a healthier nutritional

Chapter 6 - References



Chapter 6 – References

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