Understanding flavour nutrient learning: the impact of extinction and expectation

by

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Declaration

I hereby declare that this thesis has not been, and will not be, submitted in whole or in part to another University for the award of any other degree.

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DPHIL EXPERIMENTAL PSYCHOLOGY

UNDERSTANDING FLAVOUR NUTRIENT LEARNING: THE IMPACT OF EXTINCTION AND EXPECTATION

SUMMARY

Humans and other animals learn to associate flavours with aspects of consuming foods, which can result in acquired liking or aversion for that flavour. Two main processes of learning have been proposed to be critical: flavour-flavour (FFL) and flavour-nutrient (FNL) learning. This thesis addresses two main research questions primarily in the context of FNL; firstly, does liking for a flavour acquired through FNL persist once energy has been removed? It has been suggested that acquired flavour liking is resistant to extinction, but there are conflicting results within the human literature, which has concentrated on FFL. Studies One and Two explored this but failed to demonstrate acquired liking, although they tentatively suggested that extinction did not occur for acquired liking as pleasantness ratings remained stable after removal of energy. The second research question investigated whether liking acquired through FNL was modulated by expectations. Study Three manipulated viscosity of a yoghurt drink to determine if this impacted upon FNL, as thicker products have been shown to signal higher energy content. Expectations were influenced by viscosity, but with little impact upon pleasantness ratings and little evidence that FNL was enhanced. Studies Four and Five used labelling to influence expectations regarding a yoghurt-based breakfast. Study Four found that when no information was provided, liking changed as predicted from FNL. Contrary to prediction, when congruent information about energy content was provided, this acquired liking was not demonstrated, and ratings remained stable across sessions. Study Five did not replicate this finding, with pleasantness ratings in line with FNL literature. Addition of a hedonic label actually resulted in decreased pleasantness of the breakfast over time, suggesting a contrast effect with the flavour not delivering what was expected. Methodological limitations are recognised, with measurement of liking and contingency awareness discussed as potential explanations for weaker findings.

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Abbreviations

BMI – Body Mass Index

CS – Conditioned Stimulus

CTA – Conditioned Taste Aversion

FCL – Flavour Consequence Learning

FFL – Flavour Flavour Learning

FNL – Flavour Nutrient Learning

HE – High Energy

LE – Low Energy

TFEQ – Three Factor Eating Questionnaire

US – Unconditioned Stimulus

Chapter 1: General Introduction

Overconsumption of palatable foods has been widely implicated in the current obesity epidemic, and investigating the mechanisms involved in the development, and persistence, of liking for flavours may help in changing food choice behaviour. This thesis will explore the learning processes involved when cueing a certain benefit in a food product, and what happens when this benefit is removed from the food.

There is strong evidence that we show innate preference for sweet and dislike for sour and bitter flavours. Many studies have looked at newborn infants (human and non-human) and their responses to these flavours, and orofacial responses are consistently positive for sweet and negative for bitter (e.g. Berridge, 2000). However, these preferences are developed and changed through experience and exposure to a food, and the processes thought to account for these changes will be discussed in detail below. Current theories suggest that consumption of high energy foods result in an increased liking for the flavour of the food, promoting subsequent consumption. Alongside this, liking for flavours can be induced through the addition of sweet tastes, again promoting future consumption. Once a flavour becomes liked, it is likely to become over-consumed, therefore encouraging a positive energy balance, and in turn, contributing to the obesity epidemic (Yeomans, 2008).

1.1 Relevant learning principles

Before focusing on the two main contemporary theories of flavour learning, flavour-nutrient (FNL) and flavour-flavour (FFL) learning, some relevant principles of learning need to be described to put the thesis into the broader context of liking acquisition. A full evaluation of all of these ideas is beyond the scope of this thesis, and these ideas are explored more in several recent reviews (De Houwer, Baeyens, & Field, 2005; Delamater, 2012b; Pliner & Salvy, 2006).

1.1.1 Mere exposure and neophobia

Zajonc (1968) stated that preferences increase for any stimuli after repeated exposure regardless of reinforcement, a phenomena that he defined as mere exposure. Although this concept is a widely cited theory in appetite research there is surprisingly limited research exploring the role of mere exposure in the development of food preferences in adult humans. Pliner (1982) exposed participants to novel fruit juices on two sessions, where they were either exposed to the juice five, ten, or twenty times, with a no exposure control group. After exposure, liking was measured, and the more frequently the juice had been tasted, the more it was liked, although these effects were stronger in session one than they were in session two. Stevenson and Yeomans (1995) showed an increase in pleasantness of chilli burn after mere exposure, and that this was not influenced by familiarity of the intensity of the burn.

A larger body of research has demonstrated the effects of mere exposure in young children or infants (e.g., Anzman-Frasca, Savage, Marini, Fisher, & Birch, 2012; Birch, Gunder, Grimm-Thomas, & Laing, 1998; Birch & Marlin, 1982; Lakkakula, Geaghan, Zanovec, Pierce, & Tuuri, 2010; Sullivan & Birch, 1990; Wardle, Herrera, Cooke, & Gibson, 2003). In addition, mere exposure was found to be the most efficient method (compared to flavour-nutrient and flavour-flavour learning) in increasing children's acceptance of a novel vegetable puree, with effects being prolonged six months after the initial ten exposures (Hausner, Olsen, & Møller, 2012). However, as discussed in the Yeomans (2006b) review paper, mere exposure can account for familiarity but does not provide a mechanism for the changes that occur. It has also been argued that mere exposure is a form of conditioning, with the fact that there are no negative consequences acting as an unconditioned stimulus (Zajonc, 2001).

Along similar lines, neophobia and 'learned safety' refer to the behaviour often demonstrated by both non-human and humans, whereby new foods are only consumed in small amounts, and only after a time delay are they consumed in larger quantities. It is thought that this delay occurs to allow negative post-ingestive consequences to be assessed. If there are no negative consequences (which would result in conditioned taste aversion, CTA, discussed below) neophobia for that flavour becomes reduced and the flavour becomes preferred (learned safety). Neophobia itself is a widely accepted concept, but learned safety is less useful as an explanation for the development of food preferences as it does not account for why liking is not acquired for items that are neither harmful or beneficial (Yeomans, 2006b).

1.1.2 Pavlovian conditioning, latent inhibition and extinction

Pavlovian conditioning principles underlie the main theories of flavour preference learning, and therefore it is important to understand some of the critical concepts before talking in detail about these theories. As discussed in a paper by Rescorla (1988) contemporary accounts of Pavlovian conditioning still regard the associations between stimuli as basic, but recognise that these associations are not merely formed through a pairing of two stimuli (CS and US), but through representations of multiple events, and these can, in turn, be related to additional associations.

Exposure to the conditioned stimulus (CS) before it becomes paired with the unconditioned stimulus (US) is thought to have a detrimental effect on subsequent learning about that stimulus. This robust phenomena is referred to as latent inhibition, first defined by Lubow and Moore (1959), and is demonstrated across a variety of species and tasks (Lubow, 1973) It is important to consider latent inhibition in the process of flavour learning as, with many previous experiences of flavours, and other sensory aspects of food stimuli such as texture, it can be reasoned that this could slow down any subsequent learning in the laboratory setting. This highlights the importance of making the CS as novel as possible in order to maximise the learning that could occur. This will be further discussed in relation to flavour consequence learning in the following section.

A classic, frequently cited associative learning model is the Rescorla-Wagner model (Rescorla & Wagner, 1972), which conceptualises learning (from a Pavlovian conditioning perspective) as changes in associative strength between the conditioned stimulus and the unconditioned stimulus. A main component of this model is concerned with prediction error, whereby an expectation that has been generated by a US, due to associations with a particular CS, is not met. Initially, a CS will not generate much of an expectation regarding a US, but once the two have been experienced together this creates a positive prediction error as it was a surprising outcome. As the association strengthens, the US becomes completely anticipated which results in no prediction error, and subsequent learning will not occur (Delamater, 2012a). This model is particularly important when investigating extinction, which is a major focus of this thesis (covered in detail in Chapter 3). Extinction refers to the process by which a conditioned response becomes weakened, or non-existent when the CS is no longer paired with the US, so presented either in the absence of the US, or separate presentations of CS and US. According to the Rescorla-Wagner model, when extinction occurs, a negative prediction error is created, as the US, which has been strongly associated with the CS and therefore completely anticipated, does not occur. The suggestion is that as this association weakens further with subsequent trials, it may become completely redundant.

However, the way in which an association changes through extinction has been debated, in terms of whether the original associations remain intact and are masked, or whether offset by new learning. Research has shown that extinction does not result in a complete deletion, with at least part of the original associations remaining intact, with evidence from various processes such as spontaneous recovery, renewal and reinstatement (Falls, 1998; Rescorla, 2001). The key aspects of this research relating specifically to flavour preference learning will be discussed in Chapter 3. Delamater (2012a) evaluates the research and suggests that different learning processes may be affected differently by extinction, with associations weakening in some forms and not in others. In a separate paper (Delamater, 2012b), the multi-component model of Pavlovian learning is introduced, suggesting that there can be separate associations between the CS and independent sensory, hedonic, emotional, temporal and response components of the US, and therefore extinction could have differing effects on these components. This could help explain why findings vary within the literature.

1.1.3 Evaluative conditioning

Alongside the development of flavour preferences through more traditional methods of Pavlovian conditioning, evaluative conditioning is also an important concept. Evaluative conditioning occurs when a neutral stimulus (equivalent to CS) is paired with either a negative or positive stimulus (equivalent to US), and changes in the valence of the neutral stimulus are measured (Levey & Martin, 1975) Evaluative conditioning can be seen in conditions where other forms of Pavlovian conditioning are not seen, as it appears to be resistant to extinction and can be demonstrated without contingency awareness, suggesting that the underlying mechanisms may differ, or that there are procedural differences which give the appearance of differences in underlying mechanisms (as reviewed by De Houwer, et al., 2005; De Houwer, Thomas, & Baeyens, 2001). The remainder of this section will examine the two main processes of flavour learning in detail; flavour-consequence learning and flavour-flavour learning.

1.2 Flavour Consequence Learning

A large body of research has established that both humans and other animals learn to associate flavours with positive and negative post ingestive consequences, leading to the development of flavour preferences and aversions (see reviews by Gibson & Brunstrom, 2007; Sclafani, 1997; Yeomans, 2006b; 2008). This type of learning was first established as conditioned taste aversion (CTA), where co-experience of a taste and gastrointestinal illness resulted in avoidance of that taste (Garcia & Koelling, 1966). Subsequent research has established that CTA can occur after a single exposure (Bernstein & Webster, 1980; Garcia, Hankins, & Rusiniak, 1974), and also when there is a delay, even up to 24 hours, between exposure and consequence (illness) (Garcia, Ervin, & Koelling, 1966). When simultaneously exposed to shock, light and flavour cues resulting in illness, only the flavour was subsequently avoided by rats, suggesting a preparedness for taste-illness associations (Garcia & Koelling, 1966). Following the establishment of CTA as a form of learning, theorists generalised this type of learning to all situations where flavours predict post-ingestive consequences rather than just aversive events, and the generalised name of flavour consequence learning (FCL) was widely adopted (e.g. Capaldi, Campbell, Sheffer, & Bradford, 1987; Chambers, Mobini, & Yeomans, 2007; Yeomans, 2006b). FCL was proposed as one of the main underlying mechanisms of flavour preference development. In FCL, the sensory characteristics (for example, taste) of a food or drink (CS) become associated with the post-ingestive consequence of consumption (US), resulting in an enhanced preference, or avoidance, for that taste (depending upon the nature of the consequences). Therefore, FCL is conceptualised as a form of associative learning, which can be generated through ingestion of nutrients, or drugs such as caffeine.

FCL can help to understand how associations between energy content and flavour are formed, and this specific form of FCL is referred to as flavour nutrient learning (FNL). It is well established that energy density and palatability of foods are positively related (Drewnowski, 2003), with high energy dense foods often being over consumed due to their fat and sugar content, providing immediate rewards but less satiating than low energy dense foods. Therefore, increasing liking for low energy dense foods could help to reduce daily energy intake, and therefore aid weight loss. FNL may also help determine whether these associations are based upon the short term reward or long term satiating consequences.

1.2.1 Animal research

There is a wealth of animal literature investigating FNL, which has established an important basis for research with human participants. This thesis is concerned only with humans; therefore only the most relevant animal research will be drawn upon and discussed.

A common methodology adopted in FNL in animals is the administration of intragastric infusions of a nutrient solution whilst the rat consumes a cue flavour solution (CS+) (Ackroff, 2008). This combats the differing flavours of nutrients, which may lead to flavour-flavour learning (FFL) and an unconditioned response (for example, preference for sweeter tasting nutrients). Another flavour cue (CS-) is paired with intragastric infusions of water and flavour preference is then measured using a two bottle test, where after training, rats are given the choice between two bottles, one containing the CS+ and the other the CS-. Holman (1968) was one of the first to show FNL in rats, with rats displaying preference for a flavour paired with intragastric infusion of a complete liquid diet, compared to a flavour paired with an infusion of water.

Animal literature consistently demonstrates preference for a flavour paired with an intragastric infusion of nutrients compared to a flavour paired with an infusion of water (as reviewed by Sclafani, 1997). Flavour preferences can be conditioned in rats using complete liquid diets, as in the example above, or using specific reinforcers: glucose/ glucose polymers such as Polycose (Ackroff & Sclafani, 1991, 1994; Drucker, Ackroff, & Sclafani, 1993; Myers & Sclafani, 2001a, 2001b; Pérez, Fanizza, & Sclafani, 1999; Pérez, Lucas, & Sclafani, 1998) sucrose (Ackroff & Sclafani, 2007; Harris, Gorissen, Bailey, & Westbrook, 2000), alcohol (Ackroff & Sclafani, 2001, 2003), fats (Elizalde & Sclafani, 1990a; Lucas & Sclafani, 1989) and starch (Sclafani & Nissenbaum, 1988).

Nutrients differ in their reinforcing properties, and glucose seems to be the optimum nutrient in terms of flavour preference conditioning in animals (Ackroff, 2008). Glucose polymers, such as Polycose, are also strong reinforcers, with the optimal concentration at 16%. When comparing the reinforcing properties of high fat and high carbohydrate diets, rats show a preference for a flavour paired with carbohydrate infusions over a flavour paired with the fat infusions, with both being preferred over a flavour paired with infusions of water (e.g. Lucas & Sclafani, 1999a; Pérez, et al., 1999). However, it has also been suggested that, when consumed as part of a mixed diet rather than in pure nutrient form, fat is more reinforcing (Lucas, Ackroff, & Sclafani, 1998). Methodological reasons could account for those differences, as tested by Lucas and Scafani (1999b), with duration of training sessions and deprivation state influencing preference for fat or carbohydrate. If training and testing procedures were similar (short sessions and deprived), a preference for high carbohydrate was demonstrated in both mixed and pure diets. If subsequent training with the mixed diets was carried out on non-deprived subjects and in long sessions, this preference shifted to the high fat flavour. One conclusion drawn from this research is that long term preferences are not necessarily predicted by short term preferences, which influences human research as short term lab based studies are often used to draw general conclusions regarding long term preference development.

Animal research does not solely rely on intragastric infusions to condition flavour nutrient preferences, and can also demonstrate FNL independent of FFL. Mehiel and Bolles (1988) used four isocaloric solutions, differing in hedonic value (assessed by intake of each solution in a preliminary stage of the study) to condition preference for a novel flavour. A preference for the calorie-paired flavour was demonstrated in all caloric solutions, with no difference in strength of preference, indicating it was the post-ingestive consequences and not the hedonic value that conditioned this association.

An important factor that will be discussed further in Chapter 3 is the current motivational state of the animal at the time of testing. Deprivation or motivational state has been linked to learning about the post-ingestive consequences of a food through a concept known as incentive value (Benoit, Davis, & Davidson, 2010). The deprivation level of an animal in terms of food will determine the incentive that is assigned by the animal to that food, and this incentive alters in relation to the level of deprivation. A number of studies in the research group of Dickinson and Balleine demonstrate this effect (Balleine, 1992; Balleine & Dickinson, 1994; Dickinson & Balleine, 1994, 1995), where foods consumed consistently in a state of deprivation are assigned a higher incentive value by rats than foods consumed in various deprivation states, leading to increased food intake. Yiin, Ackroff and Sclafani (2005) looked at the effect of food deprivation on both preference and consumption in rats. Food deprived rats consumed more of the CS+ relative to the CS-, whereas rats that had been able to eat *ad libitum* during training consumed similar amounts of both the CS+ and CS-. However, in a two-bottle preference test, both groups displayed a stronger preference for the CS+ than the CS-, suggesting that deprivation state influences the expression but not the acquisition of flavour preferences in animals.

1.2.2 Human research

Evidence for FNL is clearer in animals than in humans, with human research complicated by prior learning and associations before exposures in a lab session, and with nutrients rarely consumed alone but as part of a mixed diet. Most studies use liking for a flavour as the outcome measure of preference.

However, the limited human studies (reviewed by Brunstrom, 2007; Yeomans, 2006b; Yeomans, 2008) show preference for flavours associated with fat (Johnson, McPhee, & Birch, 1991; Kern, McPhee, Fisher, Johnson, & Birch, 1993), protein (Gibson, Wainwright, & Booth, 1995), caffeine (Richardson, Rogers, & Elliman, 1996; Yeomans et al., 2000b; Yeomans, Spetch, & Rogers, 1998) and carbohydrates (Birch, McPhee, Steinberg, & Sullivan, 1990; Booth, Mather, & Fuller, 1982; Yeomans, Leitch, Gould, & Mobini, 2008b).

Studies using a child population provide clearer evidence for FNL than adult studies, as young children have had fewer, if any, exposures to some foods providing an opportunity to condition new associations. Children have shown acquired preferences for a flavour paired with high fat content over one paired with low fat content (Johnson, et al., 1991), which were not maintained when children were sated (Kern, et al., 1993). It has been suggested that FNL is state dependent, both in terms of motivational and appetitive state (Brunstrom, 2007). A number of studies conducted on adults by Gibson and colleagues have found that liking change for flavours differs depending upon energy requirement; with liking increases only demonstrated in a state of high energy requirement (Gibson & Wardle, 2001), or high protein requirement (Gibson, et al., 1995), or no change when in a state of high energy requirement compared to decreased liking when in a low energy requirement (Gibson & Desmond, 1999). A study by

Mobini, Chambers and Yeomans (2007) also found that a novel flavoured drink containing sucrose became more pleasant for those who consumed it in a hungry state than those who did not. Similarly, Appleton, Gentry and Shepherd (2006) found that liking increased for high energy yoghurts, but only when in a state of high energy requirement, those in the low energy deprivation state do not demonstrate pleasantness differences between energy content. The researchers note that factors other than energy, such as texture and mouth-feel, may also have influenced these deprivation state dependent liking changes for yoghurt in their study. A similar effect on liking for flavours paired with caffeine has been demonstrated when in a state of caffeine deprivation (e.g. Chambers, et al., 2007; Yeomans, et al., 2000b). These differences in motivational state may provide an explanation for why some studies find no evidence of FNL, as perhaps the positive association is reliant upon current need state (Brunstrom, 2007).

A recent review by Yeomans (2012) also identifies motivational state as an important factor in FNL, but concludes that a failure to control for this does not provide an explanation for the mixed findings of FNL in the human literature. In the review a distinction is made between flavour-nutrient hedonic learning (where an acquired liking is formed) and flavour-nutrient satiety learning (where information is learned about how satiating a food is). A number of additional key considerations are raised, including the novelty of the conditioned stimulus, individual differences and the amount of nutrients ingested in training sessions. Ensuring that the conditioned stimulus is novel is a challenge for flavour learning research in adult humans due to the amount of previous exposures and experiences with types of foods and flavours (an example of latent inhibition), with support coming from the relatively higher rate of success of demonstrating FNL in studies using child participants (as discussed above). Further support for this is shown in the study by Yeomans, et al., (1998) where liking increased for a fruit flavoured tea that contained caffeine, and decreased when caffeine was absent, but the greatest overall changes in pleasantness correlated with the initial rated novelty of the tea. However, as the authors note, a more robust experimental design would be beneficial to test this further.

Individual differences are reviewed in detail in an earlier paper by Yeomans (2010) with discussion about the effect of restrained eating on the acquisition of flavour preferences based upon nutrients. Restrained eaters are defined as individuals who restrict their intake as a method of controlling their body weight and, as a consequence, impose cognitive limitations on their intake rather than attending to physiological drives (Herman & Mack, 1975; Herman & Polivy, 1980). Research has shown that highly restrained individuals are insensitive to FNL, and fail to respond to energy manipulations (Brunstrom & Mitchell, 2007). In the study, individuals scoring low on dietary restraint showed different changes in liking for a novel dessert dependent upon energy content; liking increased for the high energy, and decreased for the low energy version. Restrained eaters, however, showed increased liking for both desserts, indicating that this is an important factor to control when researching flavour nutrient associations.

Preference for high-energy can also be conditioned using a non-flavour cue, which has recently been shown by Zandstra and El-Deredy (2011). Flavour-matched yoghurts differing in energy content were labelled using different colours (counterbalanced between energy content and label colour). Following two weeks of exposure (alternate days for each), the high-energy drink was chosen significantly more times than the low energy version when given free choice on the subsequent five days.

Along with measures of liking, intake can also be used as an indicator of flavour preference, with higher intake associated with higher pleasantness ratings (Yeomans, Blundell, & Lesham, 2004). However, intake may be influenced by conditioned satiety, the process by which sensory properties of a food become associated with the physiological signals which are present towards the end of an eating episode (Booth, 1972; Booth, Lee, & McAleavey, 1976), which could lead to a decrease in consumption. Interestingly, Gibson and Wardle (2001) found that, despite the changes in liking for a novel dried fruit bar increasing in hungry participants, intake was actually higher when sated. The authors suggest that participants learned that the bars were not satiating, and therefore greater intake could be allowed when sated, a process known as ‘conditioned desatiation’. In the study by Yeomans et al., (2008), increased liking only occurred in the condition where both FNL and flavour-flavour learning were involved (a high energy sweet drink), whereas intake was increased for both conditions where energy was added (FNL). The increased intake in the absence of increased pleasantness in one of these conditions, suggests that an association has been made independent of palatability, reinforcing the findings from Mehiel and Bolles (1988) that were discussed earlier. Capaldi and Privitera, (2007) also report FNL independent of palatability, where participants were trained with a bitter flavoured cream cheese, but those consuming a high fat version during training rated it as more pleasant than those consuming a low fat version, when tested with a flavoured (but not bitter) cream cheese cracker. Therefore, despite the aversive taste, the fat content appeared to have influenced liking. It has been suggested that there were design issues with this study that may prevent such a firm conclusion, as a non-aversive control condition was not included and therefore the impact of the bitterness on the hedonic ratings could not accurately be assessed. In fact, mean pleasantness during training was relatively high, suggesting very little impact at all. There was also no information regarding the differences in energy or the amount of cracker consumed during training (Yeomans, 2012).

FNL is an important mechanism that still requires further research, particularly in relation to human flavour preference development. A better understanding of the underlying processes, along with other factors such as cognitive expectations, will be helpful in encouraging healthier eating and reducing obesity within the population.

1.3 Flavour- flavour learning

Another mechanism implicated in flavour preference learning is flavour-flavour learning (FFL). FFL is a form of evaluative conditioning (as discussed briefly in Section 1.1) where liking increases for a stimulus that is paired with positive stimuli, or decreases for one paired with negative stimuli. Therefore, FFL involves the association of a novel flavour (CS) with a flavour that is already liked, or disliked (US), resulting in an increased, or decreased, liking for the novel flavour. The unconditioned stimulus often takes the form of tastes that are innately liked (i.e. sweet) or disliked (for example, bitter, sour) by humans and animals alike (e.g. Berridge, 2000; Desor, Maller, & Andrews, 1975).

1.3.1 Animal research

Animal research has consistently shown that rats learn to prefer a flavour paired with saccharin compared to one paired with water (e.g. Capaldi, Owens, & Palmer, 1994; Fanselow & Birk, 1982; Fedorchak & Bolles, 1987; Holman, 1975; Warwick & Weingarten, 1994). As saccharin has no nutritional benefit it can be assumed that the preference is formed through association with the sweet taste, therefore through flavour-flavour learning. Fanselow and Birk (1982) were the first to demonstrate that a flavour could also become rejected when paired with an aversive taste (for example, quinine), which expands upon the conditioned taste aversion literature, with the reinforcer an aversive flavour rather than an aversive post-ingestive consequence.

Research has proposed that, unlike FCL, motivational state does not mediate the development of flavour-flavour preferences (e.g. Fedorchak & Bolles, 1987; Harris, et al., 2000). Fedorchak and Bolles (1987) found that saccharin-paired and ethanol-paired flavours were equally preferred over those paired with water, but that deprivation state modulated only the ethanol preference, with no effect for the saccharin paired flavour. It was also observed that preferences based upon taste were weaker than those based upon nutrients. However, Harris et al., (2000) suggest that, in hungry rats it is the motivational state that determines which association (flavour-nutrient or flavour-flavour) controls preference, whereas if tested sated, the rat only learns to associate an odour with the taste not the nutrient.

1.3.2 Human research

The first study of human evaluative conditioning using flavour based learning was by Zellner, Rozin, Aron and Kulish (1983). Three studies were conducted whereby participants experienced 24 exposures to one flavour paired with a sucrose solution and one flavour paired with water. An enhanced liking developed for the flavour previously paired with a sucrose solution, although the unpaired flavour also increased in pleasantness, indicating a mere exposure effect was also having an impact. This mere exposure effect for the unpaired flavour was not demonstrated when participants were asked to rate samples in terms of bitterness during training rather than sweetness, but the enhanced pleasantness of the sucrose-paired flavour was still demonstrated. These findings suggest that attending to the sweetness of the solution impacted upon liking for both flavours, regardless of which was paired with the sucrose itself. Subsequent FFL studies in humans show mixed results (as reviewed by De Houwer, et al., 2001; Yeomans, 2006b), with aversive stimuli providing more robust results (Baeyens, Crombez, DeHouwer, & Eelen, 1996; Baeyens, Crombez, Hendrickx, & Eelen, 1995; Baeyens, Eelen, Vandenbergh, & Crombez, 1990b).

Baeyens et al., (1990b) failed to find evidence of positive conditioning for a flavour paired with sucrose, but demonstrated a strong negative conditioning effect for a flavour paired with tween (a solution which creates a soapy mouthfeel). However, it was noted that the tween was much more disliked than the sucrose solution was liked, and also that there was a large variation in liking for the sucrose which could account for the differences between the positive and negative conditioning. Equally, this could also relate to a preparedness to readily form associations for negative consequences compared to positive consequences, as also demonstrated in the conditioned taste aversion literature (Garcia & Koelling, 1966).

Capaldi and Privitera (2008) showed that both children and adults could be conditioned to increase their liking for bitter and sour tastes through pairing with a sucrose solution. In the study with children, adding sucrose to grapefruit juice over 20 exposures resulted in increased liking in those children who rated the juice as neutral or not liked at baseline. For those who already liked the juice at baseline, pairing with sucrose did not result in an enhanced liking after training. The second study attempted to replicate with adults and using bitter vegetables. Pleasantness ratings increased from baseline in the unsweetened versions of the vegetables after repeated exposure to the sweetened versions, and only three exposures were necessary for this enhancement to occur. However, conclusions could not be drawn as to whether this was due to FFL or FNL, or an interaction of both processes as sucrose is both sweet in taste, and calorific, which could facilitate both processes. In another study with children (Hausner, et al., 2012), intake of a novel vegetable puree increased across ten exposures, with a marked increase between exposures five and ten for those trained using FFL. For those in the mere exposure group, intake increased sharply between exposures one and five but this increase was smaller over the latter sessions. This suggested that more exposures were necessary for acceptance to occur through FFL than mere exposure, with evidence that these learning effects were still present at six month follow up for both groups. FFL and mere exposure were more effective in promoting acceptance of a novel vegetable than FNL. FFL and mere exposure were also found to be successful in increasing children's intake and liking for a disliked vegetable (Anzman-Frasca, et al., 2012).

The odour-taste paradigm produces clearer and more consistent evidence of FFL, where a neutral odour (CS) is paired with a taste (US), often sweet, but also bitter and sour

(Stevenson, Boakes, & Prescott, 1998; Stevenson, Boakes, & Wilson, 2000; Stevenson & Case, 2003; Stevenson, Prescott, & Boakes, 1995; Yeomans, Mobini, Bertenshaw, & Gould, 2009b; Yeomans, Mobini, Elliman, Walker, & Stevenson, 2006). As there are no known innate preferences for food odours, this paradigm provides a good basis to study flavour learning as it can be assumed that identification and liking of these odours are learned responses. In these studies (as reviewed by Yeomans, 2006a), it is commonly found that the odour takes on the properties of the taste, for example, when paired with a sweet solution, odours are rated as sweeter when experienced orthonasally without taste. These studies also allow discrimination between FFL and FNL as the solutions are usually tasted but expectorated rather than consumed.

However, a change in liking for the odour is rarely demonstrated in these studies, with many finding no change despite altered sensory ratings of the CS (Stevenson, et al., 1998; Stevenson, et al., 2000; Stevenson, et al., 1995), and some more recent studies finding a change in pleasantness but these changes were often dependent on individual differences between participants (Brunstrom & Fletcher, 2008; Yeomans & Mobini, 2006; Yeomans, et al., 2009b; Yeomans, et al., 2006). One study that has reported increased pleasantness for the CS (odour) after pairing with a sweet taste (Yeomans, et al., 2006) offered an explanation for the common failure to demonstrate such a change through the assumption that sweetness is innately pleasant, as there are individual differences in the hedonic ratings of sweet taste which could account for these mixed findings (Looy, Callaghan, & Weingarten, 1992). In the study by Yeomans et al., (2006), increased odour liking was only shown in participants classified as 'sweet-likers', based upon a pre-screening procedure where a 10% sucrose solution was rated higher than 50 on a 100 point scale for sweetness and pleasantness. The same pattern was also shown for reduced liking for an odour paired with salt or quinine, based upon 'dislikers' of these tastes. This suggests that ensuring participants rate the US as pleasant, or unpleasant depending on the nature of the target, may be crucial to the FFL paradigm.

A further suggestion for the inconsistent findings within FFL research is the effect of dietary restraint, as, in a similar pattern to FNL, it appears that unrestrained eaters are sensitive to these associations whereas restrained eaters are not (Brunstrom, 2001). In a subsequent study by Brunstrom, Higgs and Mitchell (2005), it appeared that restrained eaters were sensitive to these associations, but differed in the extent to which the US was found to be rewarding. In this study, three CS flavours were used, and each was reinforced with a sweet US in a different percentage of trials; one was reinforced in 10% of trials, one in 50% and one in 90%. Unrestrained eaters learned to like the flavour that was reinforced 90% of the time, whereas restrained eaters liked the flavour reinforced 10%. Perhaps the US is less rewarding for these individuals as it challenges their cognitive control, although these were non-dieting restrained eaters. This pattern was also observed when pictures were reinforced rather than flavours. However, some studies suggest liking is influenced by disinhibition scores rather than restraint, with high disinhibitors showing increased sensitivity to FFL (Brunstrom, 2001; Yeomans, et al., 2009b), although this is not always the case (Brunstrom, et al., 2005). Disinhibition, as measured using items such as the Three Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985), can be used to identify individuals who may over-respond to hedonic cues which they interpret as more rewarding, and as summarised in the review by Yeomans (2010), high scorers demonstrate sensitivity to the palatability of foods and choose high fat, sweet food and drinks more frequently. A study by Yeomans et al., (2009b) found that, although rating a sweet US similarly to low disinhibitors, those scoring high on the scale showed a larger increase in liking for the odour that had been paired with the US, suggesting differences on the scale could influence the development of liking through FFL.

Human research appears to contradict the animal findings regarding motivational state. Brunstrom and Fletcher (2008) show that when participants were split into high and low hunger (based upon a median split), those in the high hunger group displayed increased preference for the saccharin paired flavour and a decrease for the unsweetened flavours. A study by Yeomans and Mobini (2006) also found that pleasantness of an odour paired with sucrose showed greater increase after a low energy, or no, preload (tested hungry) than those who consumed a high energy preload (sated). Hunger state did not influence increased sweetness of the odour, or the reduced pleasantness or increased bitterness of the odour paired with quinine. This supports the notion that sweet tastes are rated as more pleasant when in a state of hunger than when sated, reflecting underlying physiological needs (Yeomans, 2006a), defined generally by Cabanac (1971) as alliesthesia, where a stimulus can be regarded as pleasant or unpleasant dependent upon internal state. Conversely, Mobini, Chambers and Yeomans (2007) found that pleasantness increased for a flavour paired with aspartame independently of hunger state, but that hunger state influenced the magnitude of pleasantness change when paired with sucrose; those tested hungry showed a larger increase than those tested sated. This supports animal research (e.g. Harris, et al., 2000) that suggests that motivational state influences FNL but not FFL.

1.4. Summary and aims

It is important to remember that the two processes of FNL and FFL are likely to interact in our every day dietary experiences and the effects of each are difficult to isolate outside of the laboratory setting, especially when sucrose is used as the reinforcer. Also, as outlined by Birch and Anzman-Frasca (2011), context is an important factor in learning for humans and influences all of the learning processes discussed above.

This thesis will be divided into two sub-sections each addressing separate research questions. Each section will include a detailed review of the relevant literature, with Part One concentrating on the extinction of flavour preferences after the removal of nutrients, and Part Two investigating the role of expectations in these learning processes and how these can be manipulated to facilitate learning. A general methodology chapter will outline the methods common to the studies in this thesis and discuss why these methodologies were selected.

The aims of this thesis are to investigate further the processes described in this review, with particular focus on FNL, what happens when the reinforcing nutrient is removed, and the role expectations play in this process. Specific aims will be outlined at the end of each detailed literature review.

Chapter 2: General Methods

2.1 Introduction

This chapter will review the different ways of measuring and manipulating the key variables that are relevant to the experiments presented in this thesis, with explanation for why certain methodologies were adopted. Additionally, common experimental features used throughout the body of experimental work in this thesis will be summarised.

2.2 Measures

The main outcome measures in this research focus on changes in liking and appetite, alongside characterising participants as low in dietary restraint. Issues and approaches in the measurement of each of these variables will be discussed in turn.

2.2.1 Acquired liking

Liking, or pleasantness, is an important factor in determining food intake, with measures of liking being sensitive to conditioning, and conditioned changes in liking proposed to play a part in changing habitual eating patterns (Appleton, et al., 2006; Rozin & Zellner, 1985). Before liking can be measured, it is important to distinguish the difference between liking and preferences. Preference refers to choosing one item over another, and liking is a hedonic response to a food, and whilst it is often assumed that the two occur simultaneously this is not always the case as other motivations, such as perceived health value, influence preferences (Rozin & Vollmecke, 1986). Generally though, preferences do reflect liking measures, and most data in the field are difficult to separate so the two are often evaluated as indices of the same outcome.

In human research, subjective pleasantness or liking ratings, and often intake (but see Section 2.2.2), are the main outcome measures to assess if FNL has occurred, and whether there has been an acquired liking for the flavour. Intake may not always be a good indicator of liking as there are many other factors which can affect it (De Graaf & Zandstra, 1999), therefore subjective ratings are often purer measures (Appleton, et al., 2006).

Scales commonly take the form of visual analogue (VAS), categorical, Likert, or magnitude scales and can be completed in paper or electronic form. A common scale used to measure liking or disliking of foods, and widely used in the measurement of food preference, is the categorical Natick 9pt hedonic scale (Peryam & Giradot, 1952; Peryam & Pilgrim, 1957), which has nine labelled intervals ranging from "dislike extremely" to "like extremely" with a marked neutral category, "neither like nor dislike". Although a number of limitations apply to this method in that it does not provide interval data as intervals are not equidistant, the neutral category can encourage complacency and individuals often avoid using the end points in case a stimuli that is more or less liked is subsequently presented, it is a simple, easy to use scale, which has been accepted across many sensory and psychological dimensions (Schutz & Cardello, 2001).

VAS were initially implemented in pain research (Ohnhaus & Alder, 1975), and consist of a continuous straight line, usually 100-150mm in length, with a question or statement written underneath. At each end of the line is an anchor for the extreme of the scale, for example, 'not at all' at the extreme left, and 'extremely' at the extreme right. Individuals then mark a cross, or move a cursor, to indicate the position on the line that best represents their rating. VAS is a more sensitive measure than categorical or Likert scales, as it allows for small changes in ratings without individuals making a forced choice. Although these forms of scales are analysed as parametric data, theoretically they may not produce ratio data. Labelled magnitude scales were developed to attempt to overcome this issue (Green, Shaffer, & Gilmore, 1993), later modified to Labelled Affective Magnitude (LAM) scales (Schutz & Cardello, 2001), and also general Labelled Magnitude Scales (g)LMS (Bartoshuk et al., 2002), and are used to rate the intensity of a sensation in relation to 'the greatest imaginable' or 'the least imaginable', sometimes with phrases or numbers placed along the scale to aid the participant in deciding where to mark their rating. The inclusion of these anchors, a point representing null feelings, and the fact that these judgements are made relative to each other for a single individual, allow ratio data to be assumed (Cardello, Schutz, Lesher, & Merrill, 2005), although training is often required to ensure that participants understand how to make ratings using this method. More recently there has been the development of a new scale, the Labelled Hedonic Scale, LHS, (Lim, Wood, & Breen, 2009) aiming to provide a reliable measure of hedonic states. This measure requires further validation, but in early evaluation this method appears to overcome issues of avoidance of extreme ratings and the distribution of such ratings at the ends of the scales, which often becomes clustered. It should be noted that in terms of hedonic ratings, it may prove difficult to measure what is the “strongest imaginable”, and to determine whether people are actually using the extremes of experience when completing these ratings or whether they are just rating at the extreme of their common experiences (Prescott, 2009).

In their review on assessing motivation to eat using VAS, Stubbs et al., (2000) report a number of advantages of this method of measurement; standardised format allowing comparison across many experimental manipulations, easy use and interpretation, allow discrimination and participants do not need to think up their own descriptions for ratings. In terms of reliability and validity, the authors conclude that experimental manipulations do seem to result in appropriate sensitivity in subjective ratings, and that these can be reproduced, although perhaps more so when in a within subject design. Also, VAS appear to have some predictive ability in terms of actual feeding behaviour making them a useful tool. Electronic rating systems have been developed in order to minimise missing data and the potential for participants to remember their previous ratings during a session. The majority of studies reported in this thesis mainly use an electronic ratings system (SIPM, discussed in detail in Section 2.4.2) to record appetite, hedonic and sensory ratings, with paper scales used only for ratings obtained outside of the laboratory as a control of intake. More recently, hand held electronic systems have been developed for ratings to be completed outside of the laboratory, as it has been demonstrated that participants may be more limited in their use of the extremes of the scales in electronic versions compared to pen and paper, perhaps suggesting they should not be used together (Stubbs, et al., 2000).

Pleasure can be interpreted as an interaction between liking and wanting, and a combination of these two elements is likely to result in a greater reward value (Finlayson, King, & Blundell, 2007b). It is often assumed that making a food more palatable will lead to an increased liking for that food which in turn will lead to increased intake (e.g. Yeomans, 1998), and vice versa. However, liking and wanting can be dissociated in the brain, suggesting this pattern of behaviour may not always occur and that they can occur as separate entities (Finlayson, et al., 2007b). Much of the seminal research separating wanting and liking was conducted by Berridge and colleagues using rats (as summarised in Berridge, 2001) and has more recently been investigated within a human food paradigm leading to the development of a methodology that attempts to dissociate liking and wanting in the human appetite laboratory (Finlayson, King, & Blundell, 2007a). Liking can influence behaviour through wanting, and this can be mediated by incentive learning (Dickinson & Balleine, 2002), and this is something that could be considered when assessing liking changes.

A large body of previous research in flavour learning has focused on changes in liking or pleasantness as the main outcome measure (for example, Appleton, et al., 2006; Wardle, Mitchell, & Lovibond, 2007; Yeomans, et al., 2000b; Yeomans, et al., 2008b; Zellner, et al., 1983), and therefore this thesis continues with this methodology. VAS were primarily used throughout this thesis, with previous research suggesting that identical results can be obtained using either gLMS or VAS (e.g. Yeomans, Tepper, Rietzschel, & Prescott, 2007), and this method has been widely used and is easy to implement.

2.2.2 Appetite

Appetite can be defined as "sensations that promote food ingestion or rejection," is likely to be important in maintaining energy balance and is usually split into three parts; hunger, satiation and satiety (Mattes, Hollis, Hayes, & Stunkard, 2005, p. 87). It is important to measure appetite alongside intake, sensory and hedonic ratings to help establish whether energy differences between high and low versions of food are sufficient, and also to give a better insight into eating behaviour than intake alone (Stubbs, et al., 2000). The concept of hunger is difficult to define in terms of subjective ratings, as external stimuli also have a part to play in feelings of hunger, for example palatability of a food can influence hunger ratings (Hill, Magson, & Blundell, 1984). Hill and Blundell (1982) therefore suggested that a multidimensional view of hunger should be adopted in order to produce a more sensitive measure, and this is a methodology which is commonly followed in appetite research.

There are some standard recommended scales for measuring self reported appetite, and as stated by Blundell et al., (2010) these have been used internationally in a wide range of research. The recommended scales record hunger, fullness, desire to eat, thirst and prospective consumption (as implemented by Hill, et al., 1984), and it has been suggested that researchers should not deviate too far away from these consistently implemented scales as they are valid, sensitive measures. In the study by Hill et al., (1984) hunger, fullness and desire to eat were found to be influenced in different ways by the palatability of a test food, suggesting that they are measuring slightly different processes, but they do co-vary. A subsequent principal components analysis (Reid, Harbron, Blundell, & Stubbs, 1998) on these rating scales produced a solution of two factors, one concerned generally with motivation to eat, and the other concerned with gastro-intestinal repletion (reported in Stubbs, et al., 2000).

It has been noted that it is difficult to accept external validity in visual analogue scales, in that the measurement of hunger may not actually be measuring hunger, and may differ in its meaning between individuals (Stubbs, et al., 2000), but it does appear to have face validity (Hill, Rogers, & Blundell, 1995) by allowing “general agreement among people in certain experiences… along a continuum, and allow for distinctions to be made within these” (Blundell, et al., 2010, p. 261). The authors also note that ratings, and changes in ratings, generally follow characteristics of the meal such as energy load and volume, suggesting sensitivity to the physiological changes that may be occurring.

Food intake and biomarkers are also commonly used as a measurement of appetite. Intake is not always a good representation of appetite, as there are many other factors that can influence it, such as social expectations, emotional eating and eating in the absence of hunger. The measurement of biomarkers are beyond the scope of this thesis, but there are a number of criteria that need to be met to ensure that this is a useful measure (see Mattes, et al., 2005 for a review). Other methods of measuring appetite include rate of eating, bite size and salivation measures, which are currently less accepted but, if validated, could provide a useful direction for future research (e.g. Yeomans, 2000).

2.2.3 Restraint

As discussed in Sections 1.2.2 and 1.3.2, there is evidence that individuals scoring high in dietary restraint do not respond to energy manipulations and are insensitive to flavour nutrient learning (Brunstrom & Mitchell, 2007). This could be attributed to a loss of sensitivity to internal cues, as restrained eaters frequently over-ride the processes of dietary control. As flavour nutrient learning is one of the major theoretical focuses of this thesis, participant's restraint scores were controlled (see Section 2.4.1 for details).

There are a variety of questionnaires designed to measure dietary restraint, a concept first defined by Herman and colleagues as the restriction of intake as a method of controlling body weight, resulting in the imposition of cognitive limits on intake rather than attending to physiological drives, and they developed the first measure; the Restraint Scale (Herman & Mack, 1975; Herman & Polivy, 1980). The construct validity of the scale has been questioned (e.g. Ruderman, 1986) leading to two subsequent questionnaires measuring restraint that have been widely used; the Three Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985) and the Dutch Eating Behaviour Questionnaire (DEBQ; van Strien, Frijters, van Staveran, Defares, & Beurenberg, 1986), although there is much debate as to which of these scales actually measure restraint (Gorman & Allison, 1995). The validity of these measures has been examined (Williamson et al., 2007), with the TFEQ restraint scale deemed to be the most valid in terms of measuring intention to diet, and actual restriction of calories (with the latter expressed as a change score in the scale). The restraint scale of the TFEQ appears to be a robust measure of cognitive restraint but both the disinhibition and hunger scales appear to be unstable (e.g. Hyland, Irvine, Thacker, Dann, & Dennis, 1989). The factor structure of the TFEQ has been re-examined using obese subjects, resulting in a revised, shortened 18 item version (TFEQ-R18; Karlsson, Persson, Sjöström, & Sullivan, 2000). This shortened version still has a three factor structure; cognitive restraint, uncontrolled eating and emotional eating, and has been validated within a general population (de Lauzon et al., 2004), and implemented in a number of studies (e.g. Keränen, Strengell, Savolainen, & Laitinen, 2011).

For the purpose of the current research, it was decided to continue with the original version of the TFEQ, where the restraint scale has been found to be robust and have construct validity, and has been used in participant recruitment for a large body of previous research in flavour nutrient learning (e.g. Yeomans, et al., 2008b). Restraint scores were used in the eligibility criteria rather than as a method of assigning individuals to conditions, therefore it was decided that the original TFEQ was adequate for this purpose.

2.3 Manipulations and design issues

In each study the energy content of the test foods was manipulated in order to assess FNL. In the final three studies (Studies Three, Four and Five), expectations were also manipulated, and this manipulation will be discussed in detail in Part Two.

2.3.1 Mixed design

In a review by Reid and Hetherington (1997), the advantages and disadvantages of using between or within subject designs in nutrition research were discussed. There are a number of issues with using within subject designs for research where learning is likely to occur, as participants can change their behaviour according to demand characteristics and this is a problem if they are experiencing different conditions within different test days. The review discussed that a benefit of using within subjects would be that the individual acts as their own control but it was suggested that a between subjects design may be more appropriate when investigating the effect of a nutrient on behaviour. This relates to the present thesis as it is looking at whether individuals learn about the nutrients and energy in a food, what happens when this nutrient is removed and how the situation can be manipulated to facilitate learning. Between subject designs have also been criticised for perhaps encouraging pseudo-conditioning, especially when caffeine is used as a reinforcer, as often participants take part in these studies after some period of withdrawal, therefore those in the CS+ condition experience some form of relief from this withdrawal upon receiving the reinforcement of either caffeine or nutrient (Brunstrom, 2005). This could result in more positive evaluations for those in the CS+ condition than those in the CS- condition as the latter condition never experience this relief from withdrawal. As discussed by Chambers, Mobini and Yeomans (2007) studies can overcome this potential pseudo-conditioning effect by training each participant with two flavours, one CS+ and one CS+, and this can show evidence for increased liking for the CS+ and no change or decreased liking for the CS- (Mobini, Elliman, & Yeomans, 2005).

It did not make sense to use within subjects for the extinction part of this thesis, as once participants had undergone extinction in one condition this would affect their subsequent exposures to new stimuli. As FNL was the focus of the research, multiple testing sessions were necessary for associations to be made, and therefore a mixed design was deemed appropriate for all of the studies, with the between subjects components being the condition that the subject was assigned to and the within subject component being exposure day. A large number of exposure trials would be required to conduct these studies as within-subject studies and this was decided against and as nutrients were being used as the reinforcer, generally conditions involved some form of consumption which may help counteract the issues of pseudo-conditioning where studies with caffeine reinforcers may not.

2.3.2 Laboratory setting

As summarised succinctly in a review by Blundell et al., (2010), laboratory studies are high in internal validity, allowing control over experimental manipulations but are difficult to generalise outside of the laboratory setting. Free-living studies meanwhile, have high external validity, but rely on self report and cannot be strictly controlled. The repeated exposure nature of the experiments in this thesis led to the decision to use the laboratory setting, allowing tight control over the manipulations and data collection. Whilst not out of the realms of possibility of conducting in the home environment (e.g. Mobini, et al., 2007), some of the studies would have been difficult in terms of preparation and intake of the relevant test stimuli. In response to misgivings set out by Meiselman (1992) regarding current laboratory methodologies, Kissileff (1992) argues that the location should be led by the research question, making both the laboratory and “real life” settings equally valid dependent upon the question under investigation, particularly amongst humans who consume food in an infinite number of individual situations and circumstances.

2.3.3 Energy content

In order to investigate FNL, a flavour-matched high and low energy version of the test food was developed, with energy manipulated using sucrose (Silver Spoon, UK) in the extinction studies (Study One and Study Two), maltodextrin (Cargill) in the breakfast studies (Study Four and Study Five) with the addition of whey protein isolate in Study Three. Sucrose was selected as the energy source for the extinction studies as Study Two was based upon a previous methodology used in the laboratory where maximum learning occurred when the stimuli facilitated both flavour-flavour (sweet) and flavour-nutrient (energy) learning processes (Yeomans, et al., 2008b). It was decided to remain consistent with this energy source across both extinction studies, and this also aided the production of the test stimuli for Study One from Unilever and International Flavours and Fragrances. The stimuli for the remaining expectation studies (Study Three, Study Four and Study Five) were also developed from previous methodologies within the laboratory. Maltodextrin was easy to conceal within yoghurt based breakfast without making the taste too sweet to consume a large portion (300g) with an adequate energy difference between low and high versions, and whey protein allowed additional energy to be added to the drinks in Study Three without making them aversive in texture.

2.4 Common methodologies in this thesis

The studies presented in this thesis all shared some general methodological procedures. For example, the studies all followed similar experimental protocol, and participants were selected using particular demographic information and exclusion criteria. To avoid repetition in subsequent chapters, common experimental features are summarised in this chapter, with study-specific methodology included in each experimental chapter.

2.4.1 Participants

Individuals who expressed interest in appetite studies were invited to complete a general appetite questionnaire, which included the TFEQ (Stunkard & Messick, 1985) and other relevant questions regarding eating behaviour. Demographic information was collected, including an estimation of height and weight and smoking behaviour. After completion of the TFEQ, there was a section asking about drinking habits, focusing on caffeine and alcohol, and a section on food habits such as current dieting status and breakfast consumption (see Appendix 1 for a copy of the recruitment questionnaire). These answers were stored on a secure database, and potential participants were selected based upon their TFEQ-Restraint scores and other inclusion criteria. These individuals were contacted via email, and those who were interested in the specific study were then sent a detailed email with the relevant information sheet attached. They were invited to arrange a time for their first session/screening session. Upon arrival at the laboratory, another copy of the information sheet was provided, along with a consent form to be completed.

With the exception of Study One, only female participants were recruited. Many studies involved ad-libitum eating, and the amount of food consumed is often considerably different between males and females, with females often socialised to eat differently to males (Rolls, Federoff, & Guthrie, 1991) and this could have resulted in a confound. Gender differences in food perception have also been demonstrated in a series of studies by Oakes and Slotterback (e.g. 2001), whereby women reported eating healthier foods, more frequent reading of nutrition labels, and more knowledge about which foods were better for them. However, research has not consistently found gender differences (e.g. Oakes, 2005) and sometimes the opposite patterns have been reported (Carels, Konrad, & Harper, 2007). Where both males and females were recruited (Study One), different patterns of behaviour were indeed demonstrated.

All participants scored less than seven on the restraint scale of the TFEQ (Stunkard & Messick, 1985), which was based upon a median split of the existing appetite lab database. Justifications for selecting individuals low in dietary restraint were discussed in Section 2.2.3.

Motivational state also plays a role in flavour preference learning and extinction in both animals and humans (discussed in detail in Chapters 1 and 3). Harris, Shand, Carroll and Westbrook (2004) manipulated hunger state of rats during training, and found that hungry rats appeared sensitive to extinction whereas sated rats were resistant. In humans, Appleton, Gentry and Shepherd (2006) found that liking increased for flavours when conditioned in a state of high energy requirement, whereas no such changes were observed in a state of low energy requirement. Similar effects have also been demonstrated with caffeinated drinks (Yeomans, Jackson, Lee, Nesic, & Durlach, 2000a), where liking only increased in caffeine deprived individuals. Consequently, participants in this thesis were required to fast before test sessions to ensure that they were hungry. In Studies One, Three, Four and Five (which all involved a breakfast component), participants were instructed to only consume water from 23.00 before each test day, and were asked if they had complied with this demand upon arrival at the laboratory. In Study Two participants were asked to consume their usual breakfast, and to then consume only water between that and reporting to the laboratory for lunch. In order to control for hunger, standard meals (either breakfast or lunch, outlined in detail in individual experimental chapters) were provided in Studies One, Two and Three, and participants were required to consume only water between this and their return to the laboratory for the actual test session. Participants were also required to refrain from eating or drinking anything except water for one hour post test session, to minimise the likelihood that associations could be made between flavours and subsequent energy intake after leaving the laboratory. Due to the Human Tissue Act (2004, which came into force in 2006), it was no longer ethical to take a dummy saliva test as had been used in previous research (Mobini, et al., 2005), therefore participants' word was all that could be taken to ensure compliance.

Body Mass Index (BMI) is an index of weight for height, whereby an individual's weight (kg) is divided by their height (m^2) and according to the World Health Organisation (WHO, 2012), a BMI of over 25 is classified as overweight, with over 30 classified as obese. As advised by the WHO, BMI should be used as a rough guide as it does not necessarily relate to the same amount of body fat between individuals. BMI was required to be in the 'normal' range (18-30) as this thesis examined behaviour within a non-obese population. The majority of participants were within the normal BMI range (18-24.9), with a few classed as overweight (25-29.9). None were classified as underweight (BMI <18) or obese (BMI > 30). BMI was included in the eligibility criteria because there has been a wealth of literature suggesting that obese and lean individuals respond differently to foods, with the obese often selecting more energy dense, savoury foods (e.g. Cox, Perry, Moore, Vallis, & Mela, 1999), reacting more to external than internal cues (Schachter, 1968; Schachter & Gross, 1968) more susceptible to emotional eating than non-obese (e.g. Schachter & Rodin, 1974). Support for the externality theory has been mixed; for example, Rodin (1981) discussed the fact that internal cues and external cues are difficult to differentiate, as both may influence each other. Other research suggested that important factors associated with BMI are restrained eating and emotional eating, with external eating not significantly related to overweight (e.g. van Strien, Herman, & Verheijden, 2009). However, if viewed as a more complex picture than the original theory it can then be viewed as a more valid basis for obesity research (Mela, 2001), and the theory was extended by Herman and Polivy (2008) by classifying external cues into two sub-components. The first, normative external cues, are proposed to affect all eaters, and the second, sensory external cues, to have a stronger influence over certain individuals (those classified as obese, and those who are dieting). Environmental cues regarding how and what should be eaten were regarded as normative cues, whereas the sensory cues were defined as properties of the food making it more or less likely to be eaten.

Age was limited to 55 years old as appetite and energy intake decreases with age (Chapman, 2004). Individuals who were pregnant or currently taking prescription medication were also excluded as these factors may alter appetite. A maximum of 5 cigarettes smoked per day was permitted as nicotine results in reduced appetite and body weight (Miyata, Meguid, Fetissov, Torelli, & Kim, 1999) along with reduced preference for sweet tastes (Grunberg, 1982).

Additional exclusion criteria were included to limit any potential risks to the participants: diabetes, history of eating disorder, aversions or allergies to a number of common food ingredients. Previous participation in a similar study also resulted in exclusion, as this may result in the formation of prior associations within the laboratory.

2.4.2 Materials

In all studies except Study One, computerised mood and appetite ratings were completed on a PC computer using the Sussex Ingestion Pattern Monitor (SIPM v. 2.0) in an air-conditioned cubicle with no windows. This system, modified from the Universal Eating Monitor (Kissileff & Van Itallie, 1980), also allowed *ad libitum* intake, and refills to be provided after a set amount of consumption had occurred, through the use of a balance (Sartorius BP4100) concealed from view by a placemat. This ensured that participants could not simply eat until they had emptied the plate/bowl, and attempted a more reliable measure of consumption. Mood ratings were mainly recorded as distracters from the appetite ratings, aiming to prevent participants from focusing too heavily on any appetite-related effects, and also to hide the true purpose of the study (learning). Mood ratings were displayed in the format: "how <mood> do you feel right now?" and measured using a 100pt visual analogue scale, with "not at all" and "extremely" used as anchors. Ratings of the food were similarly worded for example, "how <taste> is the yoghurt?" Participants were required to click a button labelled as 'rating completed' after each scale. This ensured that no comparison was made between previous ratings and the current rating.

E-prime (Version 1.2: Psychology Software Tools, Inc.) was used in Study One, where no *ad libitum* consumption occurred, and therefore no balance was required. At this stage, SIPM was in development and therefore E-prime was used as an alternative.

Mood scales were presented in a similar format to those described for SIPM. For a detailed description of this programme see Section 4.2.4.

Ethical approval for all studies was granted from the University of Sussex Ethics committee and all studies were conducted in line with the BPS Code of Human Research Ethics (2011) and the Declaration of Helsinki on ethical conduct of studies (revised 2008).

2.4.3 Protocol

All studies required reporting to the laboratory for multiple sessions (detailed in each experimental chapter). As previously discussed, all studies required participants to fast before reporting to the laboratory, to ensure that they were hungry upon arrival. For all studies except Study Two this was an overnight fast (consuming only water) from 23.00 the night before reporting to the laboratory for breakfast. For Study Two, participants consumed their normal breakfast and then only water until reporting to the laboratory for lunch. For studies that involved more than one test session per day (Studies One, Two and Three) a control meal was provided, to ensure that all participants had consumed the same meal earlier in the day and to control for hunger. Additionally, participants were required to fast for an hour post-test, to allow any appetite associations to be made with the test foods rather than subsequent consumption after leaving the laboratory. A maximum of two to three sessions were completed in any one week, to reduce over-exposure to the food which might have resulted in monotony effects and so decreased pleasantness (Hetherington, Bell, & Rolls, 2000; Hetherington, Pirie, & Nabb, 2002; Meiselman, De Graaf, & Lesher, 2000; Zandstra, De Graaf, & van Trijp, 2000). On the last session of every study, participants were asked their opinions about the purpose of the study, were fully debriefed and their height and weight was recorded before being reimbursed for their time with money or course credits.

2.4.4 Data analysis

Data were analysed using the Statistical Package for Social Scientists (SPSS) version 18.0 on a Macintosh computer using OS X version 10.6.8. Each individual study will include a detailed section regarding the methods and tests used to analyse the data. Statistical significance was assessed at $p < .05$, with $p < .09$ considered a trend. Where means are reported, the standard error of the mean (SEM) will also be reported. When sphericity could not be assumed the appropriate corrections will be applied to the test statistics (if ε < .75 Greenhouse-Geisser, ε > .75 Huynh-Feldt). Where homogeneity of variance is violated (assessed using the Levene's test), the appropriate statistics will be reported or precautions in interpretation taken. Normality was also assessed using the Kolomogorov-Smirnov (K-S) and Shapiro-Wilk (S-W) tests, histograms and boxplots. Where data were non-normal, all factors were taken into account and corrections applied where necessary (see individual method sections for specific detail).

PART ONE: Acquisition and extinction of liking for a flavour through FNL. Studies One and Two.

Chapter 3: Extinction and flavour preference learning

One of the primary research questions in this thesis is whether acquired liking for a flavour is extinguished when the functional ingredient (in this case the nutrient) is removed. Since the processes of acquired liking for nutritive products discussed in this thesis are interpreted as examples of Pavlovian learning, where the flavour acts as CS predicting the beneficial effects of the ingested nutrient, then removal of the nutrient can be viewed as an example of extinction in Pavlovian terms. As discussed briefly in Section 1.1.2, previous research has shown that extinction seems to have little effect on the original learned association, but instead occurs due to new learning that offsets the original (Falls, 1998; Rescorla, 2001). This section will discuss research specifically investigating extinction with a flavour preference paradigm, with some suggestion that acquired likes and dislikes are unique as they appear to be resistant to extinction (De Houwer, et al., 2001).

3.1 Animal research

Within the flavour learning literature in non-humans, it appears that the conditioned response persists when the flavour is no longer paired with a reinforcer (Capaldi, Myers, Campbell, & Sheffer, 1983; Drucker, Ackroff, & Sclafani, 1994; Dwyer, Pincham, Thein, & Harris, 2009; Elizalde & Sclafani, 1990b; Harris, et al., 2004; Mehiel, 1991; Sclafani, 1991). There are two methodologies that are commonly used in these studies: intake tests and preference tests. Intake tests compare the intake of the conditioned flavour against the control flavour, often referred to as conditioned acceptance (Boakes, Albertella, & Harris, 2007; Sclafani, 1991), and if extinction occurs, intake of the CS+ would decrease (e.g. Pérez, et al., 1998). Preference tests are two bottle tests where the CS+ (flavour that had previously been paired with a reinforcer) can be tested against either a CS- (flavour that had not been paired with the reinforcer), or water. However, it has been noted (Diaz & De la Casa, 2011; Harris, et al., 2000), that there may be a confound when testing preference against a CS-, in that rats learn to avoid an unpaired flavour, which may explain the persistent preference for the CS+ instead. This was illustrated in a study by Drucker et al., (1994), where extinction did weaken the preference for CS+ when compared with water, but that the preference persisted if the comparison was with the CS-. Additionally, whilst the preference persisted, absolute intake of the CS+ decreased during the extinction phase.

Harris et al., (2004) report a series of studies where rats acquired a preference for a flavour paired with ingestion of sucrose compared to water, and this preference remained intact after a number of exposures (up to 20) to the flavour without sucrose (extinction). This finding supported research by Mehiel (1991), where a preference persisted over 13 exposures to a flavour that had previously been paired with sucrose ingestion. This preference continued to persist even after one day of exposure to a reversal of paired flavour, where the preference disappeared on the next day only, returning for the subsequent five extinction tests. A set of studies by Albertella (2006) conditioned preferences for almond using either sucrose or saccharin as a reinforcer, and these preferences were not extinguished, with no effect of delaying food access after test sessions. This suggests that the reason that the preferences were resistant to extinction was not because of associations made with subsequent food consumption after the testing sessions.

This effect is not always consistent across the literature, as demonstrated in a series of studies by Delamater (2007) whereby extinction did weaken the association between flavour and nutrient. However, these studies used a relatively unique procedure, whereby preference was assessed comparing an extinguished CS+ with a non-extinguished CS+. Two of the studies also explored how flavour preferences would be affected by devaluation of the nutrient, with one CS+ paired with a nutrient that was subsequently devalued, and one that remained of value. When the nutrient had been devalued, the extinguished flavour was preferred over the non-extinguished, whereas the opposite pattern was observed when the status of the nutrient had been retained.

Sensitivity to extinction was also demonstrated in flavour-flavour learning studies by Diaz and De la Casa (2011) where a conditioned preference was reduced after the CS (citric acid) was presented in the absence of the US (saccharin). Some unique conditions also applied to these studies, as the CS was citric acid which is a typically disliked flavour, and the intensity of the US was lower than that used in other studies, perhaps suggesting that intensity could also be an important factor for persistence of flavour preferences (Diaz & De la Casa, 2011).

Tarner, Frieman and Mehiel (2004) also demonstrated extinction of flavour-nutrient preferences, which they suggest could be attributed to the fact that the conditioning trials and extinction sessions were matched in length (23h), so rats were exposed for longer to the solution without calories than in other studies (e.g. Mehiel, 1991). In a second experiment, extinction was again demonstrated, although this time those in the experimental group also showed a reduction in consumption of the flavour that had previously been paired with sucrose. In this second experiment, spontaneous recovery of the flavour preference occurred 7, 14 and 21 days later.

Other possible explanations for contradictions within the literature will be addressed later.

3.2 Human Research

As discussed, there have been a number of animal studies that have investigated persistence of conditioned flavour preferences, but relatively few studies with human subjects. Resistance to extinction has been demonstrated in humans, particularly when using olfactory stimuli, in both odour-odour and odour-taste paradigms (Boakes, 2005; Stevenson, et al., 2000; Stevenson, Case, & Boakes, 2003). In the study by Stevenson et al., (2000) a novel odour was rated as more sour after being paired with citric acid (acquired similarity), and this effect failed to extinguish after repeated trials of the odour presented alone, even after 12 exposures. Results also suggest that an acquired similarity through odour-taste learning appears to be robust even after an interference phase where the target odours are paired with other odours (Stevenson, et al., 2003).

Baeyens, Crombez, Hendrikx and Eelen (1995) also demonstrated a resistance to extinction in a flavour-flavour learning context. The CS+ flavour was paired with an existing aversive flavour (Tween) and the CS- was either paired with water, or a moderately pleasant sugar solution. There was a significant difference in liking for the CS+ and CS-, but only when the CS- was presented in sugar rather than water, and this difference persisted after eight trials of each flavour with water alone. Resistance to extinction has been demonstrated even after the number of extinction trials has exceeded the number of conditioning trials (e.g. Stevenson, et al., 2003).

In contrast, a study by Yeomans et al., (2000b) found that preference for a drink that had previously been paired with ingestion of caffeine was reversed when this caffeine was removed, although this effect was dependent on motivational state. No study has replicated that finding, and it stands out as the only published example of extinction of acquired flavour liking in humans. The studies discussed do not address extinction of flavour-nutrient learning in humans and whether liking for a flavour would persist despite the nutrient being removed. This has important implications in the current climate, with many food companies attempting to reduce the sugar, salt or fat content of products, so acceptance of the flavours without the nutrients is desired.

3.3 Context and renewal

A recent review by Bouton (2011) discusses a number of mechanisms which can help to explain the persistence of conditioned flavour preferences, and strongly supports the idea that extinction does not involve erasing the original learning, and is highly dependent upon context. This context could be, amongst others, temporal, spatial or related to motivational state, and therefore, the behaviour could reappear in a different context to which it was learned, even after extinction. Referring back to the proposal that at least part of the original associations are present, extinction results in a cue becoming ambiguous due to the presence of both the new and old associations, and the context within which the cue is subsequently presented in may help to resolve this ambiguity (Van Gucht, Vansteenwegen, Beckers, & Van den Bergh, 2008).

Rosas and Bouton (1997) have investigated contextual effects in the extinction of a conditioned taste aversion. In these studies, rats did extinguish their aversion to a flavour that had previously been paired with illness, with the context in which extinction occurred having little effect. However, if extinction occurred in a different context to the conditioning, when returned to the conditioning context, the aversion was renewed, and this was not due to dishabituation. This idea has also been extended by Diaz and De La Casa (2011) who suggest that the association itself is a context, with the second association of the CS with absence of US classed as a subsequent context.

In a study looking at chocolate craving in humans, (Van Gucht, et al., 2008), conditioned craving for a neutral cue which had been paired with chocolate persisted after extinction, regardless of whether the extinction trials occurred in the same or different context (based upon lighting of the room) to where training had occurred. Expectancy that chocolate would be consumed (US expectancy) was also conditioned alongside craving, and these expectancies did extinguish, with renewal of expectancy occurring only when the extinction trials had taken place in a different context to the training trials.

3.4 Re-exposure

It has also been suggested (Harris, et al., 2004) that extinction may not occur because of a failure to recognise that a flavour presented alone is not as pleasant as when previously paired with the reinforcer. Re-exposure to the reinforcer acts as a reminder of the initial hedonic value, therefore increasing the discrepancy and extinguishing the preference. This suggests that the CS+ becomes more palatable after being paired with a palatable reinforcer, for example, sucrose. However, acquired flavour preferences do not always result in increased palatability (Forestell & LoLordo, 2003) and can also persist without these hedonic changes (Dwyer, et al., 2009), with lick cluster size used as an indication of palatability.

3.5 Motivational state

Animal research (Capaldi, et al., 1983; Delamater, 2007; Fedorchak & Bolles, 1987; Harris, et al., 2004; Mehiel, 1991), along with the Yeomans et al., (2000b) study, has indicated that motivational state may also be an important determinant of the development of flavour preferences based upon calories and in turn this may influence whether or not extinction occurs. As discussed previously, when in a state of deprivation (for example, hungry), flavours that have previously been associated with a higher energy content tend to be preferred, whereas when sated, those associated with low energy content are preferred (Booth, 1980). Mehiel (1991) also reports similar findings, with a much higher ingestion of a calorie-paired flavour when rats were tested hungry, compared to ingestion of a flavour paired with saccharin or water, which stayed stable regardless of motivational state. When sated, ingestion of the calorie-paired flavour was greatly reduced. This finding was extended by examining the relationship between the degree of hunger and the amount of flavour consumed; the hungrier the rats were, the stronger the preference for the calorie-paired flavour over a saccharin-paired flavour. This could be influential in the extinction of conditioned flavour preferences.

Harris et al., (2000; 2004) manipulated hunger state of rats during training, and found that hungry rats appeared sensitive to extinction whereas sated rats were resistant. The calories that had become expected from the flavour were no longer received, facilitating a reduction in preference for that flavour. This may be due to the learning process involved, as for hungry subjects, extinction will weaken the association both between the motivational components (FNL) and the sensory properties of the nutrient (FFL, Delamater, 2007).

Capaldi et al., (1983) conducted five studies that counter-intuitively found a greater preference for a flavour if it had been conditioned during low deprivation rather than high deprivation, and this preference persisted over 28 test days. However, as this preference was apparent when saccharin solution was used as the reinforcer compared to sucrose solution (where only a small concentration resulted in preference), it was suggested that these preferences were formed through FFL rather than FNL. It was also noted that resistance to extinction could not be concluded from this study, as all rats were under some level of deprivation.

In conclusion, flavour learning has often been shown to be resistant to extinction, but there remain conflicting results within the literature, with very few studies in humans. The focus of extinction studies within this paradigm in humans have concentrated on FFL, with only one example of FCL investigated (Yeomans, et al., 2000b) and none investigating extinction of flavour nutrient learnt processes in humans. The first part of this thesis aims to investigate whether acquired liking formed through flavour nutrient associations will extinguish when the nutrient is no longer present as further study is needed to investigate this phenomenon within humans, with possible impact upon the dieting and obesity developments.

Chapter 4: Study One - Extinction within a drink context

4.1 Introduction

As discussed in Chapter 3 previous research has shown that extinction seems to have little effect on the original learned association, but instead occurs due to new learning that offsets the original (Rescorla, 2001). In flavour learning in animals, it appears that the conditioned response persists when the flavour is no longer paired with a reinforcer (Capaldi, et al., 1983). There have been a number of animal studies that have investigated the persistence of conditioned flavour preferences (e.g. Delamater, 2007), but relatively few studies with human subjects, with the majority focusing on FFL. Resistance to extinction has been demonstrated in humans, particularly when using olfactory stimuli (Stevenson, et al., 2000). One study where evidence of extinction was demonstrated, although using a flavour-consequence paradigm, found that increased pleasantness of a drink that had been acquired by association with caffeine ingestion was reversed when this caffeine was removed, but this effect was dependent on deprivation state (Yeomans, et al., 2000b). No study has replicated that finding, and it stands out as the only published example of extinction of acquired flavour liking in humans. The current study will attempt to address the robustness of this finding, using energy (nutrient) as a reinforcer.

The finding that a person's motivational state could influence whether or not extinction occurs (Yeomans, et al., 2000b) supports previous research that highlights a similar relationship in animals. Harris et al., (2004) manipulated hunger state of rats during training, and found that hungry rats appeared sensitive to extinction whereas sated rats were resistant. In hungry rats, extinction would weaken not just the association between the flavour and the sensory properties of the nutrient through FFL, but also the association with the motivational components of the nutrient through FNL (Delamater, 2007).

The present study aimed to investigate extinction in a FNL paradigm, using sucrose as a reinforcer, with all participants tested hungry to maximize likelihood of acquired liking, and the opportunity for extinction to occur. On eight sessions over a period of two to three weeks, participants were asked to complete a taste test of five drinks, where one drink was the target flavour. Dependent upon experimental condition, participants subsequently consumed a larger sample of the target flavour (either high energy or low energy), followed by repetition of the taste test. Those in the extinction condition consumed the high energy (HE) version for the first phase (sessions one to four) and the low energy (LE) version for the second phase (sessions five to eight), and those in the exposure control condition merely completed the initial taste test on each session. To assess acquired liking for the target flavour, pleasantness ratings were included in the taste test, and subjective ratings of appetite were recorded at the beginning and end of each session, and one hour post session.

Individuals consuming a HE drink would be expected to demonstrate an acquired liking (in terms of pleasantness ratings) for this drink, compared to those consuming a LE version. If flavour preferences are resistant to extinction, this acquired liking by consumers for the HE drink should be maintained during a second learning phase where the energy is no longer present. Furthermore, those consuming the HE version would be expected to report a larger reduction in hunger than those consuming the LE version. Those in the extinction condition would be expected to show similar patterns of hunger reduction to those in the HE condition over the first testing phase, but this would not be expected to be maintained after removal of energy in the second phase.

4.2 Method

4.2.1 Design

The study used a mixed design, with participants randomly assigned to one of four conditions, ensuring gender was equal in each condition (10 females, 8 males, except for the extinction condition where a female withdrew in the last session of the study) and all participants attending eight sessions in the laboratory. The four conditions are illustrated in Table 4.1.

Condition	Days 1-4 (learning phase)	Days 5-8 (extinction phase)
High Energy (HE) Exposure	400g HE drink	400g HE drink
Extinction	400g HE drink	400g LE drink
Low Energy (LE) Exposure	400g LE drink	400g LE drink
Control	None	None

Table 4.1: A summary of the basic design of Study One.

4.2.2 Participants

Seventy one participants (39 females, 32 males, aged 18-36, *M* = 22.0 ± 0.5) were recruited using a participant subject pool at the University of Sussex. Those who expressed an interest were directed to an online eating behaviours questionnaire that consisted of the TFEQ (Stunkard & Messick, 1985) and additional questions about eating behaviour including allergies and aversions (see Appendix 1 for a copy of the recruitment questionnaire). All participants were unrestrained eaters; scoring less than 7 on the TFEQ, based upon a median split of the existing database. For exclusion criteria see 2.4.1. Table 4.2 shows mean age and BMI of the participants in each experimental condition. There were no significant group BMI, $F(3,67) = 0.74$, $p = .530$, or TFEQ-R $F(3,65) = 1.33$, $p = .273$ differences. As homogeneity of variance was violated for age ($F(3,64) = 10.45$, $p < .001$), the Brown- Forsythe and Welch statistics were reported; $F(3,37.22) = 2.36$, $p = .087$, and $F(3,34.2) = 1.96$, $p = .138$, concluding no difference in age between conditions.

	HE exposure	Extinction	LE exposure	Control
Age	22.0 ± 0.7	21.4 ± 0.8	20.5 ± 0.5	24.1 ± 1.5
BMI	22.8 ± 0.6	23.7 ± 1.2	23.5 ± 0.8	24.6 ± 1.2
TFEQ-R	2.6 ± 0.3	3.4 ± 0.5	2.4 ± 0.3	3.1 ± 0.4

Table 4.2: Mean (±SEM) age, BMI and TFEQ-R score of participants in the four conditions of Study One.

4.2.3 Test foods

Breakfast consisted of 60g Crunchy Nut Cornflakes (Kellogg's), 160g semi skimmed milk and 200g smooth orange juice (total 402kcal). Test drinks consisted of concentrated novel flavoured syrups, which were produced for Unilever plc by a flavour house (International Flavors and Fragrances) and diluted with water as instructed (1:4 for HE and the additional flavours, or 1:9 for LE). Five flavoured LE syrups were used for the taste test, one of which (A) was also presented in a 400g serving, with energy content manipulated using sucrose (180kcal vs 18kcal per serving). Familiarity and pleasantness ratings of each sample on the initial session are presented in Table 4.3. Pleasantness varied between samples, with sample A formulated to be neutral in pleasantness and all samples neutral in familiarity.

Sample	**Familiarity**	**Pleasantness**
A	50.9 ± 2.7^{b}	$50.5 \pm 2.8^{b,d}$
B	$66.3 \pm 2.7^{a,c,d,e}$	$68.1 \pm 2.1^{a,c,e}$
C	51.2 ± 3.1^{b}	$50.9 \pm 2.6^{b,d}$
D	53.2 ± 2.3^{b}	$61.7 \pm 2.0^{a,c,e}$
E	52.9 ± 3.2^{b}	$45.7 \pm 2.6^{b,d}$

Table 4.3: Familiarity and initial pleasantness of the flavoured syrups used as test stimuli in Study One. ^{superscript} letters represent significantly different ratings between samples.

4.2.4 Electronic rating scales

VAS were used to assess liking for all samples, based on a scale with end-anchors of “Extremely unpleasant” and “Extremely pleasant”, and a marked point at the centre (50) of the 100pt scale. VAS were also used to assess the sensory characteristics (bitter, sweet, sour, fruity) and familiarity of the samples, along with participants’ current mood ratings (clearheaded, calm, hungry, lively, thirsty, tired, full, nauseous) with “Not at all” and “Extremely” as anchors and no defined neutral point. All ratings were made using computerised versions of these scales, custom- programmed using e-Prime (version 1.1: Psychology Software Tools Inc). The computerised rating was a horizontal line measuring 202 mm, with the rating marked by a small vertical bar which was initially positioned centrally, but which could be moved along the horizontal line towards either the left hand anchor (“Extremely unpleasant” for hedonic ratings and “Not at all” for sensory and mood evaluations) or the right anchor (“Extremely pleasant” for hedonic ratings and “Extremely” for sensory and mood evaluations) by pressing “1” or “5” on the keyboard respectively. Once the vertical bar was positioned correctly, participants registered their rating by pressing “4” on the keyboard. The location of the vertical bar as a percentage of the length of the horizontal bar was used to calculate a rating from 0-100.

4.2.5 Procedure

The study required participants to attend the laboratory on eight days over a two to three week period. Participants were asked to refrain from eating and drinking, except water, from 23.00 the night before each session. They reported to the laboratory for breakfast at a time between 08.00 and 10.00, after which they were allowed to leave but were required to return 3 hours later. They were only allowed to consume water during this time period. During the second session on each day, participants in the test conditions followed identical procedures, whereas participants in the control condition only completed the first stage. Computerised mood VAS were completed, after which participants were presented with five samples of drinks labelled A, B, C, D and E. For each sample (presented in a randomised order) they were instructed to take a mouthful, swill around their mouth for five seconds, and then expectorate into the funnel provided. They could repeat this as many times as necessary to complete the computerized VAS ratings for that sample. Participants were asked to rinse their mouth with water before moving on to the next sample. Once all five samples had been rated the experimenter was called.

At this stage, those in the control condition repeated the initial VAS mood ratings and then left the laboratory. For one hour post test they were asked to refrain from eating and drinking except water, and then completed a paper VAS mood rating with the same set of questions (Appendix 2), and anchored in the same way, as those rated on-line in the laboratory, which they returned at their next session.

Participants in the test conditions received a 400g serving of either the HE or LE version of sample A (according to which condition and stage of the study they were in; see Table 4.1). They were instructed to consume all of the drink, after which they repeated the tasting and rating of the five samples, and the VAS mood scales. As in the control condition, they were given a paper VAS scale to complete one hour post test, during which they could consume only water.

This procedure was identical for all eight sessions, except in the final session where no post test mood scale was administered. Participants were fully debriefed, height and weight was recorded and they received £30 to reimburse them for their time (consent and debrief forms in Appendix 3).

4.2.6 Data analysis

4.2.6.1 Preliminary analysis

As discussed in Section 2.4.4 normality of the dependant variables was assessed using Kolmogorov-Smirnov and Shapiro Wilk tests, alongside examination of boxplots for significant outliers. Change from baseline pleasantness data was the primary outcome measure, and when normality was examined three outliers were identified as problematic for the data. When these outliers were removed from the data normality issues were resolved, therefore these were removed from all subsequent analyses.

4.2.6.2 Main analysis

One-way independent ANOVAs were conducted to explore group differences in age and BMI. Two-way independent ANOVAs were conducted to explore any baseline differences between conditions and gender. To investigate pleasantness and appetite changes, a series of three-way mixed ANOVAs (day*condition*gender) were conducted separately for the training and extinction phases of the study. During the training phase, the HE exposure and extinction conditions were combined as the training at this stage was identical, and then split for the extinction phase. Unless otherwise stated, Bonferroni post hoc tests were used to examine significant differences. Where sphericity could not be assumed the appropriate corrected statistics were reported ($\varepsilon < .75$ Greenhouse-Geisser, $\varepsilon > .75$ Huynh-Feldt).

4.3 Results

4.3.1 Pleasantness data

4.3.1.1 Baseline ratings

In order to examine whether pleasantness ratings changed over time, it was important to determine whether there were any differences at baseline. There were no baseline pleasantness differences between conditions ($F(3,60) = 0.52$, $p = .672$). There was, however, a baseline pleasantness difference according to gender ($F(1,60) = 9.36$, $p =$

.003), with females (*M* = 44.0 ± 3.2) rating the drinks as significantly less pleasant at baseline than the males (*M* = 58.5 ± 3.6). There was also a significant condition*gender interaction at baseline ($F(3,60) = 5.32$, $p = .003$) with females in HE exposure and control conditions rating the target flavour at baseline as less pleasant than males (see Table 4.4 for descriptives). At this point, all participants had been exposed to the same drink so these baseline differences could not be attributed to experimental factors. Therefore, gender was factored into all subsequent analyses.

	HE Exposure	**Extinction**	**LE Exposure**	**Control**
Male	68.2 ± 6.9	47.8 ± 7.4	52.0 ± 7.4	66.1 ± 6.9
Female	40.9 ± 6.2	54.5 ± 6.5	53.1 ± 6.5	27.3 ± 6.2

Table 4.4: Mean (±SEM) baseline pleasantness of drink between genders in each condition in Study One.

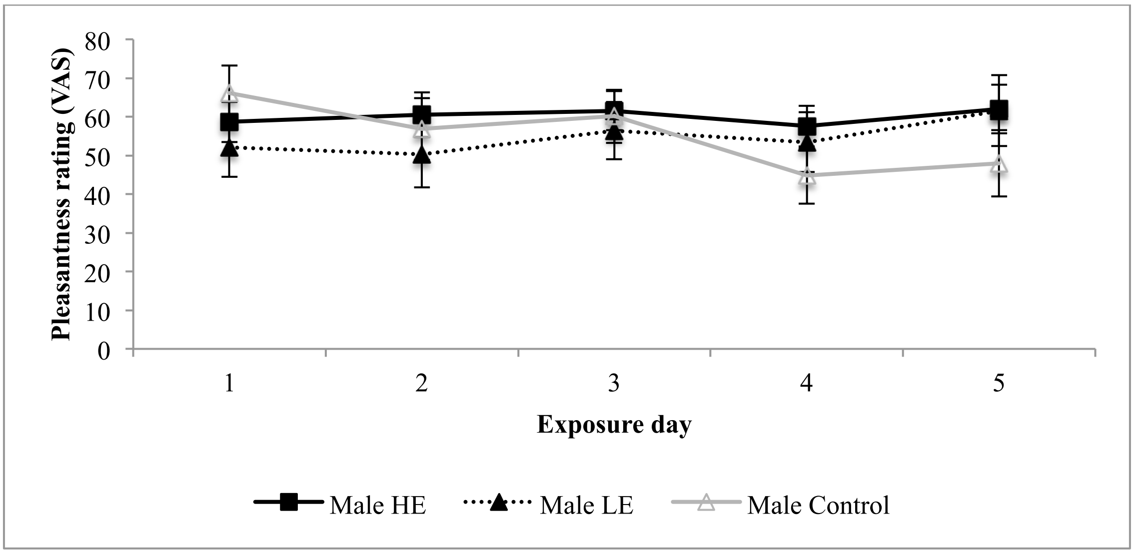
4.3.1.2 Change in pleasantness

Both absolute, and change from baseline, pleasantness ratings were analysed to determine if the HE drink resulted in an acquired liking compared to the LE drink. As described above, analysis was split into two phases: training and extinction. During the training phase, based upon the flavour learning literature, those consuming the HE drink (HE Exposure and Extinction conditions) were predicted to show increased pleasantness ratings, whereas those consuming the LE version (LE exposure) were predicted to rate the drinks as less pleasant than those in the HE, with perhaps a slight increase over the training phase as a result of exposure. In the second phase (sessions five to eight) it was predicted that those in the HE exposure condition would continue to rate the drink as pleasant, and those who had only been exposed to the LE drink (LE exposure) would continue to rate the drinks as less pleasant. Those undergoing extinction were the condition of most interest; did extinction of liking occur once the energy was removed or were pleasantness ratings maintained during the second phase? Finally, the control condition was designed to test exposure effects only, therefore pleasantness ratings were predicted to remain low and stable across sessions.

4.3.1.3 Absolute pleasantness ratings: training phase

During the training phase, there were no significant differences in rated pleasantness between conditions, ($F(2,62) = 1.50$, $p = .232$), or over days, ($F(4,248) = 1.56$, $p = .185$), with no day*condition interaction, ($F(8,248) = 0.46$, $p = .884$). Although no main effect of gender ($F(1,62) = 1.96$, $p = .167$) or condition*gender interaction ($F(2,62) = 0.52$, $p = .596$), there was a significant day*gender interaction, ($F(4,248) = 2.58$, $p = .038$), and a day*condition*gender interaction that was approaching significance, ($F(8,248) = 1.96$, $p = .053$). Separate Bonferroni corrected one way independent ANOVAs were conducted at each time point to break down the condition*gender interaction, and the only day where a significant difference was observed was day one (baseline) $F(1,66) = 9.16$, $p = .004$, a reflection of the baseline differences discussed above. Figure 4.1 plots these, and it appeared that the baseline pleasantness differences identified earlier also provided an explanation for the significant three way interaction.

a)



b)

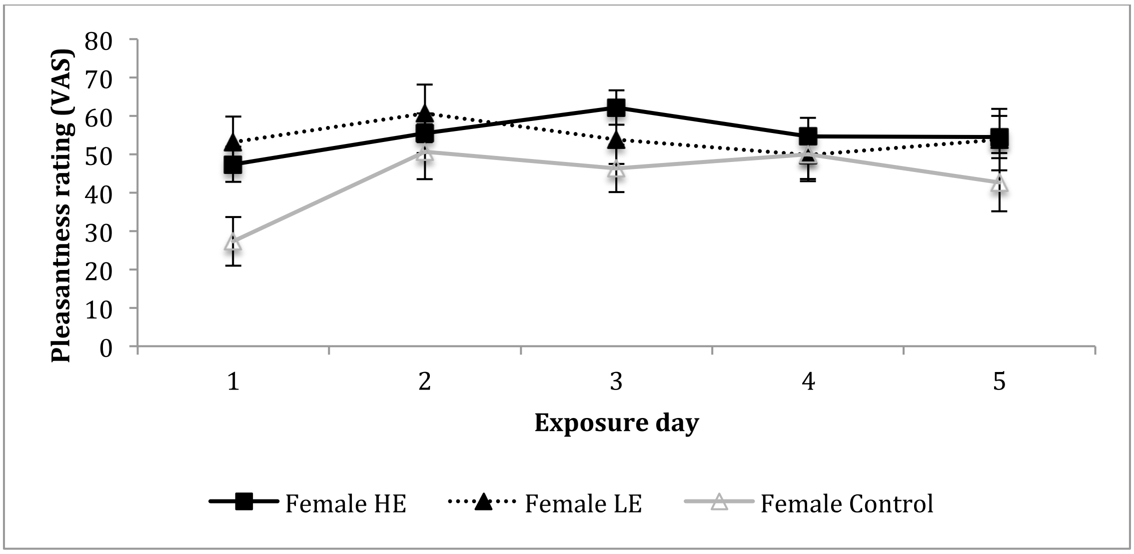
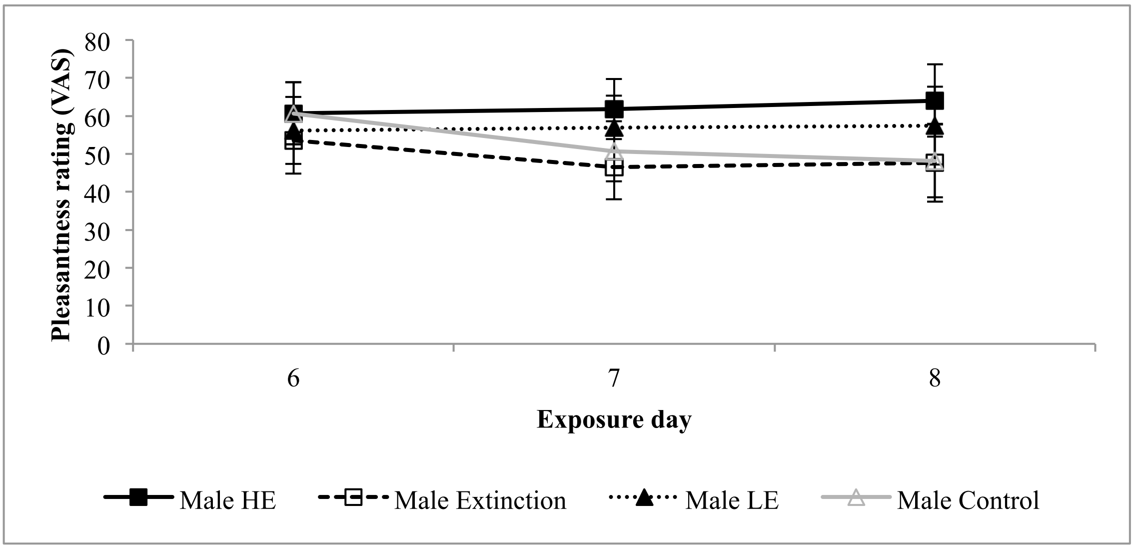


Figure 4.1: Mean (±SEM) absolute pleasantness ratings of drink over time for a) males and b) females during the training phase of Study One.

4.3.1.4 Absolute pleasantness ratings: extinction phase

Although there was no evidence of an acquired liking for the HE compared to the LE drinks, patterns of liking during the extinction phase were still examined. There were no main effects of day ($F(2,120) = 0.90$, $p = .411$), condition ($F(3,60) = 0.34$, $p = .799$) or gender ($F(1,60) = 0.02$, $p = .910$), and no significant interactions: day*condition ($F(6,120) = 0.24$, $p = .964$), day*gender ($F(2,120) = 0.04$, $p = .963$), condition*gender ($F(3,60) = 1.18$, $p = .323$), day*condition*gender ($F(6,120) = 0.76$, $p = .600$). Liking ratings are shown in Figure 4.2 and as can be clearly seen, males consuming the HE drink during the extinction phase rated the drink as more pleasant than the other conditions, with those in the extinction condition rating the drink as least pleasant. In females, this pattern was different, with those in the extinction condition rating the drink as most pleasant and all other conditions rated similarly. All ratings were fairly stable across time.

a)



b)

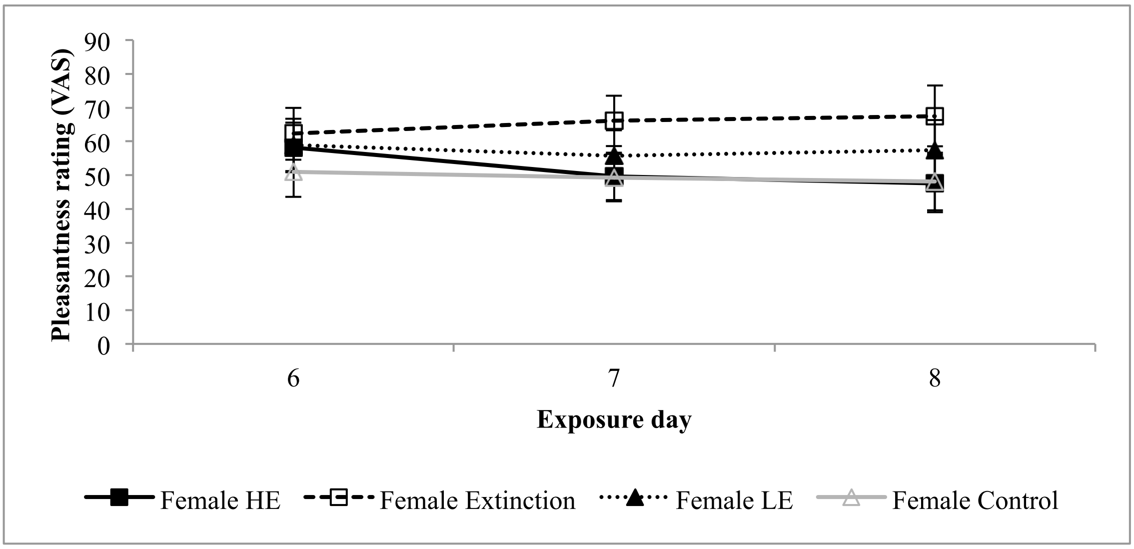
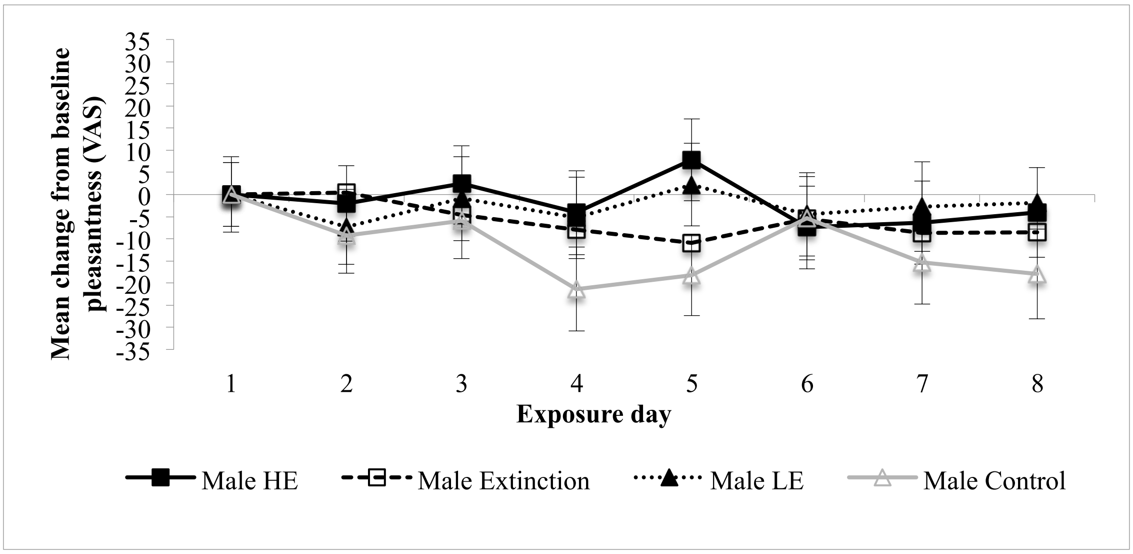


Figure 4.2: Mean (±SEM) pleasantness ratings over time for a) males and b) females during the extinction phase of Study One.

4.3.1.5 Change in pleasantness

The unexpected baseline differences could have masked any effects of learning based on analysis of all ratings. An alternative method for assessing learning is to consider change from baseline, although here again the baseline differences mean that caution might be needed in interpreting change data. Figure 4.3 shows the data for all eight days to visually examine the effect of extinction on changes in pleasantness from baseline.

a)



b)

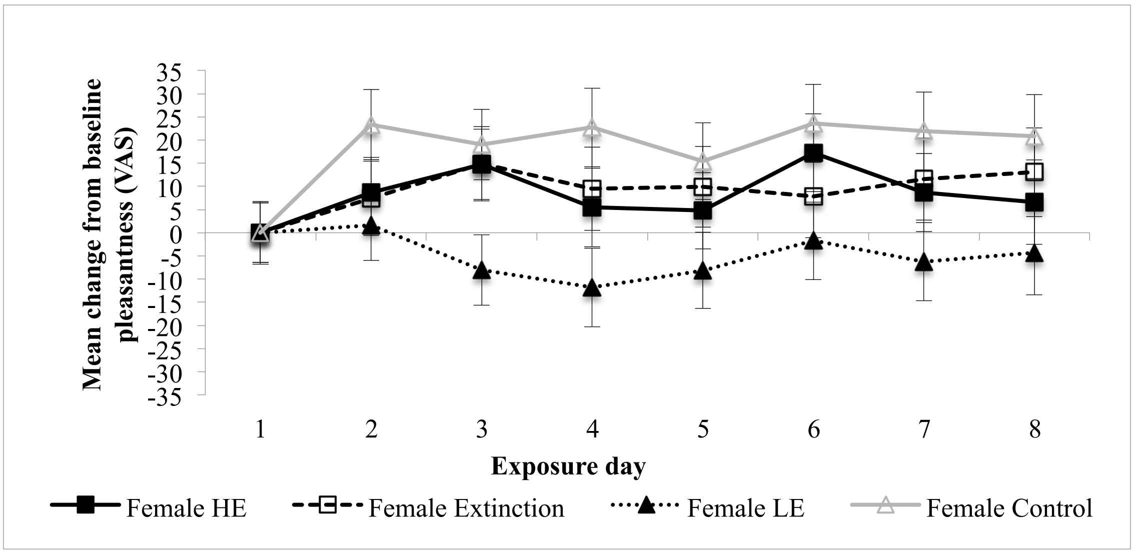


Figure 4.3: Mean (±SEM) change from baseline pleasantness ratings in a) males and b) females in Study One. Day 1 ratings have been zeroed and change from baseline plotted. Day 6 marks the first rating after removal of sucrose in the extinction condition.

4.3.1.6 Change in pleasantness over the training phase

Change from baseline (day one) scores were calculated by subtracting pleasantness on day one from each subsequent day during training (days two, three, four and five). When expressed as a change from baseline pleasantness, there was no significant change from baseline pleasantness over days ($F(3,186) = 1.13$, $p = .340$), and no interaction effects with day: condition*day (*F*(6,186) = 0.51, p = .798), gender*day (*F*(3,186) = 0.63, p = .596) and gender*condition*day interaction (*F* (6,186) = 0.93, p = .473). There was also no significant change from baseline pleasantness between conditions (*F*(2,62) = 0.25, p = .781). It was predicted that consuming the HE drink would result in increased pleasantness over time, and that consuming the LE drink would lead to decreased pleasantness. As discussed below, the difference between those consuming the HE and LE drinks in the training phase does follow this pattern but only in females.

There were gender differences in change from baseline pleasantness over the learning phase (*F*(1,62) = 10.07, p = .002). Regardless of time and condition, females showed increased pleasantness scores from baseline (*M* = 10.3 ± 2.8) whereas males showed decreased pleasantness from baseline (*M* = -2.8 ± 3.1).

There was also a significant gender*condition interaction (*F*(2,62) = 5.88, p = .005; see Figure 4.3). When consuming the low energy drink during training, there was a similar slight increase from baseline in males (*M* = 3.4 ± 6.1) and females (*M* = 1.5 ± 5.4). After consuming the HE drink during this time, females rated the drink as more pleasant (*M* = 9.4 ± 3.7) than at baseline, and males showed a very slight increase in pleasantness (*M* = 1.8 ± 4.2) than at baseline. In the control condition, females rated the drink as more pleasant (*M* = 20.1 ± 5.3) than baseline and males rated the drink as less pleasant (M = -13.7 ± 6.7). Independent t-tests were conducted to break down this interaction, and males and females only differed significantly in the control condition (*t*(16) = -4.03, $p < .001$). From Figure 4.3 it can also be observed that females appeared to be more sensitive to the energy difference during the training phase (days one-five), as there was a differentiation in change from baseline pleasantness between the HE and LE versions, whereas for males over the first section of the graph, change ratings were very similar between the LE and HE conditions.

4.3.1.7 Change in pleasantness over the extinction phase

Again, as no acquired liking was demonstrated, it was difficult to draw any conclusions regarding extinction, but the pattern of change was still of interest. Changes during the extinction phase were calculated, by subtracting pleasantness scores on day five (the final rating before extinction occurred) from the subsequent extinction pleasantness scores (days six, seven and eight). For those in the extinction condition, if sensitive to the removal of energy, pleasantness ratings were expected to decrease, but if resistant to this change ratings would follow the same pattern as those continuing to consume the HE drink. The significant gender difference was maintained during the extinction phase ($F(1,60) = 4.29$, $p = .043$) with a trend for a gender*condition interaction ($F(3,60) = 2.29$, $p = .087$). As before, females rated the drink as more pleasant ($M = 4.3 \pm 2.2$) than day five regardless of the energy content, and males rated the drink as less pleasant ($M = -2.5 \pm 2.4$) than day five.

There was no significant change from day five pleasantness over days ($F(2,120) = 0.90$, $p = .411$), and no interaction effects with days: condition*day ($F(6,120) = 0.24$, $p = .964$), gender*day ($F(2,120) = 0.04$, $p = .963$) and gender*condition*day interaction ($F(6,120) = 0.76$, $p = .600$). There was also no significant change from day five pleasantness between conditions ($F(3,60) = 1.70$, $p = .176$).

Although no conclusions could be made regarding extinction due to no evidence of acquired liking, it appeared that extinction did not influence the rated pleasantness of the drink, as there was no marked change of the pleasantness of the drink for those in the extinction condition where energy had been removed after day five.

4.3.2 Change in pleasantness ratings of drink samples

As no significant differences in pleasantness change across conditions and time in target flavour were shown, it was interesting to compare this to how the other samples changed over time. Did change in pleasantness of target flavour differ to change in pleasantness of the other flavoured samples? Change from baseline pleasantness was examined rather than the rated pleasantness of each sample on each session, as samples were initially designed to differ in pleasantness, with the target sample neutral in order to maximise FNL. It was predicted that there would be small increases in the pleasantness of non-target samples due to exposure, but that the changes would be greater in the target sample for those who consumed the larger samples (HE Exposure, Extinction and LE Exposure). Change data were only analysed during the training phase as the extinction phase should not result in differences in the other samples and changes in the target sample had already been examined in the previous analyses.

4.3.2.1 Baseline data

As with the analysis conducted purely on the target sample A, baseline differences between conditions and gender in the pleasantness ratings of each sample were examined. There were no baseline differences in pleasantness ratings between conditions or gender for any of the samples except for pleasantness of flavour B, where females ($M = 71.8 \pm 2.6$) rated it as significantly more pleasant than males ($M = 63.3 \pm 3.0$, $p = .035$), and with no interaction effects in any analyses.

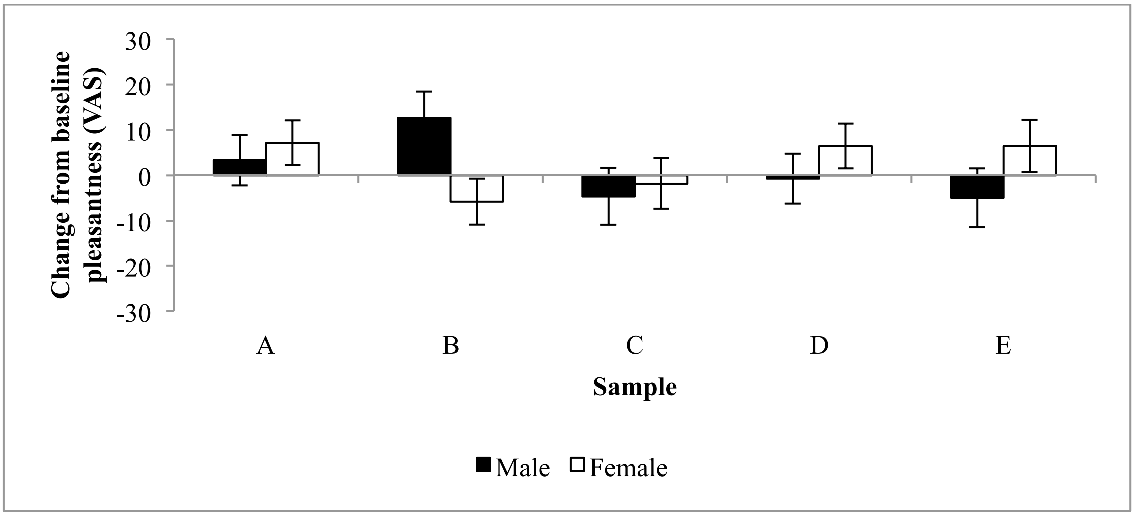
4.3.2.2 Change from baseline pleasantness

Change scores were calculated for each sample, by subtracting pleasantness at baseline from pleasantness on day five, allowing changes for each flavoured sample to be examined in the training phase.

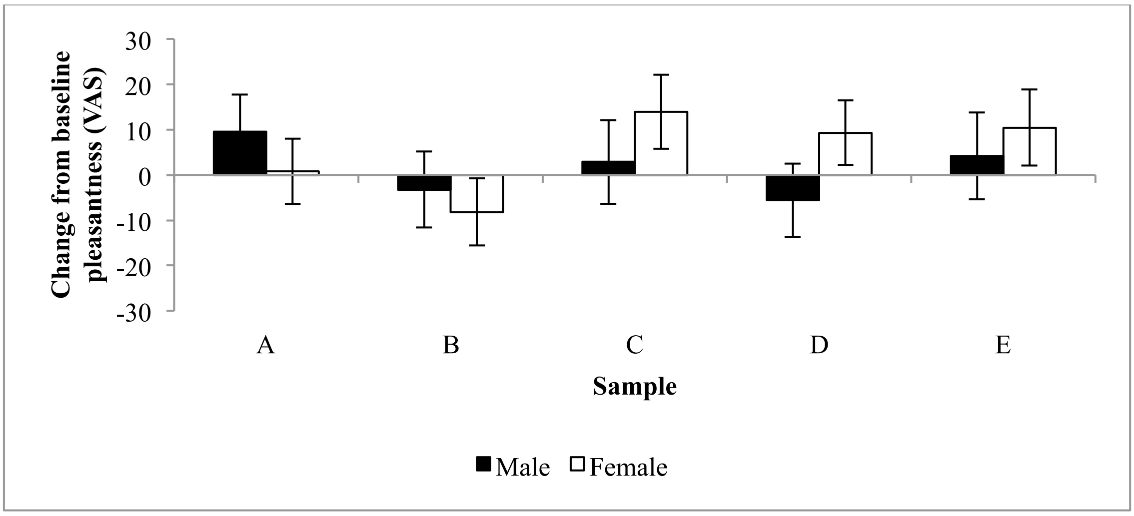
There were no significant differences in change from baseline pleasantness within the different samples $F(4,248) = 0.87$, $p = .483$, conditions $F(2,62) = 2.01$, $p = .142$ or gender $F(1,62) = 0.70$, $p = .407$. There were also no significant condition*gender $F(2,62) = 0.05$, $p = .947$ or sample*condition $F(8,248) = 0.79$, $p = .614$ interactions. There was a sample*gender interaction approaching significance $F(4,248) = 2.13$, $p = .077$ and a significant sample*gender*condition interaction $F(8,248) = 1.97$, $p = .051$.

Figure 4.4 shows the change data for the training phase, and it can be seen that there were small differences in pleasantness changes between the samples and genders, but there was little evidence of a greater change for the target flavour in those who had consumed the larger version (Figures 4a and b), or in those who had consumed the flavour paired with energy (Figure 4a). In fact, the largest difference appears to have occurred in females in the control condition, where no difference in pleasantness change was predicted as all samples were exposed to the same extent.

a) HE conditions (HE Exposure and Extinction)



b) LE Exposure condition



c) Control condition

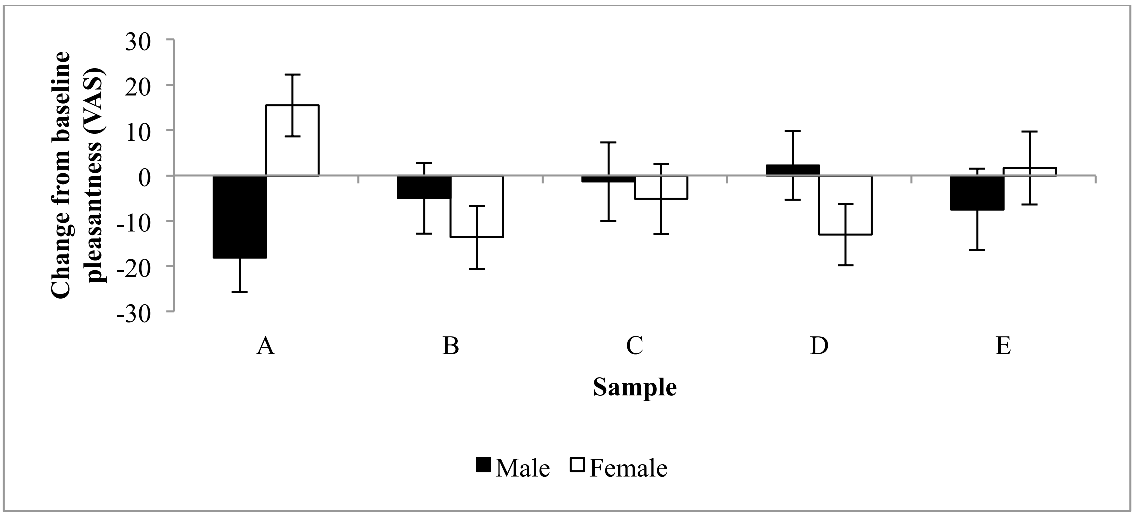


Figure 4.4: Mean (±SEM) change from baseline pleasantness for the five flavoured samples in Study One.

4.3.3 Appetite ratings: Hunger

Alongside changes in pleasantness, it was important to examine appetite and to determine whether the energy differences were sufficient between the versions to have the desired satiating effect in the HE conditions. It was predicted that those consuming the HE drink would display a greater reduction in hunger across the session compared with those consuming the LE drink. Again, the extinction group were of interest, as if there was a larger reduction in hunger during the training phase, this could have been maintained or lost after extinction, perhaps indicating either resistance, or sensitivity, to extinction.

There were no baseline differences in hunger for any of the sessions, between genders (for all $p > .256$) or conditions (for all $p > .090$) or gender*condition interaction (for all $p > .078$). Therefore change in hunger ratings across session were analysed.

4.3.3.1 Hunger changes: training phase

Change data was calculated over session and analysed for days one to four in the training phase.

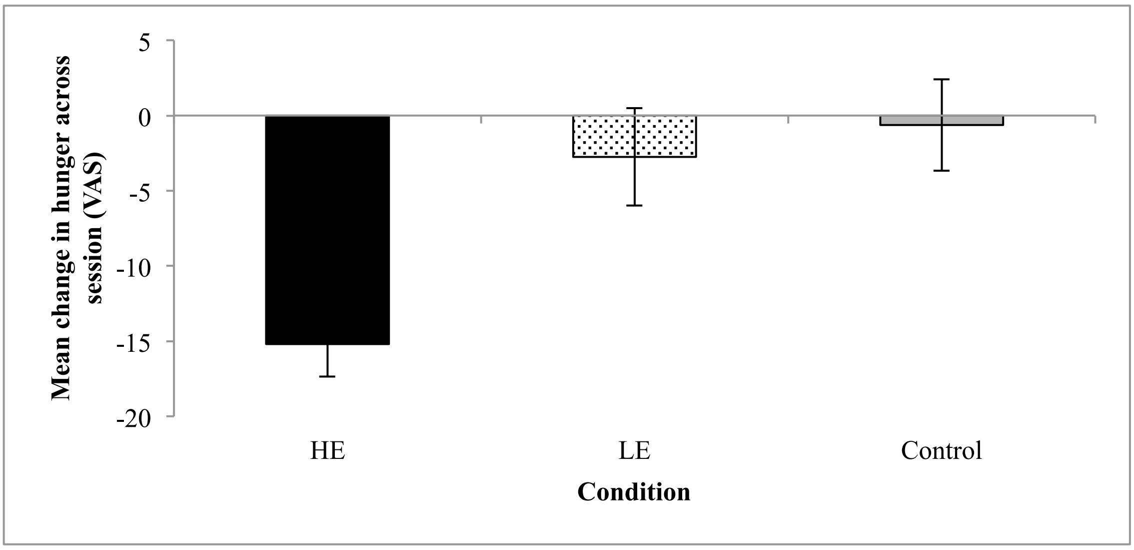


Figure 4.5: Mean (±SEM) hunger reduction across the session for each condition during the training phase of Study One.

During the training phase, the energy content of the drink consumed significantly affected the change in hunger over the session, ($F(2,59) = 9.67$, $p < .001$; Figure 4.5). Bonferroni post hoc tests confirmed that, as predicted, consuming the HE drink led to a significantly larger reduction in hunger (*M* = -15.2 ± 2.2) than consuming the LE (*M* = -2.8 ± 3.3, *p* = .007) or no drink at all (*M* = -0.63 ±3.0, *p* < .001). The difference between the LE and Control was not significant (*p* > .999)

There was no significant effect of day (*F*(3,177) = 1.33, *p* = .266) or gender (*F*(1,59) = 0.01, *p* = .693) on change in hunger, and no interaction effects: day*gender (*F*(3,177) = 0.50, *p* = .681), day*condition (*F*(6,177) = 0.91, *p* = .492), day*gender*condition (*F*(6,186) = 0.28, *p* = .947) or gender*condition (*F*(2,59) = 0.06, *p* = .946).

The difference in hunger reduction between conditions did not continue to be significant at one hour post test (*F*(2,56) = 2.31, *p* = .108), with all conditions showing an increase in hunger, although those who consumed the HE drink showed a lower increase in hunger compared to the other conditions (HE: *M* = 2.8 ± 2.9, LE: *M* = 10.5 ± 4.0, Control: *M* = 11.9 ± 3.8). All other effects on hunger change were also non significant at one hour post test: gender (*F*(1,56) = 0.31, *p* = .581), day (*F*(3,168) = 0.06, *p* = .982), day*condition (*F*(6,168) = 0.63, *p* = .707), day*gender (*F* (3,168) = 0.85, *p* = .468), condition*gender (*F*(2,56) = 0.06, *p* = .941), or day*gender*condition (*F*(6,168) = 1.28, *p* = .273).

Therefore, the energy difference did appear to be sufficient to result in differing reduction of hunger during the session, but this was not maintained one hour post test.

4.3.3.2 Hunger changes: extinction phase

In the same way as change data was calculated previously, change in hunger across session for days five to eight were calculated.

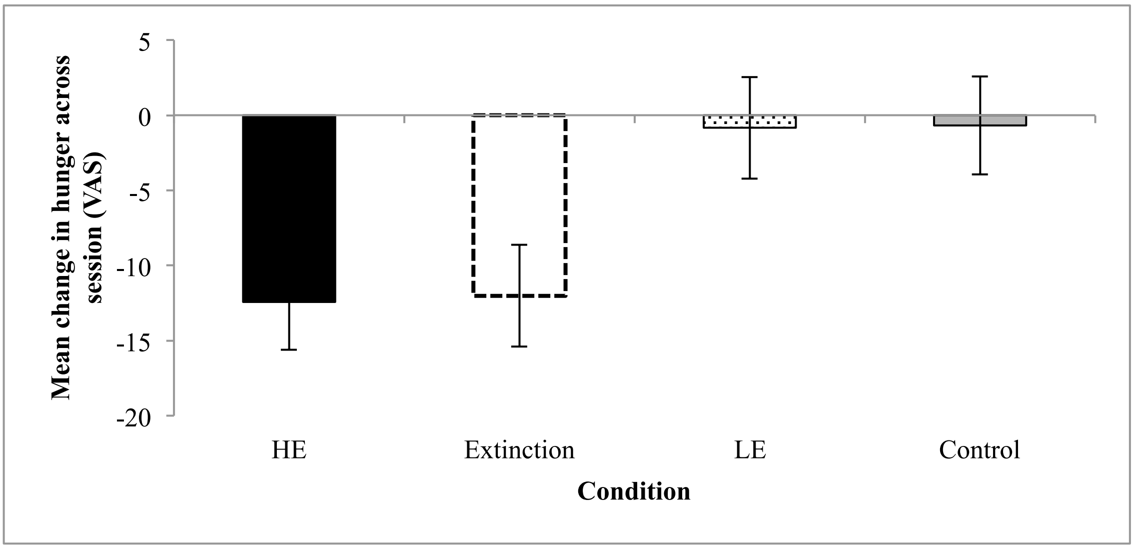
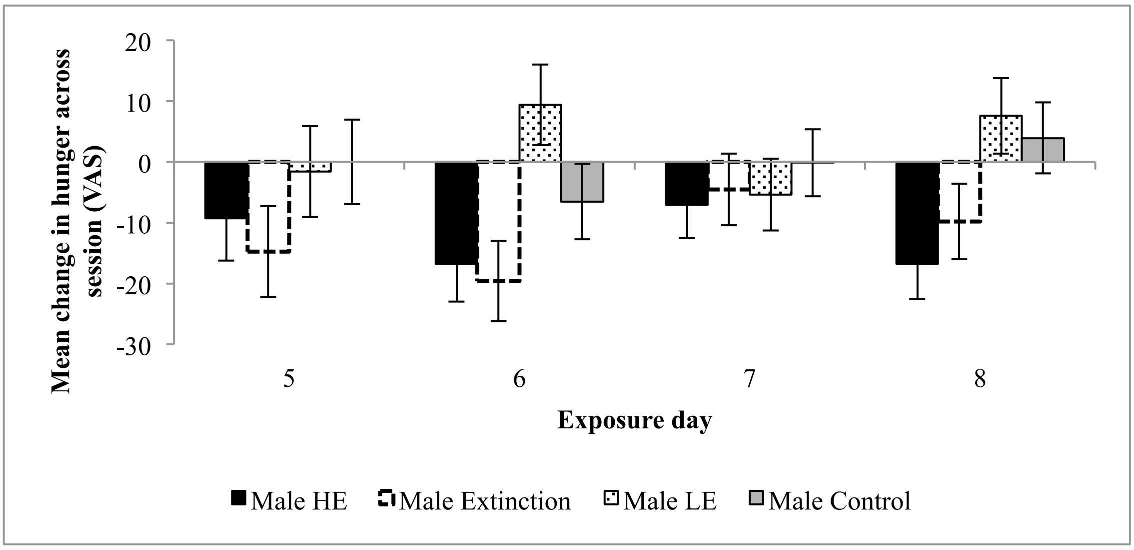


Figure 4.6: Mean (±SEM) change in hunger over the session for each condition during the extinction phase of Study One.

During the extinction phase the effect of energy content was still significant (F(3,59) = 4.03, p = .011). Post hoc tests revealed differences approaching significance between the HE exposure condition (M = -12.4 ± 3.2) and Control conditions (M = -0.83 ±3.4, p = .076). From Figure 4.6, it can be seen that those in the HE and extinction conditions were demonstrating a similar hunger reduction across session, which was a larger reduction than in the LE and control conditions. This larger reduction in hunger was predicted in those who were consuming the HE drink compared to the LE drink. It is interesting to note that this hunger reduction was also maintained in the extinction condition despite the absence of energy, suggesting the association between flavour and hunger reduction during the training phase continued even when the energy was no longer present.

As with the training phase, there were no significant effects of day (F(3,177) = 1.36, p = .255), or gender (F(1,59) = 0.23, p = .631) on change in hunger within session, and no other interaction effects: day*gender (F(3,177) = 0.07, p = .975), day*condition (F(9,177) = 0.67, p = .732) or gender*condition (F(3,59) = 0.25, p = .859. There was, however, a significant day*gender*condition interaction (F(9,186) = 2.52, p = .010). From Figure 4.7, it appears that males and females consistently showed a reduction in hunger for both the HE and extinction conditions, but that hunger differences across sessions fluctuated more for males in the LE and control conditions.

a)



b)

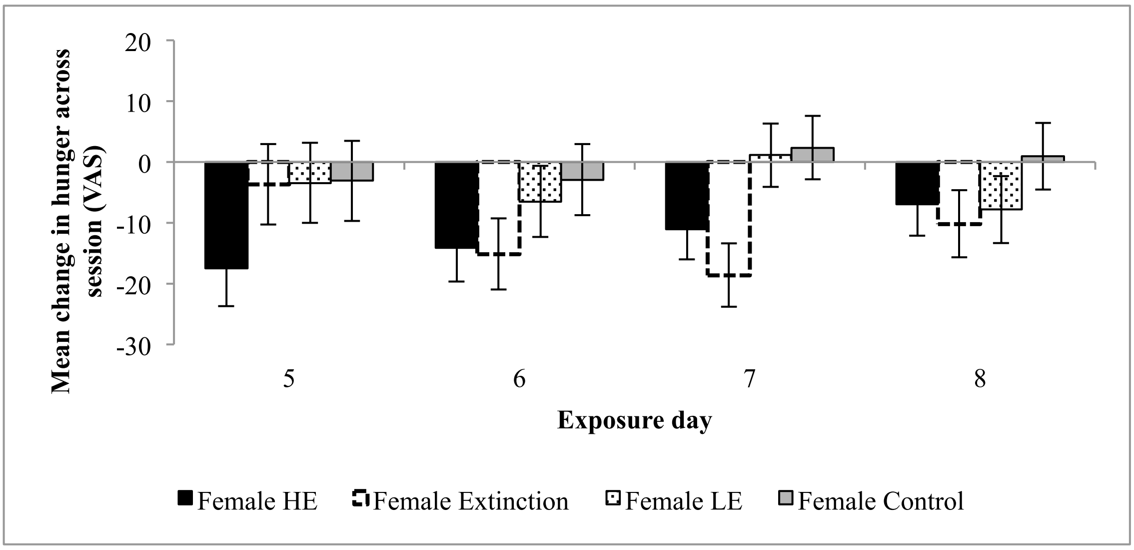


Figure 4.7: Mean (±SEM) change in hunger across sessions between conditions for a) males and b) females during Study One.

There were no significant differences in hunger change at one hour post test during the extinction phase: day (F(3.0,150.0) = 1.68, p = .174), gender (F(1,50) = 0.23, p = .635), condition (F(3,50) = 0.61, p = .612), day*gender (F(3.0,150.0) = 0.49, p = .690), day*condition (F(9.0,150.0) = 1.09, p = .374), gender*condition (F(3,50) = 2.09, p = .113), day*gender*condition (F(9.0,150.0) = 1.11, p = .363).

The results demonstrated that hunger reduction was maintained in the extinction group even though the energy had been removed from the drink, suggesting resistance to extinction. Again, these differences were not maintained one hour post test.

4.3.4 Appetite ratings: fullness

It was predicted that fullness changes would occur in an inverse pattern to the hunger ratings, with those consuming the HE drink reporting larger increases in fullness than those in the LE or control conditions. Based upon the results from the hunger data, it was also predicted that those in the extinction condition would maintain similar fullness change to those in the HE condition rather than the LE condition, despite energy no longer being present.

4.3.4.1 Fullness changes: training phase

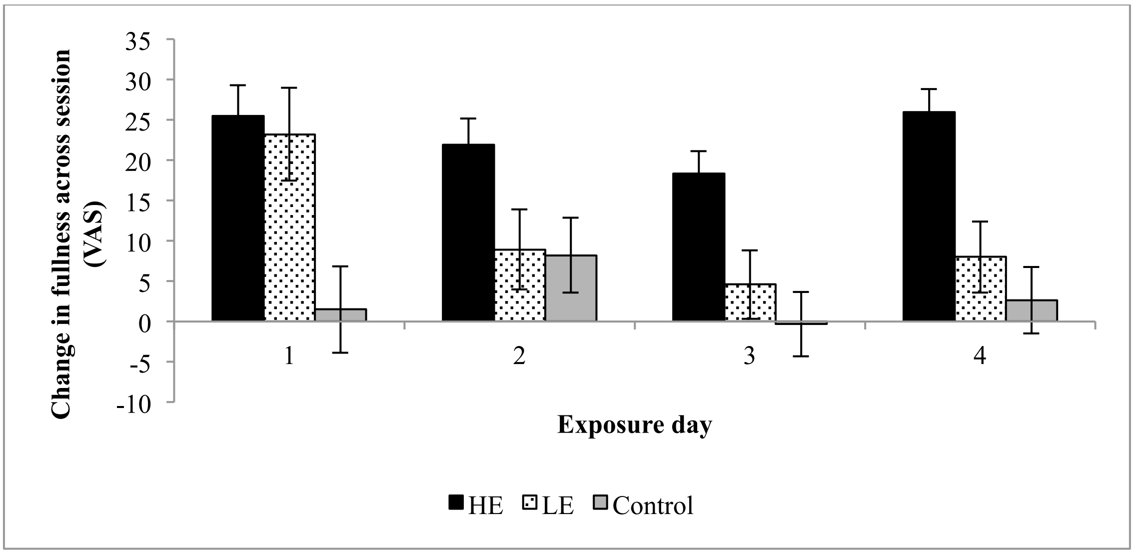


Figure 4.8: Interaction of fullness change across sessions and between conditions during the training phase of Study One (mean change ± SEM).

Homogeneity of variance was violated for three out of four days during the training phase, therefore the data should be treated with caution. Change in fullness ratings during the training phase were significantly different depending on the energy content of the drink consumed, $F(2,60) = 11.98$, $p < .001$, with those consuming the HE drink ($M = 22.9 \pm 2.4$) becoming significantly more full over the session than those consuming both the LE drink ($M = 11.2 \pm 3.7$, $p = .030$) and the control samples ($M = 3.0 \pm 3.4$. $p < .001$). There was also a significant effect of day, $F(3,180) = 4.09$, $p = .008$ although only between days one ($M = 16.8 \pm 2.9$) and three ($M = 7.5 \pm 2.2$, $p = .014$).

There was a significant day*condition interaction ($F(6,180) = 2.14$, $p = .051$ which was broken down with Bonferroni corrected ANOVAs, and as homogeneity of variance was violated for days one and four, Welch statistics were reported for those days. These tests revealed fullness change differences between conditions were significant on days one $F(2, 32.02) = 13.45$, $p < .001$, three $F(2, 65) = 8.86$, $p < .001$ and four $F(2, 36.32) = 14.67$, $p < .001$. Post hoc tests showed that those consuming the HE drink showed a significantly larger increase in fullness across the session compared to the control condition on days one ($p = .001$), three ($p = .001$) and four ($p < .001$), and to the LE condition on days three ($p = .010$) and four ($p = 004$). On day one, the LE showed a larger increase in fullness over the session compared to control ($p = .012$), but this was not significantly different on any of the other sessions. Figure 4.8 demonstrates these differences.

In contrast to the hunger data, there was a trend for a difference between gender on fullness change, $F(1,60) = 3.13$, $p = .082$, with females ($M = 15.7 \pm 2.4$) reporting a larger increase in fullness over the session than males ($M = 9.1 \pm 2.9$). This did not significantly interact with day $F(3,180) = 0.37$, $p = .775$ or condition ($F(2,60) = 0.37$, $p = .695$). Finally, there was also no significant day*gender*condition interaction $F(6,180) = 1.47$, $p = .191$.

4.3.4.2 Fullness changes: extinction phase

During the extinction phase the significant differences in fullness change between conditions ($F(3,59) = 6.60$, $p = .001$) was maintained. Those in the HE condition showed a significantly larger increase in fullness ($M = 21.97 \pm 3.7$) compared to the control condition ($M = 2.9 \pm 3.7$, $p = .004$) which in turn showed a significantly lower increase in fullness compared to the extinction condition ($M = 22.90 \pm 4.0$, $p = .003$).

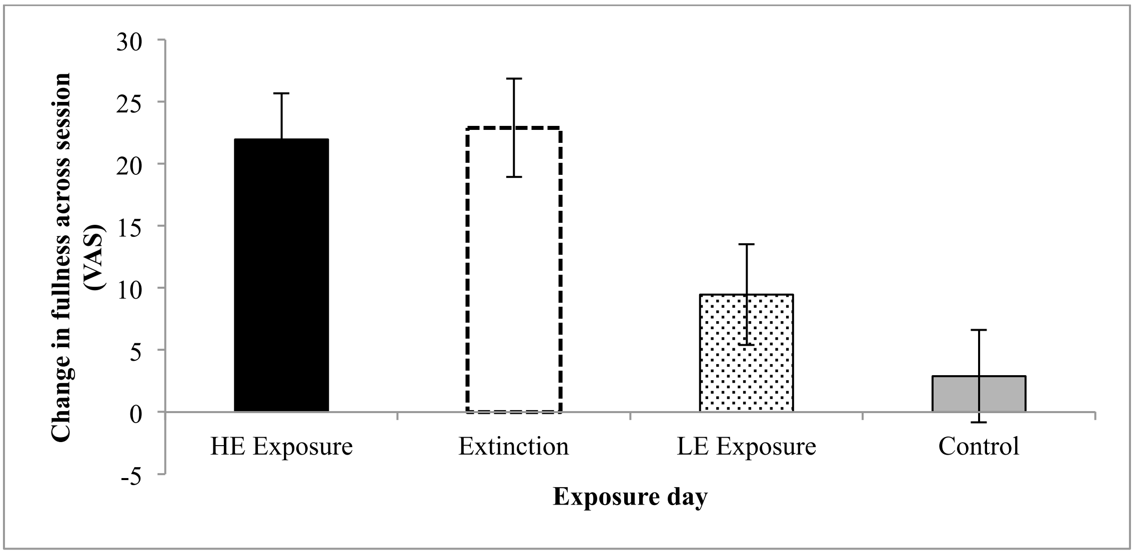


Fig 4.9: Mean (±SEM) change in fullness ratings over session for each condition during the extinction phase of Study One.

Although not significantly different from the LE condition, it can be seen from the graph (Figure 4.9) that those in the extinction condition were behaving in line with those in the HE condition, with very small difference in means between the groups. This suggested that, like with hunger changes, fullness changes remained consistent with consumption of the HE drink rather than the LE version that was being consumed during this phase.

There also continued to be a trend for gender differences ($F(1,59) = 3.35$, $p = .072$) in the same direction as during the training phase, with females ($M = 17.8 \pm 2.9$) reporting a greater increase in fullness than males ($M = 10.8 \pm 2.9$).

There were no other significant differences in fullness ratings during the extinction phase: day $F(3,177) = 1.03$, $p = .381$, day*gender $F(3,177) = 1.72$, $p = .164$, day*condition $F(9,177)=1.17$, $p = .316$, condition*gender $F(3,59) = 0.08$, $p = .972$, or day*condition*gender $F(9,177) = 1.60$, $p = .118$.

The fullness change findings supported the patterns shown in the hunger change data.

4.4 Discussion

The aim of the present study was to investigate whether extinction of an acquired liking for a flavour would occur within a FNL context, when energy was used as the reinforcer. To summarise the main findings, the predicted acquired liking for a HE version of a drink was not demonstrated, therefore conclusions regarding extinction were not possible. However, pleasantness ratings for those in the extinction condition remained relatively stable in the second phase of the study, despite the energy no longer being present in the drink, and appeared to stay in line with the ratings of those who continued to consume the HE drink. This was supported by the changes in appetite ratings, which suggested that those in the extinction condition perceived little change when the energy had been removed. The appetite changes were also in the predicted direction, which indicated that the energy differences were detected between conditions.

Evidence of FCL with humans, particularly when using nutrients as the reinforcer, is limited (as reviewed by Brunstrom, 2007; Yeomans, 2006b; Yeomans, 2008). The lack of acquired liking for HE compared to LE paired flavours in this study are in accord with other literature where flavour consequence learning has been shown to occur independently from pleasantness changes (Capaldi & Privitera, 2007; Yeomans, et al., 2008b). This could help to explain why the changes in appetite during training for those consuming the HE drink did not become associated with the flavour of the drink. In the study by Yeomans et al., (2008b), increased intake of a sorbet after training with HE versions of a drink was demonstrated regardless of taste, whereas increased liking was only demonstrated in the sweet version.

However, the failure to show evidence of acquired liking in this study is in contrast to the human studies where preferences have been conditioned based upon a number of nutrient reinforcers, such as carbohydrate (e.g. Birch, et al., 1990), fat (e.g. Johnson, et al., 1991), caffeine (e.g. Yeomans, et al., 2000a) and protein (Gibson, et al., 1995). In a review by Yeomans (2010) a number of individual differences which can influence whether FCL occurs were discussed, and this study attempted to control for these issues to maximise potential for learning. Individuals who scored low on the restraint scale of the TFEQ (Stunkard & Messick, 1985) were selected, and restriction of food intake prior to the testing session ensured that participants were similarly hungry upon arrival, as motivational state has been shown to be an influential factor in flavour preference learning and extinction (e.g. Yeomans, et al., 2000a).

There were potential methodological reasons for why acquired liking was not demonstrated. As shown in the difference between appetite changes, the energy difference between the high and low energy versions was sufficient. However, it may be that the large drink consumed was not associated with the corresponding sample during the taste test. From examining the changes in pleasantness of the target sample in comparison to changes of the other samples, the exposure to the large version of the drink did not appear to alter perceptions of the sample differentially to the other flavoured samples, indicating that no additional learning occurred. This could be overcome by explicit ratings of the large drink, to ensure that the association between the flavour and the energy is formed, an oversight of the current study. Additionally, exposure to all five stimuli may have overshadowed direct learning about the target drink compared to the non-target drinks, perhaps reflected by the increase in pleasantness of the target flavour for those females in the control condition, as a smaller number of samples were rated during each session. It is interesting to note that some samples did become more pleasant over time even with minimal exposure and no energy pairings, which is in line with the effect of mere exposure to increase liking for a novel flavour (Hausner, et al., 2012).

As sucrose was used as the nutrient in this study, another possible explanation for the failure to demonstrate acquired liking for the HE version would be sweet liker status of the participants. Although sweetness is considered to be an innately pleasant taste, there are individual differences (Looy, et al., 1992), and it has been shown that this can influence flavour learning (Yeomans, 2010). The sucrose could be acting as a US for both FFL and FNL in this study, and perhaps the lack of controlling for sweet dislikers can help to explain the findings.

Perhaps when using energy as a reinforcer, appetite ratings could be an alternative indication of whether flavour-consequence learning is resistant to extinction, as if there is no perceived change in appetite ratings it appears that the previous associations are maintained. This is of particular relevance to the food industry, as if a product continues to provide a reduction in hunger or increase in fullness when the energy that used to be paired with a flavour is removed, this is powerful for diet products.

Previous research has shown that weaker compensation is made for energy consumed in beverage compared to solid (DiMeglio & Mattes, 2000). Perhaps in this context, the appetite changes that occurred were not attributed to the drink because through evolutionary experience we have learned that liquids are not satiating, with exposure primarily to water or breast milk (Wolf, Bray, & Popkin, 2008). If the drinks were not expected to be satiating, this could explain why there was little evidence for acquired liking for the HE drink compared to the LE drink despite the differences in appetite ratings. Oro-sensory exposure and cephalic phase responses are reduced when we consume liquid compared to solids and this could impact our ability to learn about the sensory properties (De Graaf, 2011; Zijlstra, 2008), and this is probably registered in combination during a meal context rather than specifically liquid. Acquired liking has been conditioned through FNL with liquids used during training, although changes in hedonic ratings and intake were assessed using a sorbet version of the same flavour (Yeomans, et al., 2008b).

Gender differences were observed in relation to change from baseline pleasantness ratings. Females appeared to be more sensitive to the energy differences of the drinks, with those consuming the HE showing increased pleasantness and those consuming the LE showing decreased pleasantness, as would be predicted from flavour-consequence learning literature (Yeomans, et al., 2008b). Therefore, an acquired liking could have been demonstrated in the female participants but not in the male. This reflects broader gender differences that have been found with regards to eating behaviour, with females reported to consume reduced energy products more frequently than males (Fagerli & Wandel, 1999) and being more calorie conscious (Kiefer, Rathmanner, & Kunze, 2005). However, any discussion of these gender differences should be approached with caution, as it is equally plausible that these could be a result of regression to the mean (Barnett, van der Pols, & Dobson, 2005) rather than a reflection of any true gender difference. Females rated the drink as less pleasant at baseline than the males did and this may therefore be reflected in the change scores, with scores generally increasing towards the mean for females, and decreasing for males. As the baseline pleasantness of the drinks was significantly different between genders, this could not be entered into any analysis as a covariate (Miller & Chapman, 2001), which may have helped to account for any effect of regression to the mean.

In summary, due to no evidence of acquired liking relative to energy content, solid conclusions regarding extinction could not be drawn. However, appetite ratings tentatively suggest that flavour-energy associations remain after the removal of energy.

Chapter 5: Study Two - Extinction within a sorbet context

5.1.Introduction

Study One attempted to further investigate extinction of energy-based acquired liking in humans. However, as evidence of acquired liking was not demonstrated in those trained with a HE drink compared to a LE drink, conclusions regarding extinction could not be drawn. Nonetheless, appetite ratings, and pattern of pleasantness ratings, indicated that there was no evidence of extinction in those who experienced the removal of energy after the initial four training sessions. Study Two attempts to extend upon these findings, adopting the methodology from a paradigm where evidence of acquired liking has previously been demonstrated (Yeomans, et al., 2008b).

Yeomans et al., (2008b) used FFL and FNL to condition an acquired liking for a novel flavoured sorbet after training with drinks of the same flavour. Both conditions where energy was added to the drink increased subsequent sorbet intake, but pleasantness increases for the sorbet were only observed when the drink combined both learning processes (HE and sweetened). As liking and intake changes were demonstrated within that study, the present study compared those training with a sweetened HE drink with those training with a sweetened LE drink, and extended the design to include additional extinction trials, where energy was no longer present for some individuals.

Due to the different behaviour demonstrated between males and females in the previous study, it was decided that only low restrained females would be recruited for this study. Females appeared more sensitive to the energy differences between the training conditions, as those in the HE rated the drinks as more pleasant than the LE, in line with FNL. Males, surprisingly, tended to show decreased pleasantness over time in the HE condition. Also, due to the large variance in scores in the exposure control condition, which may have generated unexpected demand effects, it was decided that this would not be included in the present study. Sorbet was used as a test food in an attempt to control for some suggested issues with liquids as vehicles of learning (De Graaf, 2011; Zijlstra, 2008) that may have impacted the findings in the previous study, and this would allow manipulation of energy content in a liquid context, whilst increasing oral exposure using a solid context at test.

The present study took place over 11 days, within a three to four week period, with the procedure varying across the sessions. On days one, six and eleven, all experimental conditions consumed a LE sorbet *ad libitum* and on all other test days, a flavour matched drink was consumed (low or high energy dependent upon experimental condition). As in Study One, those in the extinction condition were switched from HE to LE drink, with the energy being removed on day seven.

It was predicted that those consuming the HE version of the training drink would rate the sorbet as more pleasant on days 6 and 11 as energy content and flavour became associated. Those consuming the LE version of training drink throughout would be expected to show very little change in pleasantness of the sorbet. For those in the extinction condition, an increased liking for the sorbet would be expected from day one to day six. Based upon patterns shown in Study One, it could then be predicted that the removal of energy after the second sorbet exposure would result in little change in pleasantness ratings of the sorbet on day 11, as liking was maintained despite energy removal. As in the previous study, pleasantness ratings were used to assess acquired liking, and subjective appetite ratings were reported at the start, end, and one hour after, each test session. In addition, intake of the sorbet was recorded, in order to examine if this differed between the energy conditions. It would be predicted that if flavour became associated with energy, those trained with the HE drink would consume more of the sorbet than those trained with the LE drink, and again, the pattern of intake in the extinction condition will be of particular interest to assess any impact of the removal of energy from the training drinks.

5.2 Method

5.2.1 Design

The study used a mixed design and participants were assigned randomly (with equal numbers in each) to one of three conditions, which varied in the energy content of the drink consumed and all attended the laboratory on 11 days. The design is illustrated in Table 5.1.

Condition	Day 1	Days 2-5	Day 6	Days 7-10	Day 11
HE	Sorbet	Drink (159kcal)	Sorbet	Drink (159kcal)	Sorbet
Extinction	Sorbet	Drink (159kcal)	Sorbet	Drink (7kcal)	Sorbet
LE	Sorbet	Drink (7kcal)	Sorbet	Drink (7kcal)	Sorbet

Table 5.1: A summary of the basic design of Study Two.

As illustrated in Table 5.1, those in the extinction condition consumed a HE drink over the first four exposure sessions (days two-five), and a LE version over the last four exposure sessions (days seven-ten). All conditions consumed a sorbet form of the drink on days one, six and eleven.

5.2.2 Participants

The original design had an intended sample size of 36 participants, however due to the discontinuation of the base drink, recruitment had to be halted prematurely. Although this unfortunately resulted in a study that lacked power, there were sufficient participants to warrant analysis. In total, the test sample consisted of 24 female participants, aged 18-27 ($M = 20.5 \pm 0.5$) who were recruited as described in Section 2.4.1. All participants were classed as 'sweet likers' as determined by a screening session (Section 5.2.4.1). All participants scored less than 7 on the restraint scale of TFEQ (Stunkard & Messick, 1985). There were a number of additional exclusion criteria (discussed in Section 2.4.1). The demographic information for each condition is presented in Table 5.2. There were no significant differences between conditions in BMI $F(2,21) = 3.14$, $p = .064$, age $F(2,21) = 0.41$, $p = .668$ or restraint score $F(2,21) = 0.75$, $p = .483$.

Condition	Age	BMI	TFEQ Restraint
High energy	21.1 ± 0.9	21.9 ± 0.9	2.9 ± 0.9
Extinction	20.1 ± 0.4	24.6 ± 1.0	3.1 ± 0.6
Low energy	20.3 ± 1.1	21.7 ± 0.8	2.0 ± 0.6

Table 5.2: Mean (± SEM) age, BMI and TFEQ-R score for each condition in Study Two.

5.2.3 Test foods

On each day, participants consumed a control lunch in the laboratory, which consisted of 40g mature grated cheddar cheese (British Mature Grated Cheddar, Sainsburys UK) and 30g cucumber in a sandwich of two slices of white bread (Great Everyday, Kingsmill UK), a packet of savoury crisps (Ready Salted Crisps, Walkers UK), five cherry tomatoes and a glass of water (total 554kcal). The test drinks were produced in house using a commercial LE cranberry and orange juice (Tesco), and flavoured with mandarin and kiwi (International Flavours and Fragrances) and was based on a product used successfully in two previous experiments in this laboratory (Yeomans, et al., 2008b plus unpublished). Sucrose was added to the HE drink (159kcal per 400g portion), and aspartame provided the sweetness for the LE drink (7kcal per 400g portion). The sorbet was a frozen LE version of the same flavour, with a small amount (37.5g per frozen block) of maltodextrin (Garnell Nutrition) added to match the sweetness of the LE drink. A Pacojet food processor (Pacojet plc, Switzerland) was used to cut 150g (around a quarter) from the frozen block, resulting in an ice cream like texture.

Table 5.3 shows the mean familiarity ratings of the sorbet and drinks on the initial exposure to each. There were no significant differences between conditions in the familiarity of the sorbet $F(2,21) = 1.44$, $p = .259$ or drink $F(2,21) = 2.54$, $p = .103$. It can be seen that the sorbet was relatively unfamiliar in flavour, and this familiarity increased on the second exposure to the flavour in the liquid form.

Condition	**Familiarity of sorbet**	**Familiarity of drink**
HE	36.1 ± 11.6	74.5 ± 9.7
Extinction	46.3 ± 5.4	83.3 ± 6.1
LE	26.3 ± 6.7	56.5 ± 9.4

Table 5.3: Mean (± SEM) familiarity of the sorbet on day one and drink on day two between conditions in Study Two.

5.2.4 Procedure

5.2.4.1 Sweet screening

Previous research has demonstrated that individuals can be classified as sweet likers or dislikers, (Looy, et al., 1992; Looy & Weingarten, 1991) so although sweetness is considered to be innately pleasant, the degree to which this is true differs between individuals. As discussed in Section 1.3.2 screening for sweet likers can maximise the potential of FFL and FNL occurring when sweet tastes are used as reinforcers (Yeomans, et al., 2008b; Yeomans & Mobini, 2006; Yeomans, et al., 2006). The training drinks in this study contained either sucrose or aspartame, and therefore screening for sweet likers was conducted. Participants who met the initial criteria (see Section 2.4.1) were invited to the lab to take part in a 10 minute screening session. Four liquid samples were presented, two of which contained a 10% sucrose solution, and two contained plain water. Participants were asked to taste and rate (pleasant, sweet, sour, familiar, bitter) the samples, and the average pleasantness for, and sweetness of, the sucrose solutions had to be at least 55 on a 100 point scale to continue into the main study, a criterion that was based previous work within the laboratory (e.g. Yeomans & Mobini, 2006). Mean pleasantness and sweetness of the sweet screening samples are reported in Table 5.4. Those who did not pass this test were reimbursed £3 for their time, and those who were eligible scheduled in the 11 sessions for the main study.

Condition	**Sweet liking**	**Sweet rating**
High energy	71.4 ± 4.6	84.0 ± 3.3
Extinction	75.3 ± 5.8	79.9 ± 4.6
Low energy	78.3 ± 4.9	86.4 ± 3.2

Table 5.4: Mean (±SEM) sweetness and pleasantness ratings of the screening session by participants in the three test conditions.

As can be seen (Table 5.4), participants in the three test conditions were matched in their screening data, with no significant differences in sweet liking, $F(2,21) = 0.46$, $p = .640$, or rated sweetness of the screening samples, $F(2,21) = 0.77$, $p = .477$ between the conditions.

5.2.4.2 Main study

Each participant was required to report to the laboratory on 11 days, over a period of four weeks, for lunch followed by a mid afternoon test session. On all days, participants were asked to consume their normal breakfast, and then to consume only water until they reported to the laboratory for their lunch, at a time between 11.30 and 13.30. They were instructed to consume the lunch in their own time, and then leave the lab until their next session three hours later. During this time they were only allowed to consume water.

5.2.4.3 Test days 1, 6 and 11

Upon arrival at the laboratory for the mid afternoon test session (14.30-16.30), participants were asked to complete a set of computerised mood and appetite ratings (calm, clearheaded, drowsy, energetic, full, headachy, hungry, lively, nauseous, thirsty, tired) using the SIPM software. They were then presented with a 150g portion of sorbet and were asked to complete a taste test (pleasant, creamy, familiar, fruity, sweet, strong, sour). *Ad libitum* eating followed, with a refill provided after every 110g consumed. Appetite ratings were taken after consumption of 25g, after a refill, and at the end of the session. Paper ratings (calm, clearheaded, full, hungry, lively, nauseous, thirsty, tired) were completed one hour after leaving the lab (after consuming only water). On day 11, participants were debriefed, height and weight was recorded, and reimbursed for their time (materials shown in Appendix 4).

5.2.4.4 Training days 2-5 and 7-10

The purpose of these test sessions was to expose participants to the relevant training conditions so that those consuming the HE drinks associated the sorbet flavour with the effects of energy, and, for those in the extinction condition, facilitated the removal of energy at day 7. Upon arrival to the laboratory for the mid-afternoon session (14.30-16.30), participants completed the same set of computerised mood and appetite ratings as in the test days. Participants then tasted and rated (pleasant, sweet, sour, fruity, strong, familiar) the appropriate 400g drink for their condition, which they were then instructed to consume in full. As in the test session, appetite ratings were completed at the end of consumption, and again one hour after leaving the lab.

5.2.5 Data analysis

One way independent ANOVAs were conducted to explore any baseline differences between groups, and to analyse change from baseline pleasantness data. Due to issues of power, analysis was conducted on HE and Extinction groups both as separate conditions and also combined within the training phase (as both had been trained with identical stimuli at this stage). Independent t-tests were used to analyse the combined data for sorbet ratings and two way mixed ANOVAs were conducted on pleasantness ratings of drink and appetite ratings, over time. Where sphericity could not be assumed, the appropriate correction was applied (ε <. 75: Greenhouse-Geisser, ε > .75 Huynh-Feldt).

5.3 Results

5.3.1 Pleasantness of the sorbet

The aim of this study was to further investigate extinction of flavour preferences and therefore a pre-requisite was that the initial training phase did result in significant increases in flavour liking in the training conditions. In order to determine whether acquired liking was demonstrated for the sorbet after training with a HE drink compared to a LE drink, and whether this would be maintained after removal of energy, the analysis focused on change data. As stated in section 4.3.1.5 when focusing on change data it is important to determine whether there were any baseline differences between conditions; there were no significant differences in pleasantness ratings of the sorbet on day 1 $F(2,21) = 1.59$, $p = .229$, means reported in Table 5.5.

Condition	Baseline sorbet pleasantness
High energy	52.9 ± 4.2
Extinction	46.0 ± 6.9
Low energy	60.6 ± 6.0

Table 5.5: Mean (±SEM) baseline pleasantness of sorbet between the conditions in Study Two.

With no baseline differences, change from baseline pleasantness was calculated, for both the training phase (difference between days one and six) and extinction phase (difference between days six and eleven).

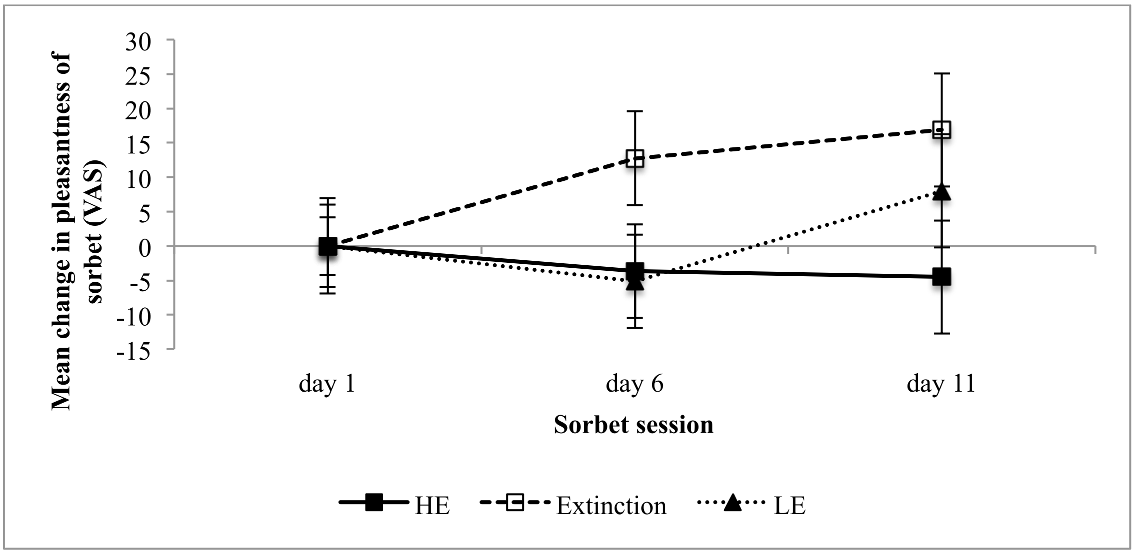


Figure 5.1: Mean (±SEM) change in pleasantness of sorbet across test days between conditions in Study Two.

5.3.1.1 Pleasantness of sorbet: training phase

As homogeneity of variance was violated, $F(2,21) = 4.10$, $p = .031$, Welch and Browne-Forsythe statistics were reported. According to the Browne-Forsythe statistic there were no significant differences in change from baseline pleasantness between energy conditions $F(2,13.33) = 2.12$, $p = .159$. However, the Welch statistic was approaching significance, $F(2,11.86) = 3.77$ $p = .054$, and as can be seen in Figure 5.1, those in the Extinction condition ($M = 12.8 \pm 3.1$) show the predicted increased pleasantness after training with the HE drink, whereas those in the HE ($M = -3.6 \pm 6.0$) and LE ($M = -5.1 \pm 9.7$) conditions showed a decreased pleasantness. Despite consuming the same drink at this stage, the HE and Extinction groups were behaving in a different way, and this will be explored further in Section 5.3.1.4.

5.3.1.2 Pleasantness of sorbet: HE and Extinction groups combined

When HE and Extinction groups were combined there was no significant difference between those trained with the HE drink and those trained with the LE drink, $t(22) = 1.12$, $p = .277$. However, with these groups combined, the means suggest that overall, those trained with the HE drink rated the sorbet as more pleasant ($M = 4.6 \pm 3.9$) on day 6 than baseline, whereas those trained with the LE drink rated the sorbet as less pleasant ($M = -5.1 \pm 9.7$). This was in line with the predictions, but as seen in Section 5.3.1.1, different behaviour was demonstrated within the HE exposure groups that had been combined.

5.3.1.3 Pleasantness of sorbet: extinction phase

The change in pleasantness of the sorbet from day 6 (where energy was removed from the training drink in the extinction condition) to day 11 was examined. It was predicted that the pattern of change in pleasantness would continue in the same way as the training phase for those in both the HE and LE conditions. For those who had undergone extinction of energy, pleasantness of the sorbet could continue in the same pattern if there was no evidence of extinction, or become less pleasant as the taste became associated with the difference in energy content. Change from day 6 pleasantness did not significantly differ between conditions $F(2,21) = 0.75$, $p = .487$. The pattern of change data suggested that those who had consumed the LE drink throughout showed an unexpected increase in pleasantness from day 6 to 11 ($M = 13.1 \pm 12.4$), as did those in the extinction condition ($M = 4.1 \pm 3.2$). Conversely, those in the HE condition showed a slight decrease in pleasantness between day 6 and 11 ($M = -0.9 \pm 6.2$). It therefore appeared that the sorbet became more pleasant between days 6 and 11 for those consuming the LE training drink, but less pleasant for those consuming the HE. Figure 5.1 shows that the extinction of energy did not result in a decrease in pleasantness for the sorbet, but these ratings were now in line with the LE rather than the HE condition.

5.3.1.4 Pleasantness of sorbet: exclusion of non-responders

As highlighted previously, the HE and extinction conditions showed a different pattern of change from baseline pleasantness of the sorbet, which was unexpected as an identical drink was consumed during the training phase (days 2-5). When the raw data were examined, three individuals who received the HE drink (all in the HE condition rather than extinction condition) demonstrated a marked decrease in liking for the sorbet between days 1 and 6 (all showing a decrease between 15 and 25 points). Therefore, pleasantness data were re-analysed with the exclusion of these individuals (shown in Figure 5.2).

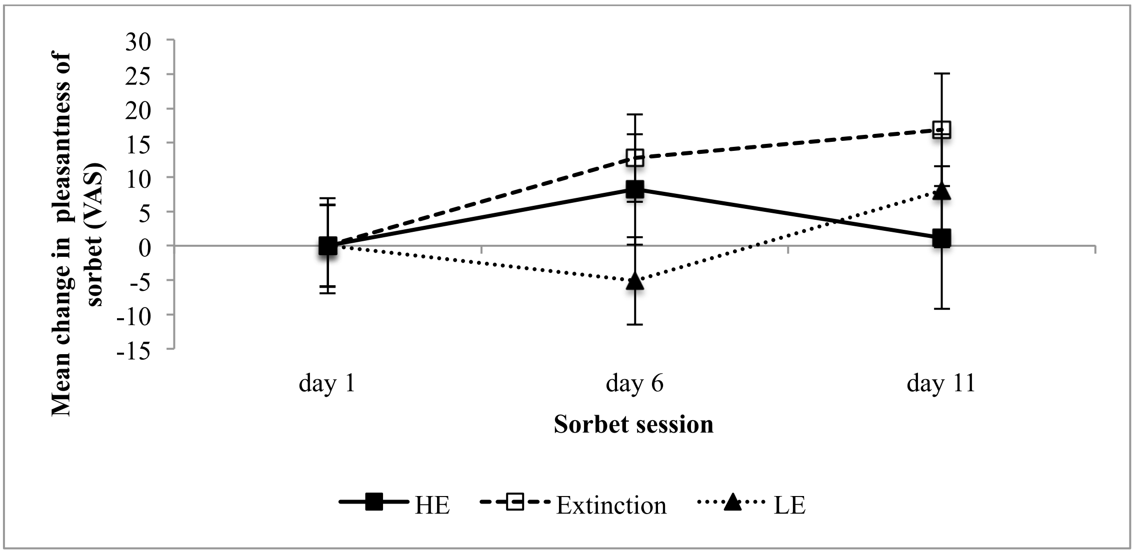


Figure 5.2: Mean (±SEM) change in pleasantness of sorbet across test days between the 3 conditions in Study Two, with the removal of those not meeting the training requirements.

Again, homogeneity of variance was violated, $F(2,18) = 6.97$, $p = .006$, so Browne-Forsythe and Welch statistics were reported. Both statistics showed there were no significant differences between conditions in change from baseline pleasantness of sorbet; $F(2,8.56) = 2.62$, $p = .130$ and $F(2,10.27) = 1.85$, $p = .206$. As can be seen in Figure 5.2, excluding these individuals brought the HE condition ($M = 8.2 \pm 2.3$) more in line with the mean increased pleasantness demonstrated in the extinction condition, and in line with predictions based upon the flavour nutrient learning literature. Thus there was some evidence that flavour nutrient learning had occurred during training.

After extinction, there remained no significant differences between the conditions; Browne-Forsythe $F(2,9.55) = 1.38$, $p = .297$, and Welch $F(2,9.67) = 2.12$, $p = .172$. Examining the means, those in the HE condition showed a reduction in pleasantness from day 6 to 11 ($M = -7.0 \pm 4.9$), whereas those in the Extinction ($M = 4.1 \pm 3.2$) and LE ($M = 13.1 \pm 12.4$) showed an increase in pleasantness across this period. Thus with the non-responders removed, the pattern of data suggested that flavour nutrient associations were learned, but that removal of nutrients failed to impact upon pleasantness ratings.

5.3.2 Pleasantness of the training drink

If the energy content of the training drink became associated with the flavour, it was predicted that those consuming the HE version would rate it as more pleasant over time, and that those consuming the LE version would show a smaller increase due to exposure and FFL, or decreased pleasantness due to an aversive hunger state. For those in the extinction condition, if flavour preferences were resistant to extinction it was predicted that pleasantness would reduce during the second phase of exposure. Again, there was no significant difference in baseline pleasantness of the drinks, $F(2,21) = 0.51$, $p = .610$, means presented in Table 5.6.

Condition	Baseline drink pleasantness
High energy	70.3 ± 4.7
Extinction	66.9 ± 6.3
Low energy	61.4 ± 7.5

Table 5.6: Mean (±SEM) baseline pleasantness of the training drinks between conditions in Study Two.

5.3.2.1 Pleasantness of the training drink: training phase

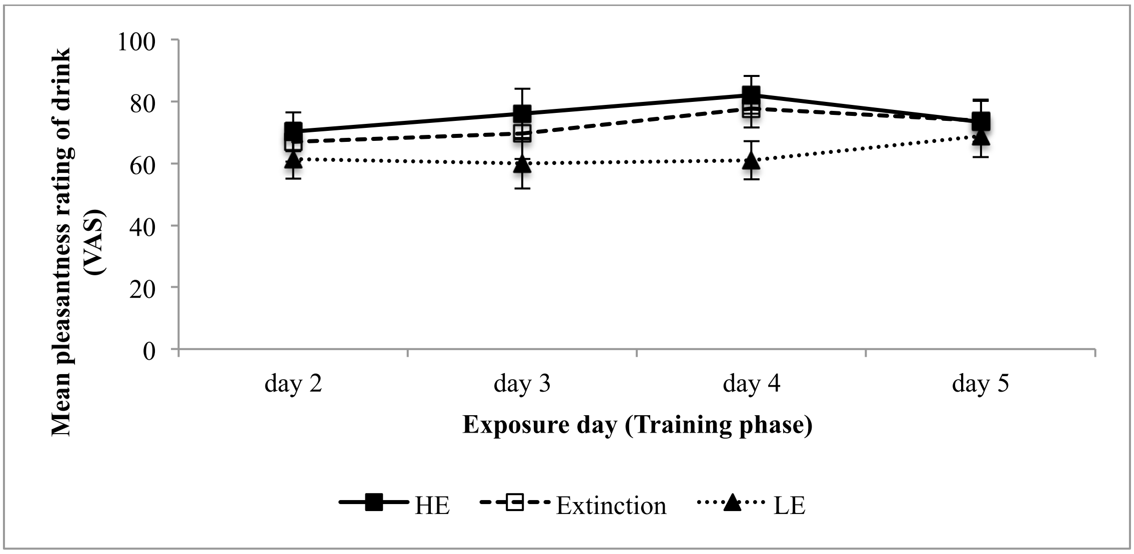


Figure 5.3: Mean (±SEM) pleasantness ratings of the drinks across the training phase in Study Two.

There was no significant difference between the four training sessions $F(2.25,47.16) = 1.59$, $p = .213$, between energy conditions $F(2,21) = 1.34$, $p = .283$, and no significant condition*day interaction, $F(4.49,47.16) = 0.72$, $p = .598$. As shown in Figure 5.3, the HE and extinction condition showed increased pleasantness across the first four exposure sessions, and this was at a higher level than those consuming the LE version, who remained relatively stable. On day 5 there was a peak in the LE pleasantness ratings and an unexpected drop in the HE pleasantness. It is interesting to note that the differences in pleasantness change demonstrated for the sorbet between the HE and extinction conditions were not reflected in the changes in pleasantness ratings of the training drinks.

5.3.2.2 Pleasantness of the training drink: HE and Extinction conditions combined

When both conditions consuming the HE drink were combined, there were no significant differences between those trained with HE and LE versions of the drink $F(1,22) = 2.59$, $p = .122$, across sessions $F(2.25,49.55) = 1.20$, $p = .312$ and no significant day*condition interaction $F(2.25,49.55) = 1.30$, $p = .283$.

5.3.2.3 Pleasantness of the training drink: extinction phase

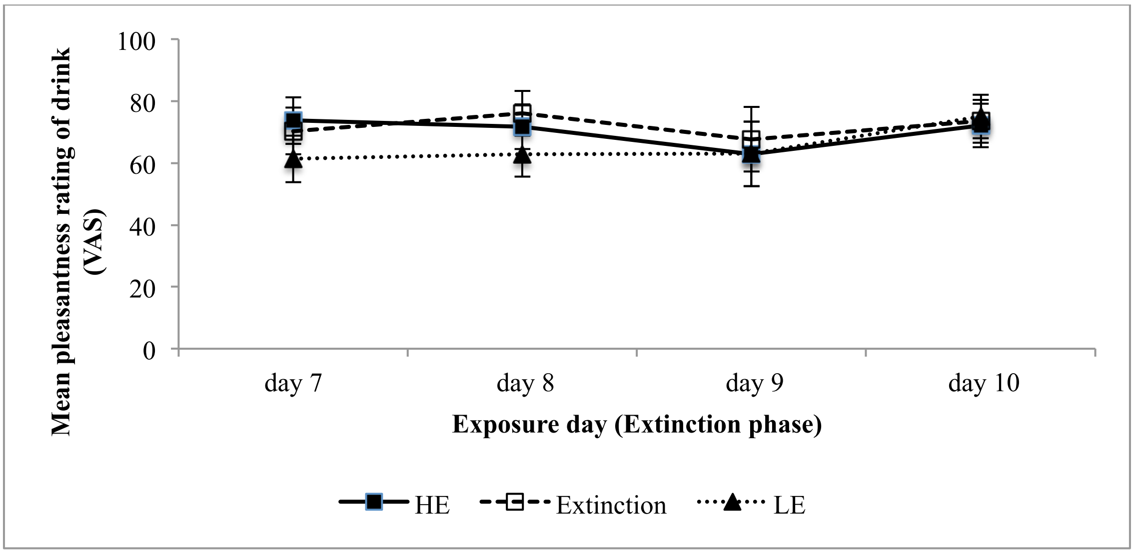


Figure 5.4: Mean (±SEM) pleasantness of drinks across the extinction phase of Study Two.

After extinction (Figure 5.4), if sensitive to the removal of energy, those who had now switched consumption from HE to LE would show decreased pleasantness ratings of the drink. There remained no significant effect between the exposure sessions $F(1.78, 37.45) = 1.53$, $p = .230$, condition $F(2,21) = 0.23$, $p = .795$ or day*condition interaction

F (3.57, 37.45) = 0.64, *p* = .620. As can be seen in Figure 5.4 pleasantness ratings were maintained in the extinction condition, therefore indicating no extinction effects, and those in the LE condition also remained relatively stable until day 10, when again there was a peak in pleasantness. This pattern in the LE condition was consistent with the unexpected change in pleasantness for the sorbet (Figure 5.1).

5.3.2.4 Pleasantness of the training drink: exclusion of non-responders

In order to remain consistent with the analysis of sorbet changes, and to examine if these individuals were impacting upon the pleasantness change ratings for the drinks, subsequent analysis continued to exclude non-responders. Non-responders remained categorised as those trained with the HE drink who did not demonstrate the predicted increased liking for the sorbet between days 1 and 6 (so the same individuals who were excluded from the analysis of sorbet pleasantness).

5.3.2.4.1 Training phase

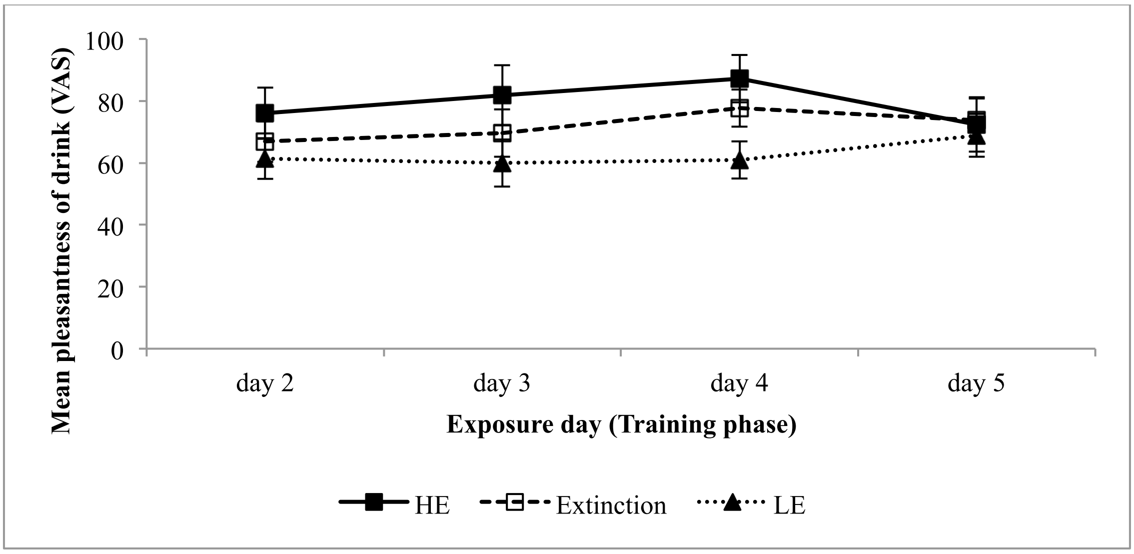


Figure 5.5: Mean (±SEM) pleasantness rating of drink across training phase of Study Two, with exclusion of non-responders.

During the training phase there was no significant difference between test days *F*(2.12,38.23) = 1.12, *p* = .346, between conditions *F*(2,18) = 1.76, *p* = .200 or day*condition interaction *F*(4.25,38.23) = 1.07, *p* = .394. As can be seen in Figure 5.5 those in the HE and extinction conditions showed an increase in pleasantness across the first three exposure days, although this increase was larger in the HE condition, and those in the LE condition remained relatively stable in their pleasantness ratings until exposure day 5. The exclusion of non-responders had very little impact on the pattern of pleasantness change of the training drinks during this phase, with a small increase in baseline pleasantness.

5.3.2.4.2 Extinction phase

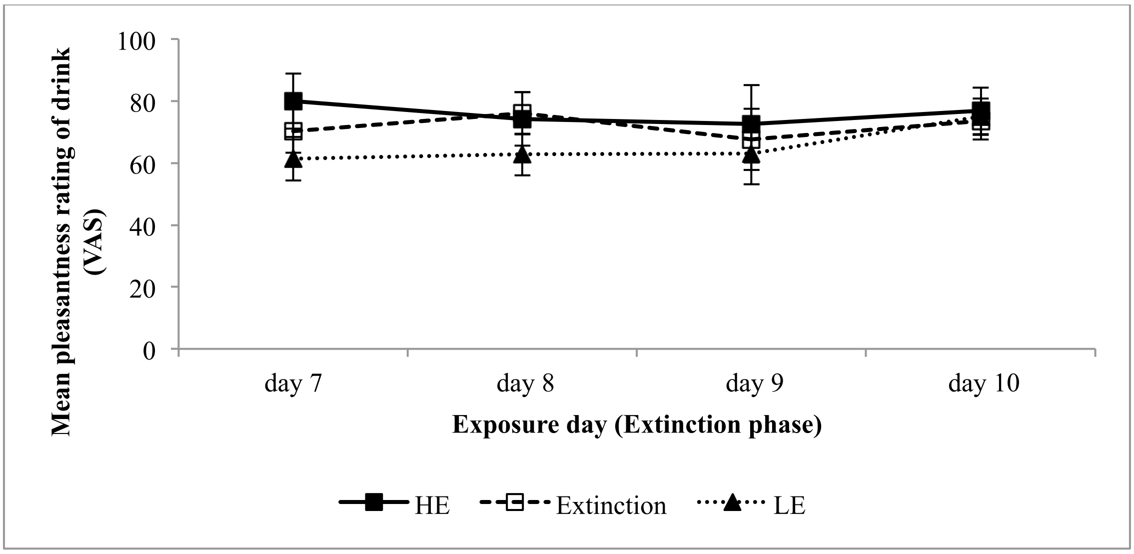


Figure 5.6: Mean (±SEM) pleasantness ratings of drink across the extinction phase of Study Two, with exclusion of non-responders.

There was no significant difference between exposure sessions $F(1.70,30.55) = 0.84$, $p = .424$, condition $F(2,18) = 0.61$, $p = .553$ or day*condition interaction $F(3.39,30.55) = 0.58$, $p = .652$. Figure 5.6 shows that pleasantness ratings of the drink remained relatively stable for all conditions across the extinction phase, with those in the extinction phase showing no reduction despite the removal of energy from the drink. The exclusion of non-responders resulted in the pleasantness ratings of the drink remaining marginally more stable over this phase than when they were included in the analysis, but interestingly the sorbet pleasantness data shown in Figure 5.2 indicates a much sharper decline during this phase after their removal than when included (Figure 5.1).

5.3.3 Sorbet intake

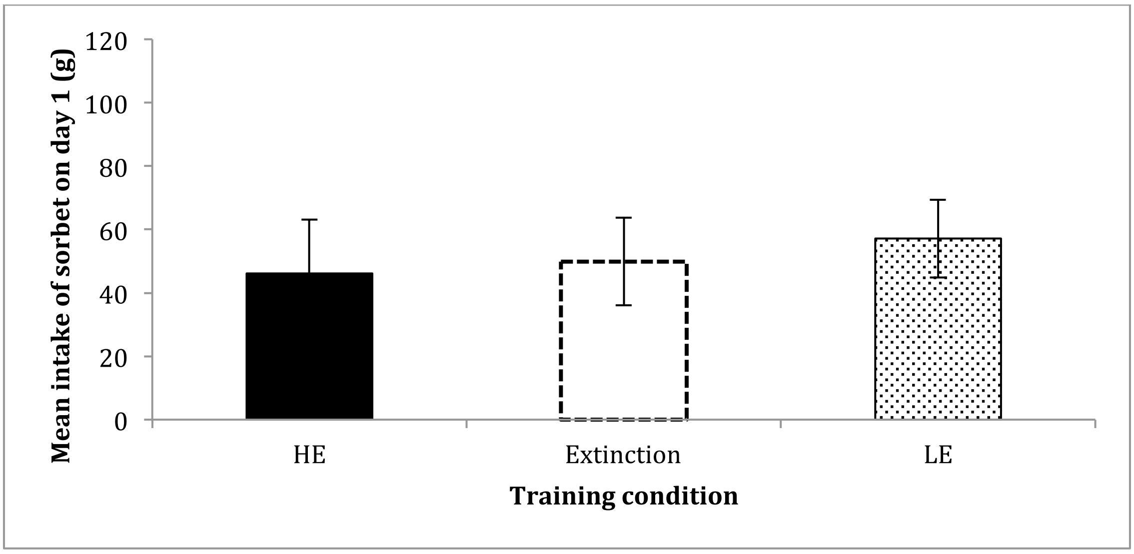


Figure 5.7: Mean (±SEM) intake of sorbet at baseline in Study Two, with exclusion of one outlier.

If an association was made between the energy content and flavour of the training drink, an increased intake of sorbet was predicted for those trained with the HE drink, with minimal change or a decrease for those trained with the LE drink. As with the pleasantness predictions, if flavour preferences persisted, those in the extinction group were predicted to also increase their intake in line with those continuing with the HE training drink. Although not significant $F(2,21) = 0.82$, $p = .454$, those in the LE condition ($M = 75.4 \pm 21.1$) consumed a considerably larger amount of sorbet on day 1 than those in the HE ($M = 46.1 \pm 17.0$) and extinction ($M = 49.9 \pm 13.8$) conditions. One individual in the LE condition was identified as an outlier, and when removed from the analysis brought the mean more in line with the baseline for other conditions ($M = 57.2 \pm 12.3$) and due to issues with power this person was excluded from further analysis. Figure 5.7 shows the baseline means after exclusion of this individual. Therefore, change scores were calculated for day 1 to 6, and day 6 to 11, to examine differences between the three exposure conditions.

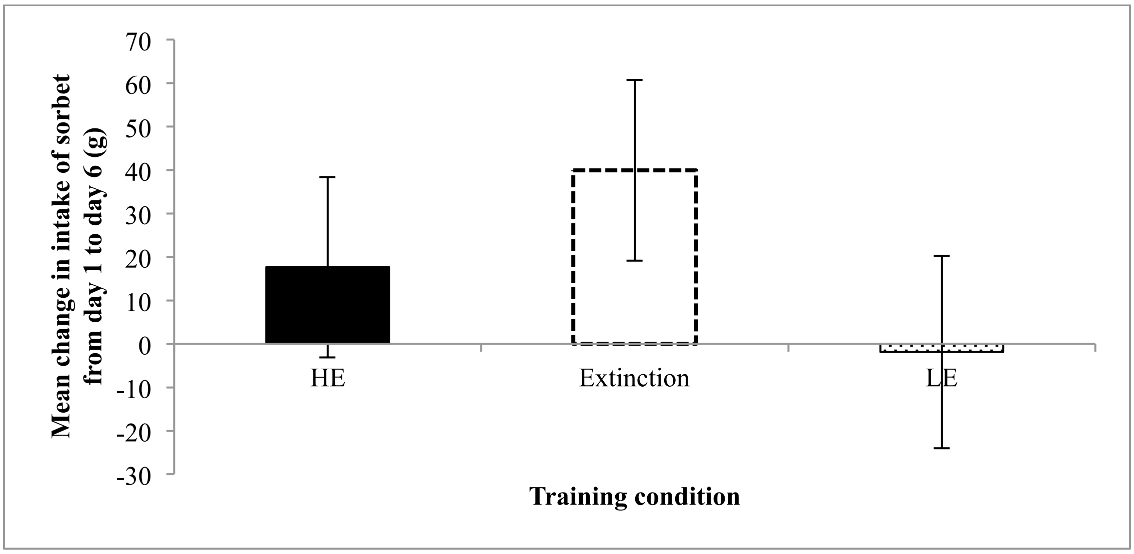


Figure 5.8: Mean (±SEM) change in sorbet intake between days 1 and 6 (training phase of Study Two).

5.3.3.1 Sorbet intake: training phase

Between conditions, there was no significant difference in change from baseline intake of sorbet on day 6 $F(2,20) = 0.95$, $p = .403$. Figure 5.8 shows that both HE ($M = 17.7 \pm 20.8$) and extinction ($M = 39.9 \pm 20.8$) conditions, who had been trained with the HE drink, increased their intake of sorbet between day 1 and 6, whereas those who had been trained with the LE drink decreased their intake ($M = -1.9 \pm 22.2$). Thus although the overall changes were not significant the data pattern was similar to what was predicted.

5.3.3.2 Sorbet intake: HE and Extinction combined

When both HE exposure groups were combined, there were still no significant differences in intake between those trained with the LE drink and those trained with the HE drink, $t(21) = 1.16$, $p = .258$. Those trained with the HE drink increased their intake of sorbet from day 1 to 6 ($M = 28.8 \pm 16.4$) whereas those trained with the LE drink decreased their intake of sorbet during this time ($M = -1.9 \pm 12.7$), although the means reflected the larger increase in intake for the HE trained conditions.

5.3.3.3 Sorbet intake: extinction phase

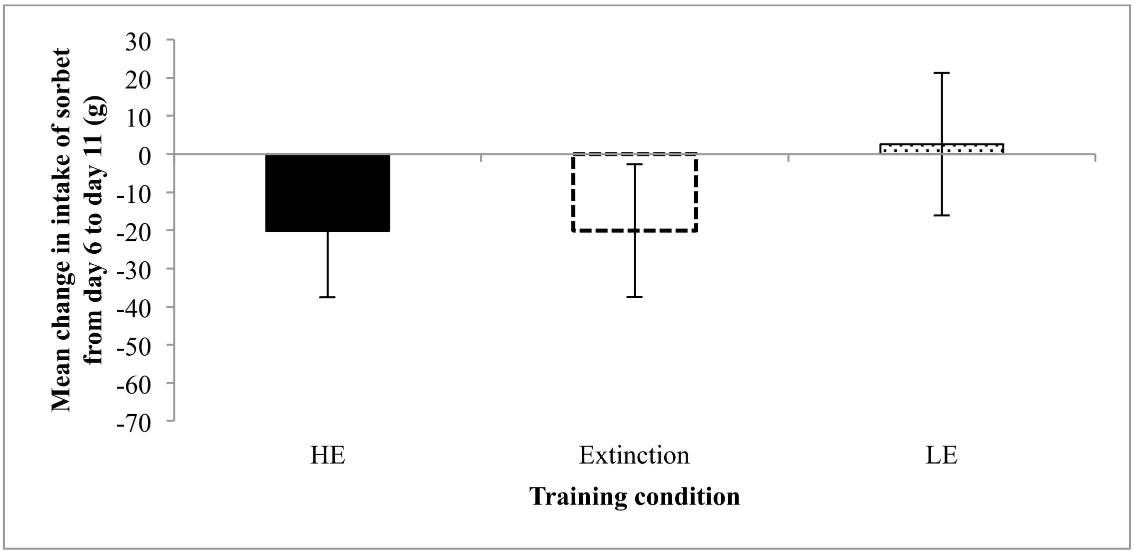


Fig 5.9: Mean (±SEM) change in sorbet intake between days 6 and 11 (extinction phase of Study Two).

On day 11, after the extinction phase, there was no significant difference between the conditions in change from day 6 intake $F(2,20) = 0.51$, $p = .606$. As seen in Figure 5.9, contrary to prediction, both the HE ($M = -20.1 \pm 17.5$) and extinction ($M = -20.1 \pm 17.5$) conditions slightly decreased their intake from day 6, whereas the LE ($M = 2.6 \pm 18.7$) had a very small increase in intake. This indicated that those in the extinction condition were behaving in a similar way to the HE condition, despite the removal of energy in the training drink. It is interesting that the pattern of change was the reverse of that from the training phase, although again changes were relatively small and did not reach significance.

When analysed separately by day and condition, there were no significant differences in intake between days $F(1.83,36.61) = 1.73$, $p = .194$, conditions $F(2,20) = 0.54$, $p = .591$ and no day*condition interaction $F(3.66,36.61) = 0.80$, $p = .526$. However, it should be noted that, although decreasing intake of sorbet between days 6 and 11, those in the extinction condition were the only condition that consumed more on day 11 ($M = 69.7 \pm 12.2$) than on day 1 ($M = 49.9 \pm 14.4$).

5.3.3.4 Sorbet intake: exclusion of non-responders

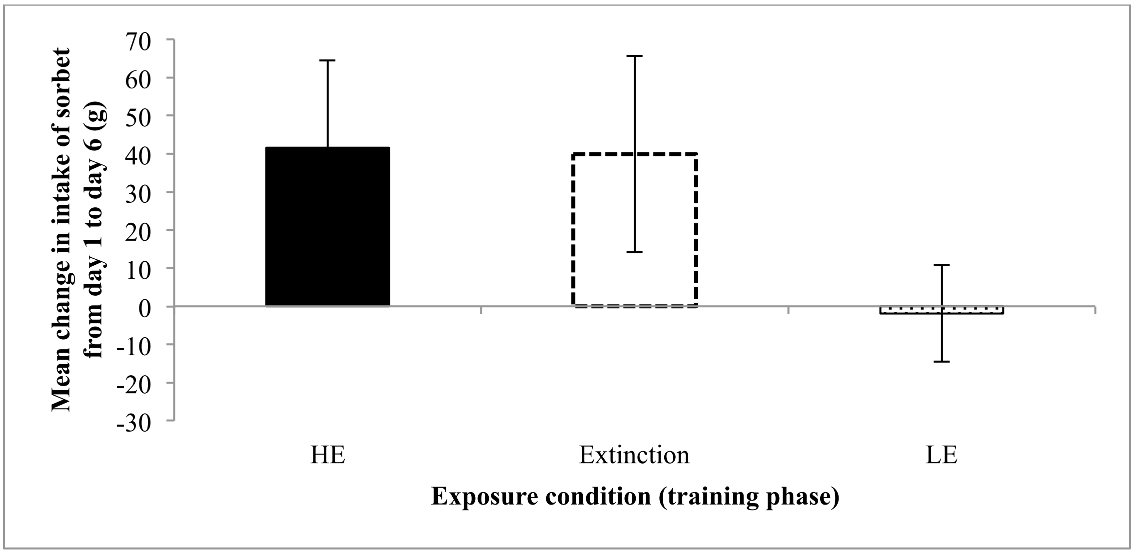


Figure 5.10: Mean (±SEM) change in intake of sorbet from day 1 to 6 in Study Two, with non-responders and one outlier excluded.

When those who did not meet the training requirements were excluded, although still no significant differences in change from baseline sorbet intake, $F(2,17) = 1.28$, $p = .303$, there was now a much larger increase in intake in the HE condition ($M = 41.6 \pm 22.9$), than when these individuals had not been excluded, as shown in Figure 5.10. This suggested that the energy content of the training drink was being associated with the flavour of the sorbet.

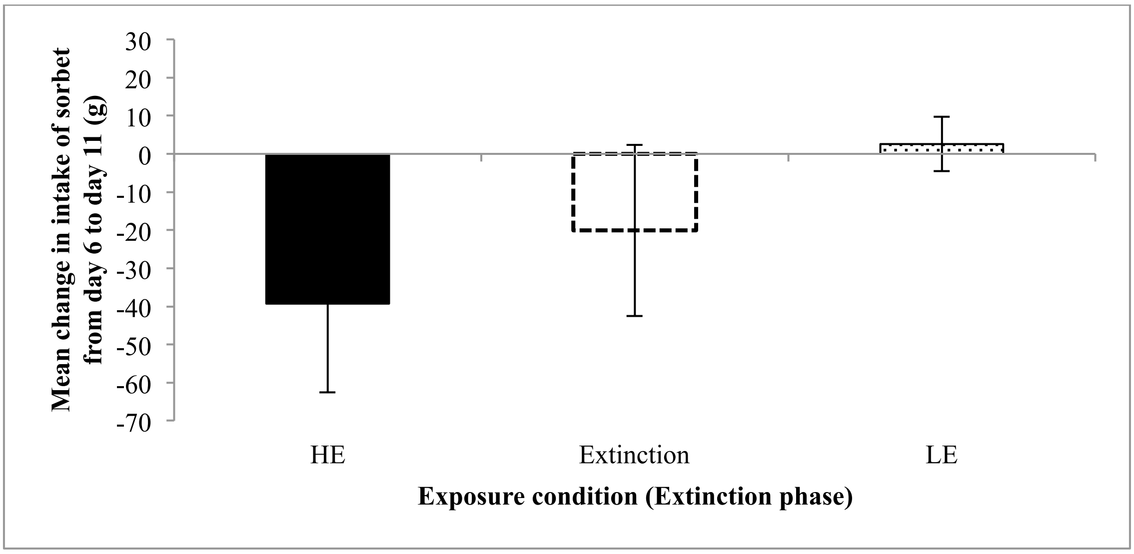


Figure 5.11: Mean (±SEM) change in intake of sorbet from day 6 to day 11 in Study Two, with exclusion of non-responders and one outlier.

After extinction (Figure 5.7), the reduction in intake of sorbet from day 6 to 11 was also much larger in the HE group once these individuals were excluded, although there were still no significant differences in change in intake between training conditions, $F(2,17) = 1.08$, $p = .362$.

5.3.4 Appetite Ratings: hunger

As in Study One, it was interesting to analyse any differences in appetite between the energy conditions in training and extinction, and to examine whether there was an impact of extinction on appetite ratings.

5.3.4.1 Baseline hunger ratings for each session

There were no significant baseline hunger differences between conditions on any of the 11 sessions (all $p > .122$), with the means displayed in Table 5.7.

Session	**HE**	**Extinction**	**LE**
Day 1	54.0 ± 8.9	56.0 ± 6.4	40.0 ± 9.9
Day 2	65.8 ± 6.7	44.5 ± 8.6	44.8 ± 9.7
Day 3	59.8 ± 7.7	46.1 ± 8.2	43.5 ± 10.3
Day 4	52.5 ± 7.3	37.6 ± 8.9	58.0 ± 8.4
Day 5	46.5 ± 10.6	39.3 ± 9.7	56.4 ± 8.6
Day 6	53.0 ± 7.4	40.9 ± 10.3	64.6 ± 8.2
Day 7	50.1 ± 9.4	53.5 ± 6.9	57.9 ± 7.9
Day 8	64.3 ± 8.6	44.1 ± 9.0	62.4 ± 8.8
Day 9	54.9 ± 8.9	45.6 ± 6.5	48.1 ± 11.5
Day 10	55.6 ± 9.9	41.9 ± 10.6	43.9 ± 12.2
Day 11	54.8 ± 10.2	28.3 ± 7.7	55.9 ± 12.3

Table 5.7: Mean (±SEM) baseline hunger ratings for each session of Study Two.

5.3.4.2 Hunger change after sorbet

Hunger change for each session was calculated, from the ratings taken upon arrival at the laboratory and those taken after sorbet consumption. It was predicted that there would be no differences between conditions in the change in hunger rating across the first sorbet session, as all conditions were consuming the same low energy sorbet, with no prior flavour associations. However, on day 6, if energy exposure (in terms of the test drink) had become associated with the flavour of the sorbet, those trained in the HE and extinction conditions may show a larger reduction in hunger across the session compared to the LE condition. On the final sorbet exposure session (day 11) all conditions would have consumed the low energy sorbet on two prior occasions, therefore no difference between conditions in hunger change across this session were predicted.

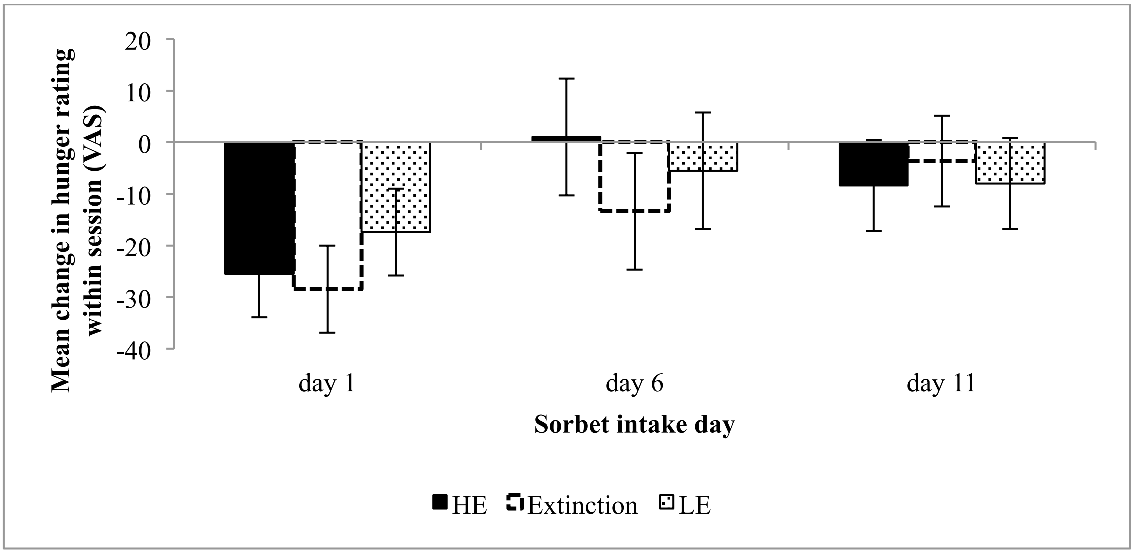


Figure 5.12: Mean (±SEM) change in hunger within each sorbet session in Study Two.

Hunger change within the sorbet session was significantly different between days $F(2,42) = 5.91$, $p = .005$, with a significantly larger reduction in hunger on day 1 ($M = -23.79 \pm 4.9$), than on days 6 ($M = -6.0 \pm 6.5$, $p = .035$) or 11 ($M = -6.7 \pm 5.1$, $p = .019$). The hunger reduction was not significantly different between days 6 and 11 ($p = 1.00$). There was no significant difference between conditions $F(2,21) = 0.12$, $p = .886$, or day*condition interaction $F(4,42) = 0.69$, $p = .606$. In a similar pattern to the pleasantness data, Figure 5.12 shows that the HE ($M = 1.0 \pm 11.3$) and Extinction ($M = -13.4 \pm 11.3$) conditions were demonstrating a different change in hunger on day 6, despite having been trained with the same drink at this stage.

5.3.4.2.1 Exclusion of non-responders

As the pattern of hunger change on day six was different between the HE and extinction group, it was interesting to analyse these changes when the previously identified non-responders were excluded.

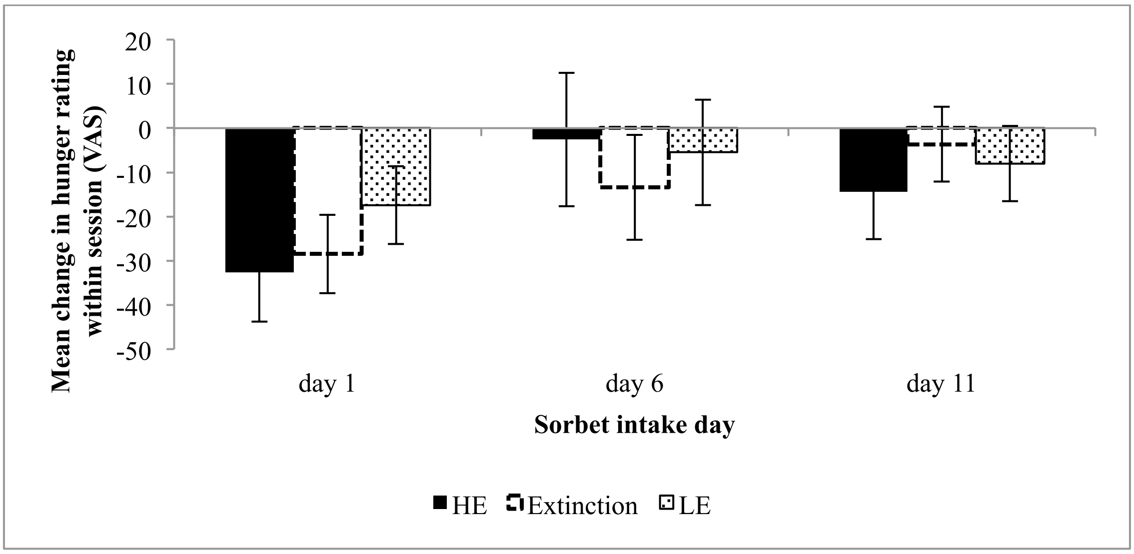


Figure 5.13: Mean (±SEM) change in hunger within sorbet sessions in Study Two, with exclusion of non-responders.

Exclusion of non responders did not have much impact upon change in hunger rating within sorbet session, with a slightly larger reduction on day 1 for the HE group, and a small reduction in hunger on day 6, compared to a small increase when these individuals were not excluded. There remained a significant difference between days, $F(2,36) = 5.50$, $p = .008$, with a significantly larger reduction in hunger on day 1 ($M = -26.2 \pm 5.6$) compared to day 11 ($M = -8.7 \pm 5.4$, $p = .031$), and approaching significantly larger reduction compared to day 6 ($M = -7.2 \pm 7.5$, $p = .058$).

There were no significant differences between conditions, $F(2,18) = 0.15$, $p = .864$ or day*condition interaction $F(4,36) = 0.73$, $p = .578$.

It therefore appeared that any association made between the energy content of the training drink between days one and six did not impact upon the hunger change over the sorbet session.

5.3.4.3 Hunger change per gram of sorbet consumed

As sorbet was consumed *ad libitum*, hunger changes across the session were likely to be influenced by actual sorbet intake. Therefore, the relative satiating effect of the sorbet was analysed, by calculating hunger change per gram of sorbet (hunger change/g intake).

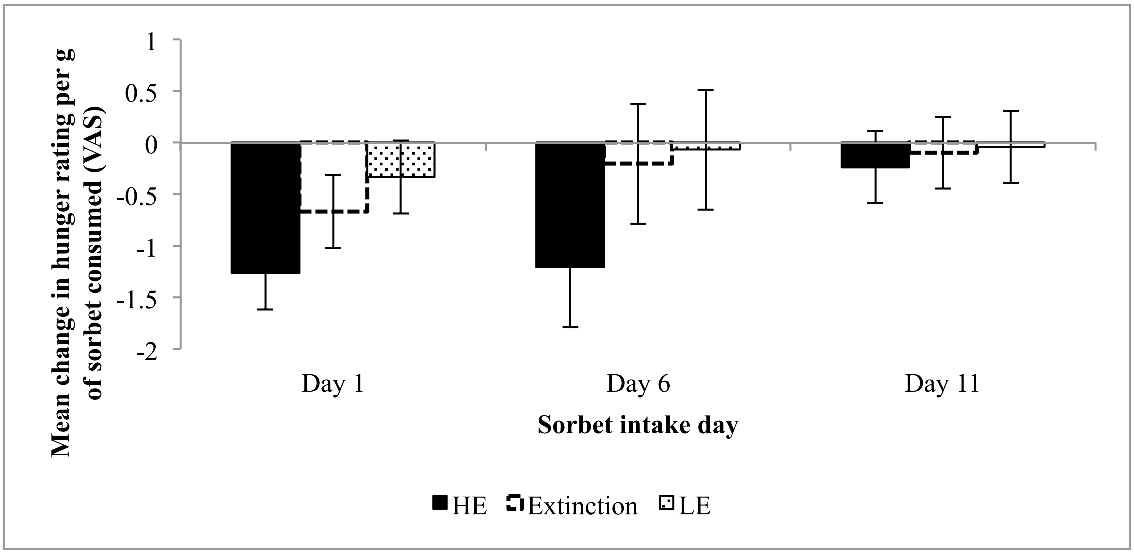


Figure 5.14: Mean (±SEM) change in hunger rating per gram of sorbet consumed in Study Two.

There were no significant differences in hunger change per gram between sorbet sessions $F(2,42) = 2.23$, $p = .120$, between conditions $F(2,21) = 1.49$, $p = .249$, or day*condition interaction $F(4,42) = 0.56$, $p = .692$. From Figure 5.14, it can be seen that on day six, once again, those in the HE and extinction conditions showed differences in their hunger change despite having trained with the same drink.

When non-responders were excluded there remained no significant differences between sessions $F(1.39,24.97) = 2.31$, $p = .134$, conditions $F(2,18) = 1.24$, $p = .313$ and no day*condition interaction $F(1.39,24.97) = 0.22$, $p = .870$.

5.3.4.4 Changes in hunger during the exposure sessions: training phase

Again, hunger change across the session was calculated, using ratings taken upon arrival at the laboratory and after consumption of the drink. A larger reduction in hunger would be expected in those consuming the HE drink compared to the LE, and this pattern could change in the extinction condition dependent upon whether sensitive to the removal of energy.

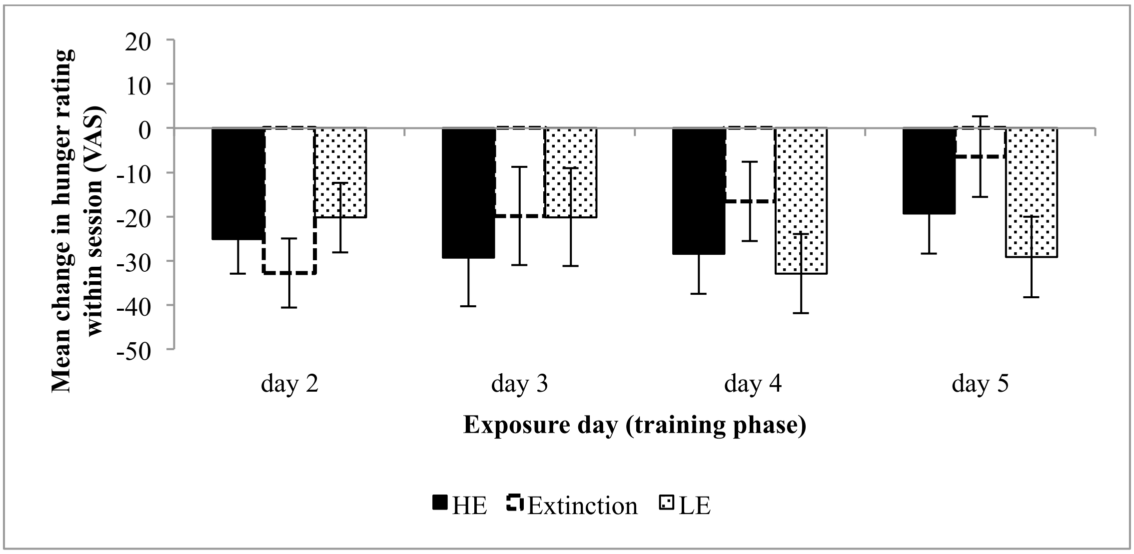


Figure 5.15: Mean (±SEM) change in hunger within each exposure session during the training phase of Study Two.

There were no significant differences in hunger change across session across day $F(2.86,60.10) = 1.27$, $p = .293$, between conditions $F(2,21) = 0.23$, $p = .796$ with a day*condition interaction approaching significance, $F(5.72,60.10) = 2.22$, $p = .056$. As can be seen in Figure 5.15, the extinction condition appeared to show different patterns in hunger reduction than the HE condition, despite having consumed identical drinks during this phase.

5.3.4.4.1 HE and extinction combined

When combined, there remained no significant difference in hunger reduction within time points $F(2.72,59.80) = 0.72$, $p = .533$ or between conditions $F(1,22) = 0.12$, $p = .730$, and the day*condition interaction became significant $F(2.72,59.80) = 2.91$, $p = .047$. When analysed separately for each condition using Bonferroni corrected one-way repeated ANOVAs, there was only a significant difference for those that consumed the HE drink $F(3,45) = 3.49$, $p = .023$, and this difference was between days one ($M = -28.9 \pm 4.9$) and four ($M = -12.88 \pm 5.79$, $p = .047$). Those consuming the HE drink appeared to show a smaller reduction in hunger as the training progressed.

5.3.4.5 Changes in hunger during the exposure sessions: extinction phase

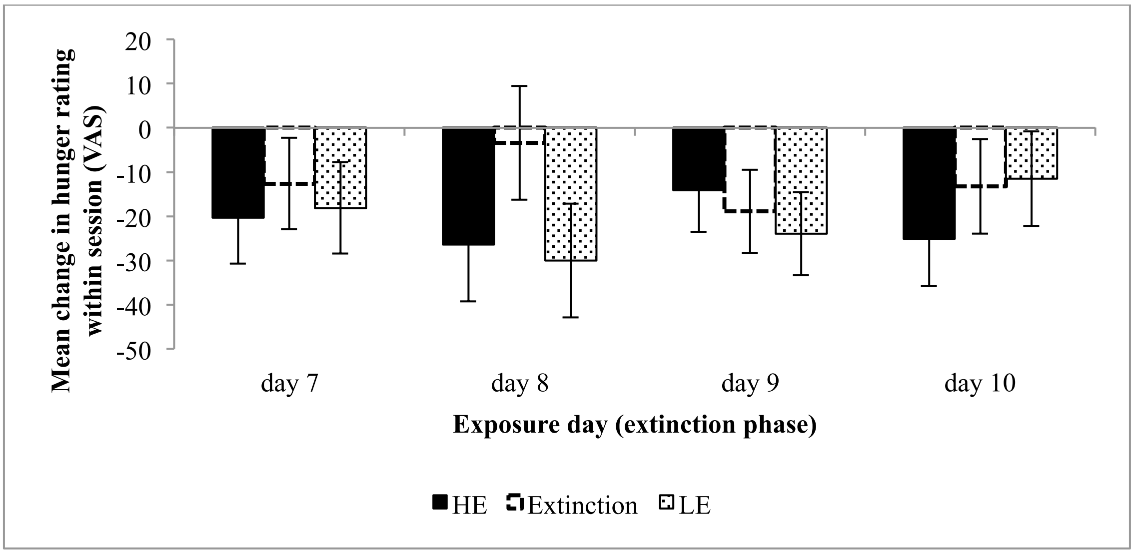


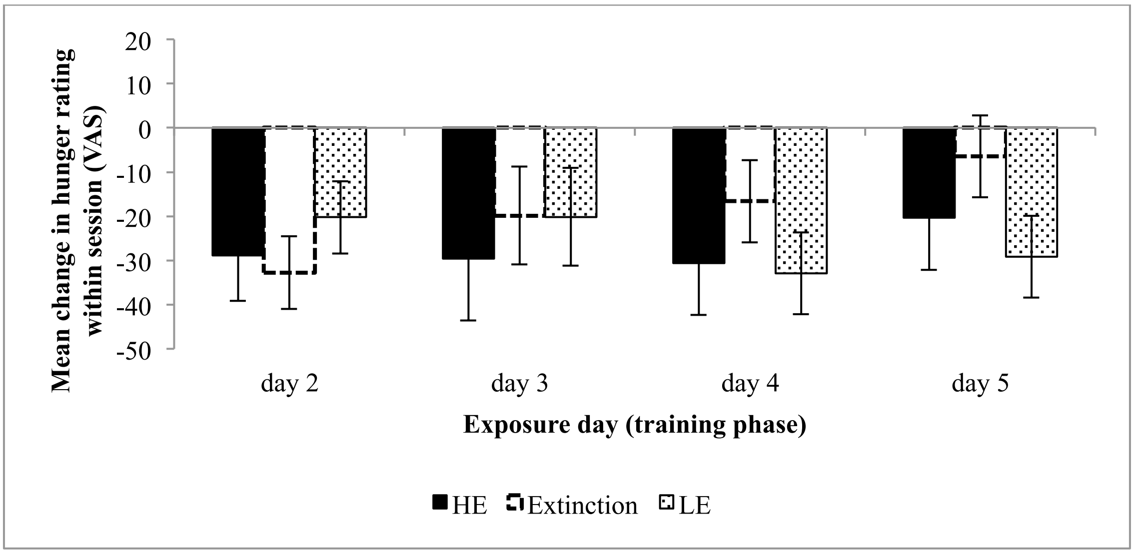
Figure 5.16: Mean (±SEM) change in hunger within each session during the extinction phase of Study Two.

During the extinction phase there were no significant differences in hunger reduction within day $F(3,63) = 0.14$, $p = .935$, between conditions $F(2,21) = 0.36$, $p = .705$, or day*condition interaction $F(6,63) = 1.21$, $p = .312$. All conditions showed a relatively similar reduction in hunger after consuming the drinks, regardless of the energy content, therefore suggesting the energy content of the drink did not influence subjective appetite.

5.3.4.5.1 Exclusion of non responders

As with the sorbet sessions, exclusion of the non-responders made very little difference to the means in the HE exposure group, with marginally higher reductions in hunger. During the training phase there were no significant differences across sessions $F(3,54) = 1.13$, $p = .345$, between conditions $F(2, 18) = 0.27$, $p = .768$ and no condition*day interaction $F(6,54) = 1.90$, $p = .098$. This was also reflected in the extinction phase; day $F(3,54) = 0.15$, $p = .927$, condition $F(2, 18) = 0.40$, $p = .679$ and day*condition interaction $F(6,54) = 0.83$, $p = .555$. Figure 5.13 shows the hunger ratings within each session for this data, and very little difference can be observed from Figures 5.15 and 5.16.

a)



b)

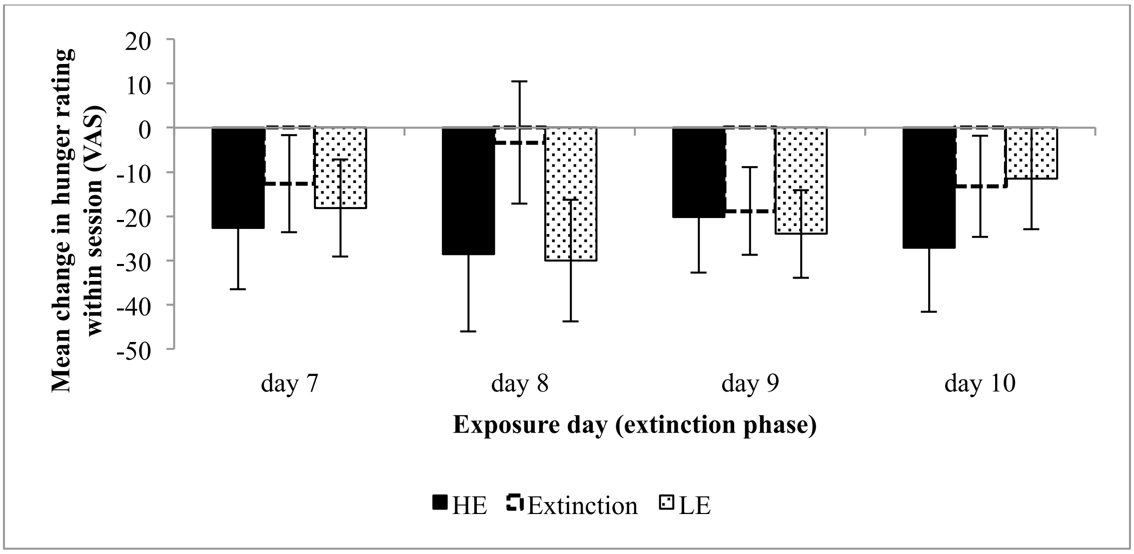


Figure 5.17: Mean (±SEM) change in hunger within each exposure session during a) the training phase and b) the extinction phase of Study Two, with the exclusion of non-responders.

5.4 Discussion

The aim of Study Two was to extend upon the tentative findings from Study One, and further investigate the extinction of flavour preferences where energy was used as the reinforcer using a paradigm where flavour preference learning had previously been demonstrated (Yeomans, et al., 2008b). Unfortunately due to lack of power in this study, firm conclusions could not be made regarding FNL or extinction but patterns of data will be discussed.

There was some evidence that acquired liking did occur for the sorbet between days 1 and 6 when trained with the HE drink compared to the LE drink, also supported by increased intake of sorbet within this period and in line with the findings by Yeomans et al., (2008b). Pleasantness ratings were maintained between days 6 and 11 for those where extinction occurred, but the pattern was less clear for the other conditions; those trained with the HE drink throughout actually showed decreased pleasantness for the sorbet during this phase, whereas those trained with the LE drink showed an increased pleasantness. Unexpectedly, intake of the sorbet during this period decreased for both the HE and extinction conditions with a small increase for the LE condition. Finally, unlike in Study One there were no consistent effects of the energy content of the training drink on changes in hunger ratings across each session, with all conditions demonstrating a similar reduction in hunger within session.

As pleasantness ratings of both the sorbet and pleasantness of the drink were maintained in those who underwent extinction, this could suggest resistance contradicting the findings with caffeine as reinforcer by Yeomans et al., (2000b) but is in line with the body of research suggesting resistance to extinction in other types of flavour learning (e.g. Boakes, 2005; Stevenson, et al., 2000; Stevenson, et al., 2003), and also supporting the conclusions from Study One. However, it is interesting to note that those in the extinction condition behaved in line with the HE condition in terms of intake changes, but pleasantness ratings appeared to switch after extinction to be more in line with those from the LE condition making it difficult to draw any potential conclusions regarding extinction. This opposite pattern of pleasantness increase and intake decrease of the sorbet in the extinction condition suggests that palatability and intake were occurring independently of each other, which has been demonstrated previously (Capaldi & Privitera, 2007; Yeomans, et al., 2008b).

Spurious group differences were identified in sorbet pleasantness change and intake between HE and extinction conditions during the training phase, but no differences were observed between these conditions for the pleasantness of the actual training drinks, where energy had been manipulated. When the sorbet data were examined three individuals were identified to report a marked decrease in pleasantness for the sorbet and as the study was underpowered, these individuals were removed to explore the impact upon the data. Inevitably, the sorbet pleasantness changes became similar between the conditions, as did the intake, however there was little impact upon drink or appetite ratings. One possibility is that these individuals did not generalise the flavour of the drinks to the flavour of the sorbet, so the drinks were pleasant during the training in the same way as with the other individuals trained with the HE drink, but this liking did not transfer to the context of sorbet in the same manner as for the other individuals. What is also interesting is that removal of these individuals impacted upon pleasantness ratings for both the sorbet, and to a smaller extent, drinks during the extinction phase. Again these effects are different between the sorbet and drinks, when these individuals are removed there is a sharp decrease in sorbet pleasantness over the extinction phase when this was fairly stable with their inclusion, whereas the pleasantness of the drinks became more stable when they were removed but compared to a general decreased pleasantness when included. This could also support a lack of generalisation between the contexts, or equally that these individuals were less sensitive to the differences that the group as a whole were responding to in terms of contrast and context (discussed below).

Contrast effects could explain the unexpected change in pleasantness and intake between days 6 and 11, as expectations can influence hedonic and behavioural factors when they are not reached (discussed in detail in Chapter 7). Those consuming the HE drink have learned that the flavour predicts energy between day 1 and 6, and on day 6 expect that the sorbet will also provide this energy. However, after this second experience of the sorbet, where the expected energy is not delivered, the perceived difference between the drink and sorbet may increase. The sorbet and the drink may no longer be perceived as comparable as participants consistently consume less sorbet than the fixed amount of drink, providing less energy. This difference may promote an expectation about the effects of the sorbet rather than the previous associations with the training flavour, resulting in decreased intake and liking on day 11. For those in the LE condition, the training drink had predicted no energy, so perhaps consuming the drink in a different context (sorbet) results in an increase in pleasantness due to reduction in a monotony effect. Additionally, perhaps two flavour associations were being made dependent upon the context (flavour and sorbet, flavour and drink) in which it was delivered. These associations may not have become generalised to both the solid and liquid versions of the flavour but remained as separate associations within each context.

FFL could have contributed to the increased liking that occurred in the LE condition over time, as the training drink was sweetened with aspartame and according to the FFL literature, neutral flavours that are paired with a flavour that is already liked or disliked will result in a shift in liking to match that of the US (e.g. Anzman-Frasca, et al., 2012). There were increases in pleasantness ratings in the LE condition for both the sorbet and the drink during the extinction phase. However, FFL would also be expected to contribute in the HE condition, with the prediction that the effect would be stronger as operating in combination with FNL (Yeomans, et al., 2008b), and pleasantness decreased for the sorbet across the extinction phase for the HE condition, suggesting that the differences observed in the sorbet pleasantness for the LE condition can be more readily explained by the contrast literature, although perhaps this had more impact for those who received the energy than those who did not. Perhaps including a flavour exposure control group would have helped clarify some of the mixed results within this study, but after the variable results demonstrated in Study One it was decided not to include one in this design.

Alongside individual differences discussed in detail in Chapter One, there has also been some research investigating differences in sensory responding, and its impact upon regulation of subsequent intake (Shaffer & Tepper, 1994; Tepper & Farkas, 1994; Tepper, Mattes, & Farkas, 1991) which could be of some relevance. In the work by Tepper and colleagues, some individuals that were identified as sensory responders, were more sensitive to sensory cues, such as flavour, rather than energy cues, so when flavours signalling a high or low energy preload were switched, they were misled into altering their subsequent intake according to the flavour cue rather than the energy content. This could be an interesting future avenue to explore in terms of extinction, as if some individuals were responding to the flavour cue rather than energy, it would be expected that these individuals would be resistant to the extinction as their responses are guided by the flavour rather than the energy content.

In summary, no firm conclusions can be drawn from this study due to power issues, and extinction resulted in different patterns of ratings dependent on whether intake, pleasantness of sorbet or pleasantness of training drink was observed.

Chapter 6: General Discussion for Part 1

It is difficult to draw conclusions about extinction from Studies One and Two due to a combination of a lack of study power (Study Two) and a lack of evidence for acquired liking (Study One). Study One failed to show evidence of FNL, with pleasantness ratings remaining fairly stable across all exposure days, although there was some suggestion that females did acquire a liking for the HE compared to the LE version. In Study Two, again there were mixed findings, with some evidence of an acquired liking after training with HE stimuli, but this pattern was not consistent amongst all individuals, and the forced early completion of the study limited power. Despite these shortcomings in both studies, removal of the nutrient had relatively little impact upon the pleasantness changes of training drinks suggesting a resistance to extinction, but the pattern of change was less clear when sorbet was introduced. Pleasantness changes during the second phase of the study were not in line with predictions from the flavour nutrient learning literature, those trained consistently with HE drinks actually showed decreased pleasantness and intake of the sorbet, whereas those consistently trained with the LE versions showed an increased pleasantness. Extinction resulted in increased pleasantness but a decreased intake, suggesting some behaviour remained in line with the HE condition and other behaviour switched to what was demonstrated in the LE condition.

An important issue that has been raised from these studies is that of food context and whether a mismatch between the context of the target food and training food disrupts FNL. In Study One, appetite changes suggested that the energy content of the HE drink was perceived to reduce hunger/increase fullness more than the LE drink (which is what would be expected), but this did not result in an increase in pleasantness of the drinks that would be in line with FNL. In Study Two, there was some evidence that certain individuals responded differently to the CS flavour in the liquid (drink) compared to solid (sorbet) form, also suggesting an influence of context. There has been a wealth of literature exploring differential effects of liquids and solids and their impact upon appetite and learning, with suggestions that the satiating effects are different (as reviewed by Almiron-Roig, Chen, & Drewnowski, 2003), that viscosity influences intake (Bennett et al., 1999) and that the sensory properties of liquids can impact upon learning (Mars, Hogenkamp, Gosses, Stafleu, & De Graaf, 2009). The study by Mars et al., (2009) found that FNL outcomes were different when yoghurts had a low viscosity compared to when they were a higher in viscosity, with facilitation of this learning in the more viscous versions, perhaps an explanation for why evidence of acquired liking may have been weak in Studies One and Two here.

Delamater (2012a) proposes three interpretations of how flavour nutrient associations can be formed between a flavour CS and nutrient US, and this could help provide an explanation for how resistance to extinction is demonstrated in flavour learning contexts. The first association is between the flavour and a post-ingestive reinforcing signal (as defined in this thesis as FNL), and this association can easily be separated from the other associations through infusing nutrients directly into the stomach when a flavour cue is in the mouth (e.g. Elizalde & Sclafani, 1990b). The second association is between the flavour and a specific sensory quality of the nutrient (for example, the sweet taste of sucrose, a similar process to FFL). The third association is between the flavour and a positive hedonic response to the nutrient, which results in the flavour evoking its own positive response rather than a response to a representation of the nutrient. There is certainly evidence in humans that both FNL and FFL type associations can be formed at the same time (Yeomans, et al., 2008b), although past studies in humans cannot readily dissociate Delamater's first and third association discussed above.

With regards to the current studies discussed in the first half of this thesis, these alternative associations may have competing effects on learning during the acquisition phase of Studies One and Two, and may have an impact upon persistence of liking after extinction. In both Studies One and Two, the training drinks themselves remain stable in pleasantness after the removal of energy, which could suggest that an association was formed based upon either or both of the latter associations rather than through a reinforcing postingestive signal as that signal would no longer be present. Perhaps pleasantness persists because the association was made between nutrient and a specific sensory quality (such as the sweet taste), or with a positive hedonic reaction, and this was then evoked by the flavour itself in the absence of the nutrient. This would be similar to the process that may be occurring in the LE conditions, where FFL may be influencing the stable pleasantness ratings demonstrated within that condition despite no nutritional value. Table 6.1 highlights which associations could potentially have been formed within each condition.

Training phase	**Flav + postingestive**	**Flav + sensory**	**Flav + hedonic**
HE	Yes	Yes	Yes
Extinction	Yes	Yes	Yes
LE	No	No	No
Control (in Study 1)	No	No	No
Extinction phase			
HE	Yes	Yes	Yes
Extinction	No	Yes	Yes
LE	No	No	No
Control (in Study 1)	No	No	No

Table 6.1: Possible ways in which associations could be formed in Studies One and Two.

In terms of intake in Study Two, perhaps an additional association was developed between the flavour and nutrient, or post ingestive consequences during training with the drink, and when given the opportunity to consume the flavour *ad libitum* (in sorbet form) the expectation that energy would be delivered led to an increased intake during the first phase. After this second consumption, if the flavour did not deliver the rewarding nutrient that had come to be anticipated over the previous learning phase, participants may have reduced their intake of sorbet on the third exposure on day 11 if they had learned that the nutrient would not be present in this sensory context (sorbet vs. drink), which could be interpreted as some form of extinction in both HE trained conditions. Those in the LE condition would have learned that sorbet and drink were equally as rewarding as neither contained energy, therefore both delivered what was expected and the sorbet provided a context change from the monotony of test drinks.

The associations discussed above are difficult to disentangle from each other, as a positive hedonic reaction to a flavour could be a result of the association with the flavour, or with the representation of the nutrient. These processes could be dissociated by a devaluation procedure, so for example, the sweet taste of sucrose could be made unpalatable by separate pairings with nausea (aversive conditioning), and if the flavour had been associated with its own hedonic response this should remain intact despite the devaluation of the taste of sucrose itself (Delamater, 2012a). However, implementing this procedure in humans would be very difficult.

Previous learning about sweet tastes throughout an individual's lifetime will inevitably have occurred before laboratory exposure and this can impact upon energy associations formed. Some attempt is made to limit this confound in FNL studies, as novel flavours are commonly used, plus for those in the LE conditions these learned associations would not be reinforced. However, there is some research in animals suggesting that the learned predictive relationship between a sweet taste and post-ingestive consequence can be degraded through experience with non-calorific artificial sweeteners (Davidson & Swithers, 2004), and this can lead to increased body weight and intake (Swithers, Baker, & Davidson, 2009, 2010). As is often the case, human research looking at artificial sweeteners and subsequent intake is not as clear, with review papers tending to conclude a lack of evidence for a relationship (Mattes & Popkin, 2009). Nonetheless, a recent paper by Rudenga and Small (2012) has shown that the use of artificial sweeteners may result in different brain responses to sweet taste in humans, in areas that are involved in signalling post-ingestive effects of flavours, although more work is needed to clarify whether there is a causal relationship. Perhaps there is a need to be screening for, or taking a record of, artificial sweetener use in participants as an indicator of past experiences and associations, which may have been formed, and investigating further how these relationships impact upon future learning.

As addressed in each separate study, there were methodological issues that may have influenced the conclusions about extinction. In addition to what has already been discussed in individual Study discussions, a possible confound may have been that the nutrient used in the HE stimuli was sucrose, and therefore all training stimuli were sweet in order to be matched. This makes it difficult to dissociate FFL and FNL, and could explain the lack of acquired liking in HE compared to LE. However, as reported in the Yeomans et al., (2008b) study, sucrose would be predicted to produce a stronger association as it can condition liking through both flavour learning processes in tandem, therefore an enhanced liking would still be predicted.

In summary, although no firm conclusions can be made, there was very little evidence that extinction of liking occurred within either study, which is in line with the majority of research in flavour preferences with humans (e.g. Boakes, 2005; Stevenson, et al., 2000) but contradicts the only known published example of extinction within a FCL paradigm (Yeomans, et al., 2000b). Context was suggested as an important factor in the development and persistence of acquired liking, and a point to consider in future studies of this nature.

PART TWO: The role of expectation in flavour nutrient learning. Studies Three-Five.

Chapter 7: Expectations and Learning

The second part of this thesis concentrates on the role of expectations in FNL and explores how manipulating these expectations influences acquired liking and how this process could be facilitated. To date the FNL literature has focused on the nature of the suggested associations between a flavour (CS) and nutrient (US), with little consideration of how such learning processes might be influenced by more explicit knowledge about the consumed items. In particular, how might explicit expectations about how sating a food or drink might be influence acquisition of liking through FNL? Such questions are critical to understand liking development since it is becoming increasingly clear that consumer expectations at the point of ingestion have important effects on the subsequent experience of appetite, and even on the physiological response to the ingested food, as the following review will explain.

Satiation refers to the processes that are involved in the termination of a meal, and influences the size of meals and snacks, whereas satiety is the state that occurs after the eating of a food or meal has ended, inhibiting further eating and influencing the frequency of meals and snacks. However, the satiating effect of food and drink is not totally dependent on its energy content; pre-absorptive cognitive and sensory cues interact with the post-ingestive consequences to determine its satiety value (as described in the satiety cascade, developed by Blundell, Rogers, & Hill, 1987). The concept of conditioned satiety proposes that associations are made between sensory properties of a food or drink and the physiological signals that occur towards meal termination, allowing us to anticipate the amount of energy that will be provided (Booth, 1972; Brunstrom, 2007; Gibson & Brunstrom, 2007). These processes all contribute to the acquired liking, and preferences, for flavours developed within the FFL and FNL paradigms discussed in Chapter One.

There are a number of sensory properties that are learned predictors of energy, for example sweetness and viscosity (Davidson & Swithers, 2004), with more viscous stimuli considered to be more desirable and higher in fat content (Drewnowski, 1992). It has been hypothesised that associations between these characteristics and corresponding energy content are formed at an early life stage, when it is learned that thicker substances (i.e. milk) provide higher calorie content than thinner substances, and that viscosity of breast milk varies with nutritional content (Davidson & Swithers, 2004; Picciano, 1998). Davidson and Swithers (Davidson & Swithers, 2004, 2005; Swithers & Davidson, 2005) have shown that the oro-sensory cues provided by viscosity are a good predictor of calories. Such associations may lead to explicit expectations about the satiating effect of foods and drinks, based on textural and flavour cues.

7.1 Expected satiety and expected satiation

Recently, researchers have defined expected satiety as the extent to which a food will stave off hunger until the next meal, and expected satiation as the anticipated amount of food needed in order to feel full (e.g., Brunstrom, Collingwood, & Rogers, 2010a; Brunstrom & Shakeshaft, 2009). Brunstrom et al., suggest that such expectations vary across a range of food items and that they may be learned over time. The 'method of constant stimuli' was introduced in an attempt to assess satiety expectations (Brunstrom, Shakeshaft, & Scott-Samuel, 2008) as it allows quantification of differences in expectations through presentation of a 'standard' food (presented in pictorial form) which is of a known energy content, and another food presented in varying quantities to be used as a comparison. Observers are asked to state which food will be more satiating, allowing statistical analysis to determine an expected satiety score which can be used to compare foods (see Brunstrom, et al., 2008, for more details). This method has then been used to measure expected satiety across a range of studies (e.g., Brunstrom, et al., 2010a; Brunstrom & Shakeshaft, 2009; Wilkinson & Brunstrom, 2009) and to explore the role of such expectations in appetite regulation and in particular, decisions about portion size. The study by Wilkinson and Brunstrom (2009) explored whether FNL could influence the expected satiation of a novel dessert, and in a between subjects design, asked participants to attend the laboratory on two sessions, where the dessert was tasted on day one and a measure of expected satiety taken, and on day two, a second measure was taken before intake was recorded. They found that expected satiation increased between time points one and two for a HE dessert but did not change for a LE version. This suggested that associations were being made between the energy and the post-ingestive consequences, and as a result, the HE dessert was expected to be more satiating on day two. There are a number of factors that can influence expected satiation and some will be discussed through the rest of this section.

7.2 Context: liquid vs. solid form

It has been proposed that calories consumed in liquid form are not perceived to be as satiating as calories consumed in solid form, for example 1kg of apples is perceived to be more satiating than the equivalent apples presented in juice form (see Almiron-Roig, et al., 2003 for a review). They conclude that the evidence is inconsistent, with some studies reporting liquid is more satiating (nine studies in the review paper, e.g., Rolls, Fedoroff, Guthrie, & Laster, 1990 who found that a soup preload was more satiating than melon or cheese and cracker preloads), and some that liquids are less satiating (seven studies in the review paper, e.g., Tournier & Louis-Sylvestre, 1991). Tournier and Louis-Sylvestre (1991) conducted two experiments, the first involved consumption of an identical food in either solid or liquid form, and the second involved consumption of test meals comprised of both solid and liquid foods, with the major amount of calories in either the liquid or solid part. Subsequent consumption was higher after the liquid than solid food, and over a 24 hour period, intake was higher after a meal where the majority of calories were in the liquid part of the meal. The second experiment controlled for the amount of masticatory movement involved, which the researchers claim rules this out as an explanation for the overconsumption. However, a number of criticisms can be raised about many of these studies, as often the foods being compared are dissimilar, and differ in other characteristics such as energy content, palatability, and cognitive information (such as a beverage vs. a meal item) which could also account for the discrepancies between studies.

It is important to consider what it is about liquids that make them less satiating than equi-caloric solid foods. In their review, Almiron-Roig et al., (2003) identified several important factors which influenced whether a solid or liquid version was more satiating, including the volume consumed, and the time period between preload and test meal; studies with a long time delay suggest solids are more satiating, whereas short time periods favour liquids as more satiating. In a more recent review, de Graaf (2011) also discusses the rate at which liquids are consumed compared to solids, and how this impacts upon their satiating qualities. Liquids are consumed at a much faster rate of up to 200g per minute, whereas solid foods tend to be consumed at less than 100g per minute (Viskaal-Van Dongen, Kok, & de Graaf, 2011). These differing eating rates will therefore result in different cephalic and gastric phase responses which initiate satiation signals (Almiron-Roig, et al., 2003), and it has been suggested that reduced gastric and post-gastric responses may be the reason that liquids are not as satiating as solids (Spiegel et al., 1997; Spiegel, Kaplan, Alavi, Kim, & Tse, 1994). Cephalic phase responses are predominately learned responses to sensory signals from food and can be stimulated by the five senses and also by the thought of food, and play a role in expected satiety (De Graaf, 2011).

Soup is an important exception to the rule, as it is consumed at an eating rate that is similar to many solid foods, and has been shown to reduce energy intake at a subsequent meal (Flood & Rolls, 2007). One reason suggested for this exception is the enhanced oro-sensory exposure time, making it easier to associate the sensory signals generated when eating with the metabolic consequences (De Graaf, 2011). In addition, the duration of olfactory stimulation may also be influenced by oro-sensory exposure, as it has been shown that more aroma molecules are released for much longer in a solid food compared to a liquid food (Ruijschop, Burgering, Jacobs, & Boelrijk, 2009), although it should be noted that these were not solid and liquid versions of the same food. A key study by Mattes (2005) compared the consumption of whole apples, apple juice and apple 'soup' (juice consumed using a spoon) and found that the soup was equally satiating as the whole apples, whereas the juice was much less satiating. Another explanation for this effect could be the cognitive effect of a 'meal context', which could be generated through the use of the spoon, leading to the 'soup' being more satiating than the juice alone. So differences in the context within which calories are consumed appear to influence subsequent appetite and intake, and ultimately this could impact upon FNL and pleasantness of a product.

7.3 Influences of viscosity on intake, appetite and weight gain within a liquid context

A wealth of research explores the impact of low viscous, high energy drinks upon subsequent meal intake, energy compensation and weight gain. Research suggests that the calories consumed in beverages can encourage positive energy balance because they are not well compensated for in later meals. In other words, the consumption of energy-yielding beverages is often in addition to, rather than substitution of, total energy consumed throughout the day (Mattes & Rothacker, 2001). Viscosity influences subsequent intake, with higher energy intake after a liquefied version of a solid diet, (Bennett, et al., 1999) and both immediate and prolonged hunger reduction reported after a thicker beverage compared to a thin beverage (Mattes & Rothacker, 2001).

Viscosity induced appetitive differences were also observed in a study by Mattes and Rothacker (2001) where thick and thin preload shakes were consumed with a minimum of a 24 hour period between versions, and were presented in cans to disguise viscosity. Food diaries were recorded throughout the study, providing intake data for days where shakes were and were not consumed. Hunger was reduced after both the thick and thin shakes but this was prolonged, and to a larger extent, after the thicker version. There were also significant correlations with the fullness, desire to eat and prospective consumption after the thick shake, but not the thin, which supports the finding that the thicker drink had a stronger effect on appetite. There were no significant differences in the time until, or energy content of, the first meal consumed after the shake, and daily energy intake was no higher on days when the shake was consumed than on the days that it was not. There was, however, a trend for daily intake to be higher on days when the thin shake was consumed compared to days when it was the thick shake. A strength of this study was that it assessed the effect of textural differences within one food context, a beverage, and the samples were matched for a number of characteristics which are often unbalanced between thick and thin versions, such as energy content, cognitive expectations and palatability. The authors suggest that the appetitive differences demonstrated in this study were primarily due to oro-sensory factors because the differences were greatest within ten minutes of consuming the preload. It was also suggested that this timing indicates a relationship between viscosity and gastric/postgastric feedback. Crucially, in the context of this thesis, learning is also implicated in these findings, as texture is an important associative cue for energy (Blank & Mattes, 1990; Sclafani, 1997), therefore if the more viscous preload signals energy this could lead to lower hunger ratings irrespective of actual energy content due to previously learned associations (Wooley, Wooley, & Dunham, 1972). Furthermore, expected satiation also increases as thickness increases across a range of dessert products (Hogenkamp, Stafleu, Mars, Brunstrom, & De Graaf, 2011) which suggests that we have learned foods with a thicker texture will be more satiating.

Oro-sensory cues can affect the satiating value of a beverage, and thick texture generates the expectation that a food product will be more satiating, so maybe one reason why beverages have a weak satiety value may be because their liquid texture does not predict the delivery of energy. An interesting study by Cassady, Considine and Mattes (2012) combined cognitive and sensory manipulations of a preload and assessed the impact upon both subjective and physiological measures of appetite. The sensory cues were manipulated through the form of preload (liquid versus solid) consumed and the cognitive beliefs were manipulated by the information participants received about what form the preload would be once it reached the gut (liquid versus solid). A visual representation of preload contact with 'gastric acid' was shown to participants in order to manipulate expectations but in reality all preloads would be in liquid form upon reaching the stomach. It was found that the sensory (oral liquid vs oral solid) and cognitive (perceived gastric liquid vs. perceived gastric solid) elements influenced different aspects of appetite and intake. Sensory elements appeared to be more influential over physiological measures of appetite: when experienced as a liquid in the mouth, gastric emptying, orocecal transit times, and insulin release, were quicker than when experienced as a solid in the mouth. The cognitive element of how the preload would be in the gut significantly affected intake, with lower intake after the preloads expected to be solids in the stomach than those expected to be liquids. There were also some trends for cognitive effects on gastric emptying. Anecdotal comments from the participants conveyed the power of the cognitive manipulation, as perceptions of how the preload felt were greatly influenced with the liquid preload perceived to be a solid in the gut feeling "like I swallowed a rock" compared to the liquid perceived to be liquid in the gut seeming "less filling than a normal drink". A similar reaction occurred between the solid preload forms, with the one perceived to be liquid in the stomach said to "hardly feel like I ate anything" and the solid felt "like I just ate an entire buffet". This study highlights the combined impact of the sensory properties of the food ingested and the expectations of the consumer on the effects of ingestion on appetite. Critically it is one of the first studies to provide clear evidence that expectations (here about whether a product remains liquid or becomes solid) altered the physiological response to consumption and this could help to explain the lack of caloric compensation often seen after energy consumed in liquid rather than solid form.

If we learn that more viscous foods and beverages provide us with more energy and this cues cephalic and gastric responses, what happens when the energy delivered is incongruent to the oro-sensory characteristics, or when these characteristics are inconsistent with explicit expectations about energy? Characteristics can be manipulated to be incongruent or congruent with the actual energy content of a food or drink. In an early study by Wooley (1972) preloads which were high and low in calorie content were manipulated to either appear congruent or incongruent to their actual energy level using viscosity (with thicker preloads assumed to signal higher energy contents), with participants believing they differed by an average of 247 calories. In addition, participants were explicitly alerted to the sensory differences, either through labelling or experimenter instruction. Intake was found to be higher after the preload that was believed to be low calorie (thinner), with no differences between preloads that actually differed in energy content. Viscosity differences also influenced fullness ratings, with higher ratings after the preloads believed to be high calorie. Manipulating the consistency between cue and actual calorie content influences subsequent energy intake (Davidson & Swithers, 2005; Swithers, Doerflinger, & Davidson, 2006). Swithers et al., (2006) explored how potato chips that were either consistent or inconsistent predictors of calories influenced intake and the body weight of rats. Some rats were given chips that were consistently high fat high calorie, and other rats sometimes received these chips and other times were given chips that were low fat lower calorie (light chips with an added fat substitute). Animals that were given the chips with sensory characteristics that consistently predicted calories consumed significantly less than rats given inconsistent exposure or no exposure at all. In a second study, inconsistent exposure also resulted in overconsumption of a high fat chip premeal and lack of compensation for this consumption in subsequent intake. There was no evidence of long term weight gain in any of the rats. There are limited human studies investigating the role of consistency between cue and predictors of energy, although recently a study has shown that artificial sweeteners (where increased usage has resulted in sweet tastes no longer consistently predicting energy) can lead to different neuronal responses in areas linked to the signalling of post-ingestive effects (Rudenga & Small, 2012). More research is needed in this area as this could have a huge impact upon the diet industry and the promotion of weight loss (although there is mixed evidence for whether consumption of artificial sweeteners impacts upon weight status, see Mattes & Popkin, 2009 for a review).

In a study looking at the effect of viscosity on weight gain, Davidson and Swithers (2004) manipulated the viscosity of a supplement for rats, with some consuming a thin version and others a thick (energy matched) version, alongside *ad libitum* chow. Over thirty days, rats that received the thin supplement gained more weight than those consuming the thick, which the authors suggest indicate a reduced ability to compensate for the additional calories. Davidson and Swithers (2005) expanded upon these findings with four studies which consistently demonstrated that intake was higher in rats that were fed a low viscosity premeal compared to a high viscosity, and this was not dependent upon the thickening agent used or the level of viscosity used as the 'high' version. Additionally, in line with the previous study, rats gained more weight after daily consumption of a low viscosity supplement over 10 weeks. The researchers discount the fact that the high viscosity supplement may have produced greater satiation due to the inclusion of a no supplement control condition in one of the studies, where calorie intake was equal to that of the high viscosity group. Intake in the low viscosity group was much higher, supporting the view that lower viscosities are less able to induce satiety (Mattes, 1996) as opposed to the higher viscosities being more able.

Manipulating satiety relevant cues of a preload (i.e. thickness and creaminess) has been shown to result in enhanced satiety and higher energy compensation in a subsequent test meal in humans (Yeomans & Chambers, 2011), but the actual energy content of the preload was also important. When satiety relevant cues were present there was an 87% compensation for the additional energy in the HE preload, compared to 18% compensation when these cues were not present. Interestingly, there was also some evidence of a rebound hunger effect when the satiety cues were incongruent to the actual energy received (so a low energy thick and creamy preload), with both higher hunger ratings before lunch and increased intake.

7.3 How does viscosity impact upon flavour nutrient learning?

So, if the viscosity of a food or drink influences expectations with regard to how satiating/ how much energy will be provided, this association would be predicted to impact upon changes in liking of flavours through FNL (Brunstrom, 2005; Mars, et al., 2009). It could be predicted that more viscous high energy stimuli would lead to more positive post-ingestive consequences and therefore become more pleasant, although there is not always a relationship between hedonic and appetitive ratings (De Graaf, De

Jong, & Lambers, 1999; Russell & Delahunty, 2004). In the study by Mars et al., (2009), repeated exposure to high viscosity and low viscosity yoghurts in both high and low energy versions revealed no significant interaction between time, viscosity and energy on pleasantness ratings, with pleasantness ratings showing a general increase over time. However, when analysed separately, low viscosity yoghurts decreased in pleasantness whereas there was no change in the high viscosity. For the low viscosity versions, the low energy was more pleasant than the high energy, but the opposite pattern was observed in the high viscosity yoghurts. There were no effects of viscosity or energy on appetite ratings or *ad libitum* intake at lunch but there was evidence that the difference in energy content impacted upon *ad libitum* yoghurt intake in the high viscosity versions only; with greater compensation demonstrated in the high energy version compared to the low energy version. This indicated a lack of learning regarding energy content in the low viscous yoghurts, but facilitation of this learning in the high viscous yoghurts. The lack of learning in the lower viscosity yoghurts could have been as a result of lower oral processing time in comparison to the high viscosity versions, and could also be attributed to expectations that were generated by the sensory properties, where thinner products have been learnt to predict fewer calories.

In summary, the sensory properties of a product and associated consumer expectations appear to impact upon learning and appetitive responses in both animals and humans. However, in humans there are a variety of sources of information, including packaging, advertising and the opinions of other consumers that can enhance explicit expectations about the likely energy content, sensory qualities and post-ingestive consequences of new foods and drinks. These expectations may then be confirmed or rejected once a product is consumed, and may be further moderated by previous experiences and associations, such as those discussed previously.

7.4 Labelling and information

Expectations are powerful as they influence liking and perception of a food with regards to fat (Solheim, 1992; Tuorila, Cardello, & Lesher, 1994; Wardle & Solomons, 1994), sugar (Kuenzel, Zandstra, El Deredy, Blanchette, & Thomas, 2011) and salt (Liem, Aydin, & Zandstra, 2012; Liem, Miremadi, Zandstra, & Keast, 2012). Also, the taste of products such as coke is liked more when consumed from a branded-named cup (McClure et al., 2004) and giving information on ingredients (such as soy content in a cereal bar) can change perceived pleasantness of a product (Wansink & Park, 2002). Solheim (1992) found that, when sensory attributes such as texture were matched between regular and reduced fat sausages, false information indicating that the regular sausages were reduced in fat content actually resulted in increased liking for the product. When the sensory attributes were not matched, information on fat content had no influence over hedonic ratings, with higher liking of regular fat sausages regardless. Eiser, Eiser, Patterson and Harding (1984) found nutritional information did not always influence pleasantness ratings of foods, which was attributed to the fact that knowledge that a food is high or low in particular nutrients doesn't necessarily provide information about pleasantness; participants can draw upon individual experiences of the food with no nutrient information needed.

7.4.1 Calorie information

One way in which expectations can be generated is through the provision of calorie information about a product. A range of literature explores the difference in appetite and intake between those informed of calorie content and those who are uninformed, with mixed findings of some finding a trend for a difference (Mattes, 1990) and others finding no effect (Rolls, Hetherington, & Laster, 1988; Rolls, Laster, & Summerfelt, 1989). Mattes (1990) investigated the difference between sucrose and aspartame sweetened breakfasts, and found that, whilst information regarding content did not influence subsequent hunger ratings, it did appear to influence intake. Moreover, when informed of the different sweeteners, intake was higher following the aspartame breakfast, although these findings only indicated a trend in this direction.

Wooley, Wooley and Dunham (1972) replaced one meal a day with a liquid meal for a baseline of 5-10 days before a test phase of 14-21 days. During the baseline period, the meals were equicaloric to their usual meal, but during the test phase they were either higher or lower calorie meals (matched in taste). In the test phase, participants were told that some of the meals would be high or low calorie and were asked to make a judgement at various intervals after each meal as to whether the meal had been low or high in calories, and rate their appetite. A large percentage of initial judgements were incorrect, and where these judgements remained unchanged across the intervals after a meal, hunger was influenced by the judgements rather than by the actual calorie content. This highlights how cognitive beliefs about the calorie content of a food can influence subjective appetite, and providing explicit information through labelling could therefore enhance these effects and impact upon learning processes.

7.4.2 Beliefs about the satiating effects of food

As well as looking at the effect of information, a number of studies have also manipulated participants' beliefs through the labelling of a preload, followed by the measurement of appetite and consumption of a subsequent test meal (Shide & Rolls, 1995; Yeomans, Lartamo, Procter, Lee, & Gray, 2001). Shide and Rolls (1995) manipulated information regarding the fat (but not total energy) content of a yoghurt preload and found this influenced subsequent energy intake, with women consuming more following the 'low fat' preload compared to the 'high fat', despite similar actual energy content. Furthermore, if no information was provided, less energy was consumed following the low fat, high calorie preload than the high fat, high calorie preload. This indicated that information and expectations regarding fat content affected responses to physiological signals.

Conversely to the study by Shide and Rolls (1995), Yeomans et al., (2001) found that intake at the test meal was dependent upon the actual fat content of the preload, not the labelled content, with unrestrained males eating less following the (actual) high fat preload. Whilst the labels did not affect the appetite of participants, the sensory ratings of the soup preload were altered, with the soups labelled as 'high fat' rated as more pleasant and more creamy, than those labelled as 'low fat'.

Labelling can be used to influence the mindset with which a food is consumed, demonstrated in a study by Crum, Corbin, Brownell and Salovey (2011) where individuals consumed an identical milkshake on two sessions, where one was labelled with "indulgence: decadence you deserve" and the other was labelled with "sensi-shake: guilt free satisfaction". The 'sensible' shake was rated as healthier than the 'indulgent' shake, but there was no difference in perceived tastiness or subjective hunger after consumption. Interestingly, the physiological responses (measured using ghrelin) to the milkshakes differed depending on the label; anticipation of the 'indulgent' shake caused a significantly steeper rise in total ghrelin, which is a biological marker of hunger, and after consumption there was a significantly steeper reduction. In the 'sensible' shake condition ghrelin levels remained stable or showed a slight increase over consumption, suggesting lack of physiological satisfaction despite an identical nutrient content to the 'indulgent' shake. This study suggests that expectations generated by labels could have a significant effect on appetite regulation, and the lack of difference in subjective hunger ratings is interesting, although authors note that this could be due to the timing of the ratings (10 minutes prior to the ghrelin changes rather than at the same time or afterwards).

Generally, in terms of appetite, individuals report higher levels of fullness after consuming what is believed to be a high calorie preload, and greater hunger after consumption of what is believed to be a low calorie version (e.g., Provencher, Polivy, & Herman, 2009; Wooley, Wooley, & Woods, 1975), although this is not always the case (Wardle, 1987). Ogden and Wardle (1990) who manipulated information about calorie content of a preload, found an effect on hunger ratings, but only in those classified as restrained eaters, with lower ratings after the preload believed to be high in calories. There were no differences in subsequent intake regardless of restraint or believed calories. In other studies, restrained eaters have been shown to consume more after what is perceived to be a high calorie preload which is referred to as counter-regulatory eating (e.g., Knight & Boland, 1989; Polivy, 1976). This could suggest that certain groups of people may be more susceptible to labelling than others.

Individual attitudes regarding food and health issues can also change the effect of nutritional labelling (e.g., Aaron, Mela, & Evans, 1994; Engell, Bordi, Borja, Lambert, & Rolls, 1998; Kähkönen, Tuorila, & Rita, 1996; Westcombe & Wardle, 1997). In the study by Kähkönen et al., (1996), participants were divided into 'concerned' and 'unconcerned' based upon their ratings of how concerned they were with various health and food related issues. When information was provided before exposure to a low fat spread those classified as 'concerned' rated the spread as more pleasant, and showed an increased pleasantness over time compared to the 'unconcerned' group.

From the research discussed above it appears that labelling may be a good way to generate expectations about a food or beverage, and indicates that these expectations could have an important impact on subjective changes in appetite, hedonic ratings, and also the physiological responses to food. However, it is not clear how strong these effects are as the results are not always consistent, and little is known about how explicit labelling influences the learned associations between the sensory properties of a food. It is important to consider how explicit information about the calorie content of a novel food influences satiety when a product is repeatedly consumed, and this will be addressed in the second set of studies in this thesis.

7.5 What happens when expectations are not met?

The research discussed above has demonstrated that expectations of satiety can be generated through oro-sensory cues and through explicit calorie information and food labelling. But what happens when these expectations are not in line with the energy that is delivered after consumption of a food or drink? Four main theories have been proposed regarding the effect of disconfirmed expectations on acceptance and liking for a product (as reviewed by Anderson, 1973); assimilation, contrast, assimilation-contrast and generalised negativity. Assimilation proposes that when the expectation is not met, the perception of a product is brought in line with that expectation, in order to reduce the discrepancy (related to cognitive dissonance; Festinger, 1957). Contrast proposes the opposite; disconfirmed expectations result in an exaggeration of the discrepancy (Schifferstein, Kole, & Mojet, 1999). These two elements can be combined into the assimilation-contrast model, where the size of the discrepancy between expected and actual experience determines the direction of the shift in perception. If the discrepancy is small, assimilation will occur, but if it is too large to be deemed an acceptable level, this will result in a contrast effect. Finally, generalised negativity proposes that the effect will always be a negative evaluation of the product when expectations are disconfirmed, regardless of whether the product was better or worse than expected.

Research in food perception finds evidence to support the assimilation model (Kähkönen, et al., 1996; Schifferstein, et al., 1999; Tuorila, et al., 1994; Wansink, van Ittersum, & Painter, 2005b). For example, when a menu used descriptive food names rather than just the standard (succulent Italian seafood fillet vs. seafood fillet), the food was rated as more appealing, tasty and caloric, and these evaluations were assimilated with expectations prior to exposure (Wansink, et al., 2005b). However, some research provides evidence for a contrast effect (e.g., Yeomans, Chambers, Blumenthal, & Blake, 2008a; Zellner, Strickhouser, & Tornow, 2004). A strong contrast effect has been shown when the expectation and actual experience of a food are very different, with rejection and dislike of the food as the end result (Yeomans, et al., 2008a). This was achieved through labelling a novel smoked salmon flavour ice cream as either ice cream or a savoury mousse. The ice cream label created the expectation of a sweet, fruity flavour resulting in a large contrast effect when the actual product was tasted.

Strength of the expectation has been suggested as a determinant of whether assimilation or contrast effects occur (Zellner, et al., 2004). In this study, information was manipulated in order to generate expectations about two novel foods, which were then evaluated by participants. It was found that whether a contrast or assimilation effect was demonstrated depended upon the type of expectation that had been generated. When the expectation generated was based upon another's rating (for example, other participants disliked this product) assimilation occurred, which the authors suggest was down to the certainty of the expectation, as others ratings led them to believe it will certainly have this hedonic value. When the only expectation generated was concerned with an average rating (for example if informed that overall the food was rated as liked this still meant some uncertainty remained as to the hedonic value), a contrast effect occurred when this expectation was not met.

Another suggested modulator of whether assimilation occurs is the nature of the stimuli itself (Kuenzel, et al., 2011). In this study, symbols were paired with high and low concentrations of sweet and salty yoghurt drinks so that a symbol became a learned cue for the drink it had been paired with. The drinks had previously been rated in terms of how sweet/salty (the sensory condition) or liked/disliked (the hedonic condition). These cues were then presented either supra- or subliminally and participants were told the cue would predict the drink that they would then taste (so based upon their previous associations), but that they should rate how much they liked the actual taste. When predictive cues were presented supraliminally and drinks were initially liked, assimilation occurred, with no influence of whether the cue was hedonic or sensory or the size of discrepancy between the cue and actual drink. Therefore, when expecting the drink was going to be pleasant (from the cue) liking increased. When a disliked drink was used, the effect was only observed in the most disliked drink, not in drinks that were only mildly or moderately disliked.

7.6 Expectations and flavour-based learning

In summary, learned associations between the sensory properties of a food and both pre and post ingestive factors generate expectations about the pleasantness and satiating quality of a product and impact upon many areas such as intake and portion size. These expectations can be enhanced through information and labelling, all contributing to eating patterns and acceptance of new foods and beverages. Although there have been a number of studies exploring the role of expectation in the modification of human based learning beyond the flavour-learning literature (e.g., Ziori & Dienes, 2008), at present there have been no similar studies using flavour based learning, and the impact of expectations on flavour preference acquisition remains unclear. Based upon the research discussed in this section, the second part of this thesis aimed to investigate the apparent influence of oro-sensory cues in the modulation of subsequent responses to nutrients, the influence of viscosity and the expectations generated by such characteristics, focusing on whether these cues can be manipulated to facilitate liking acquisition through FNL. Furthermore, this second part of the thesis explored how the introduction of labels can influence acquisition of liking through FNL, firstly through providing explicit information about calorie content, and secondly by using a hedonic description to encourage this liking to occur.

Chapter 8: Study 3 - Manipulating oro-sensory cues to be predictive of energy

8.1 Introduction

As discussed in Chapter 7, there are a number of sensory properties that are learned predictors of energy, for example sweetness and viscosity (Davidson & Swithers, 2004), with more viscous stimuli considered to be more desirable and higher in fat content (Drewnowski, 1992). As infants, this association is often formed through breastfeeding, with thicker liquids (i.e. milk) indicating a larger number of calories than thinner liquids, and also through variations in the nutrient content and viscosity of the milk which is dependent upon factors such as maternal diet (Picciano, 1998).

Davidson and Swithers (2004) gave rats a high calorie dietary supplement in addition to an *ad libitum* supply of chow. The sensory properties of the supplement were manipulated so that one was thin (water was added) and the other thick (guar), whilst maintaining identical calorie and nutritional content. Those rats consuming the thin supplement gained more weight than the rats consuming the thick supplement, suggesting weaker compensation for the calories. Manipulating satiety relevant cues of a preload (i.e. thickness and creaminess) has also been shown to result in enhanced satiety and higher energy compensation in a subsequent test meal (Yeomans & Chambers, 2011), with the actual energy content of the preload being an important factor. When high sensory preloads signalled high energy content, satiety was enhanced, whereas if the signal was incongruent with actual energy received a rebound hunger effect was seen. These studies indicate that oro-sensory information about the expected energy content of a food or drink can influence subsequent eating behaviour.

If these oro-sensory cues predict actual calories received and positive post-ingestive consequences, FNL could be enhanced. Viscous stimuli impact upon appetite through delayed (although only minimally) gastric emptying which leads to increased fullness (Marciani et al., 2000), and this may in turn trigger learning about the energy content. Additionally, if the oro-sensory cues predict calories that are not present, this may lead to reduced hunger regardless of the actual energy content, as found in the study by Wooley, Wooley and Dunham (1972). Hunger ratings remained in line with the initial judgements made by participants after consumption of a liquid diet that they had been informed would sometimes be high in calorie and sometimes low, regardless of actual calorie content. These effects on appetite may transfer into changes in pleasantness with high viscosity paired with high energy expected to be rewarding and therefore become more pleasant (Mars, et al., 2009). However, there is not always a relationship between hedonic and appetitive ratings (De Graaf, et al., 1999; Russell & Delahunty, 2004).

On a similar note, Mattes and Rothacker (2001) investigated beverage viscosity and the impact upon hunger. After consuming a thick shake and a thin shake (with 24 hours between), there was a greater reduction in hunger after the thick shake, which was sustained for four hours post consumption, compared to the thin shake which led to a significant immediate reduction but this was not sustained.

The present study aimed to examine how manipulating the oro-sensory characteristics of a yoghurt drink influenced acquired liking, focusing on viscosity and creaminess. In a mixed design study, participants consumed either a low energy (LE) or high energy (HE) version of a drink on six occasions, with half consuming a thick (high sensory) version and half a thin (low sensory) version. It was predicted that those consuming the HE version would demonstrate an acquired liking over time, and that this would be enhanced for those consuming the thick drink, as this signals energy. Those consuming the LE thick version may also demonstrate an acquired liking initially, but this may be reduced if the actual energy content is recognised. A similar pattern in appetite ratings would be expected. As drinks were used as the test stimuli, thirst ratings were also analysed, as expectations regarding energy in a beverage context are different to those generated in a food context, particularly in terms of satiety, as discussed in Part One (DiMeglio & Mattes, 2000). Perhaps the high sensory drinks would be expected to be less thirst quenching than the low sensory versions, as highlighted in a study by McEwan and Colwill (1996) where focus groups were used to identify a range of thirst quenching drinks that were then assessed by a sensory panel; thickness was considered a negative attribute for a thirst quenching drink.

8.2 Method

8.2.1 Design

Participants were assigned randomly to one of four conditions, which varied in the energy content (low energy; 78 kcals vs high energy; 279 kcals) and sensory characteristics (thick vs thin) of the drink. This resulted in the following four combinations; low energy high sensory (LEHS), low energy low sensory (LELS), high energy high sensory (HEHS) and high energy low sensory (HELS).

8.2.2 Participants

Forty eight female participants, aged 18-29 ($M = 21.3 \pm 0.4$) with a mean BMI of 22.5 ± 0.4 were recruited, mainly from the Psychology subject pool and course credits database. All participants scored less than seven on the restraint scale of the TFEQ (Stunkard & Messick, 1985) and met the additional exclusion criteria discussed in Section 2.4.1.

The demographic information for participants in each condition is displayed in Table 8.1. There were no significant differences in BMI, $F(3,44) = 2.07$, $p = .118$ or TFEQ restraint score $F(3,44) = 1.64$, $p = .193$ between conditions. As homogeneity of variance was violated for age, Welch and Brown-Forsythe statistics were reported: $F(3,23.81) = 1.97$, $p = .146$ and $F(3,31.87) = 3.45$, $p = .028$, so those in the LEHS condition may have been significantly older (by chance) than those in the other conditions.

Condition	Age	BMI	TFEQ Restraint
LEHS	23.5 ± 1.2	22.9 ± 0.9	4.3 ± 0.5
LELS	20.8 ± 0.6	23.9 ± 0.9	3.3 ± 0.6
HEHS	20.6 ± 0.5	21.4 ± 0.8	2.6 ± 0.6
HELS	20.2 ± 0.8	21.7 ± 0.6	3.1 ± 0.6

Table 8.1: Mean (± SEM) age, BMI and TFEQ-R score for each condition in Study Three.

8.2.3 Test foods

On each day, participants consumed a control breakfast that consisted of 60g Crunchy Nut Cornflakes (Kelloggs, UK), 160g semi-skimmed milk and 200g smooth orange juice (Sainsbury's, UK; total 402kcal). The test drinks were produced in-house, using recipes developed for other studies within the laboratory (Yeomans & Chambers, 2011). The base for the drinks consisted of Pomegranate juice (Sainsbury's, UK), Orange and Mango squash (Robinson's, UK), natural Normandy fromage frais (Sainsbury's be good to yourself range, UK), with rhubarb flavouring (International Flavours and Fragrances) and red and yellow colouring (Silver Spoon, UK). The LE versions also had yoghurt flavouring (International Flavours and Fragrances). Maltodextrin (Cargill) and whey protein isolate (myprotein.co.uk) were added to the base for the HE versions. The sensory characteristics were manipulated using tara gum (Kalys Gastronomie, France) to increase viscosity and milk caramel flavouring (Th.Geyer, Germany) and vanilla extract (Nielsen-Massey Vanillas Int. NL) to enhance creaminess. Tables 8.2 and 8.3 show the composition of the test stimuli.

Ingredient	**LE base (77.7 Kcal)**		**HE base (279.3 Kcal)**	
	Amount	***Kcals***	***Amount***	***Kcals***
Juice	220g	48.3	185g	38.9
Squash	30g	2.4	30g	2.4
Fromage frais	50g	25	25g	12.5
Whey protein isolate			25g	92.5
Maltodextrin			35g	133
Rhubarb flavour	4 drops		4 drops	
Colouring	8 red, 4 yellow		6 red, 4 yellow	
Yoghurt flavour	10 drops			

Table 8.2: Composition of the two base energy drinks in Study Three.

Ingredient	**LELS**	**LEHS**	**HELS**	**HEHS**
Tara gum	0.3g	0.9g		1.5g
Milk caramel		0.3g		0.3g
Vanilla		60 drops		60 drops

Table 8.3: Manipulation of the sensory properties of the drinks in Study Three.

8.2.4. Procedure

The study occurred over six days during a two to three week period. Participants were asked to refrain from eating and drinking, except water, from 23.00 the night before each session (see Appendix 5 for consent forms). They reported to the laboratory for breakfast at a time between 08.15 and 10.00, after which they were allowed to leave but were required to return three hours later. They were only allowed to drink water during this time period. During the second part on each day, all participants followed identical procedures. Upon arrival, participants completed a set of computerised mood (lively, clear-headed, tired, nauseous, energetic, headachy, drowsy, calm) and appetite (hungry, thirsty, full, desire to eat, how much they could eat) ratings, using the SIPM software (see Section 2.4.2. for more information). Participants were then given a 300g serving of the appropriate drink (dependent upon condition) and were asked to complete a taste test (pleasant, thick, novel, sweet, filling, familiar, creamy) before consuming all of the drink, through a straw. Once they had finished the drink, they completed short appetite ratings (hungry, thirsty, full, desire to eat, how much they could eat) and were asked to sit in the waiting room for an hour, consuming only water. The mood and appetite ratings were repeated at 30 and 60 minutes after finishing the drink, and participants were then free to leave the lab. On the final day, participants were debriefed, height and weight recorded and reimbursed for their time.

8.2.5 Data Analysis

8.2.5.1 Preliminary analysis

K-S tests, boxplots and histograms were used to explore normality within this data set. There were a number of incidences where normal distributions were compromised. Significant outliers were removed but with no change to normality, therefore these were included in the analyses. As Homogeneity of variance was met across all levels of all analyses (except hunger change 60 minutes post test on days five and six, change in fullness 30 minutes post test for day one, and change in fullness 60 minutes post test for days one and five) it was decided to continue with analysis on the full data set. As most of the variables were change data and involved within subject variables, transformation of one set of data would require transformation of all other variables, which would lead to other normality issues, it was considered appropriate not to transform the data.

8.2.5.2 Main analysis

One-way Independent ANOVAs were conducted to assess baseline pleasantness differences between conditions, and between appetite ratings on day one, before any exposure to the stimuli. Three-way Mixed ANOVAs were used to investigate differences in pleasantness, appetite ratings and subjective ratings of how filling the drink was, over time and between energy and sensory versions. Where significant interactions were found, one-way ANOVAs were conducted to explore where the interaction was, and Bonferroni corrections were applied where appropriate. Where sphericity could not be assumed, the appropriate corrected statistics were reported ($\varepsilon < .75$ Greenhouse-Geisser, $\varepsilon > .75$ Huynh-Feldt).

8.3 Results

8.3.1 Pleasantness

The aim of this study was to explore how the manipulation of expectations based upon oro-sensory cues influenced FNL. In order to assess acquired liking, changes in pleasantness were analysed.

8.3.1.1 Baseline differences

To assess changes in pleasantness, it was important to determine whether there were any baseline differences between conditions on the initial session of the study. There were no significant baseline pleasantness differences between conditions, $F(3,44) = 0.28$, $p = .838$. Table 8.4 shows the mean ratings for each condition.

Condition	**Baseline pleasantness**
LEHS	56.3 ± 8.8
LELS	61.2 ± 8.3
HEHS	61.6 ± 7.8
HELS	66.8 ± 7.4

Table 8.4: Mean (±SEM) baseline pleasantness ratings across conditions in Study Three.

8.3.1.2 Pleasantness ratings

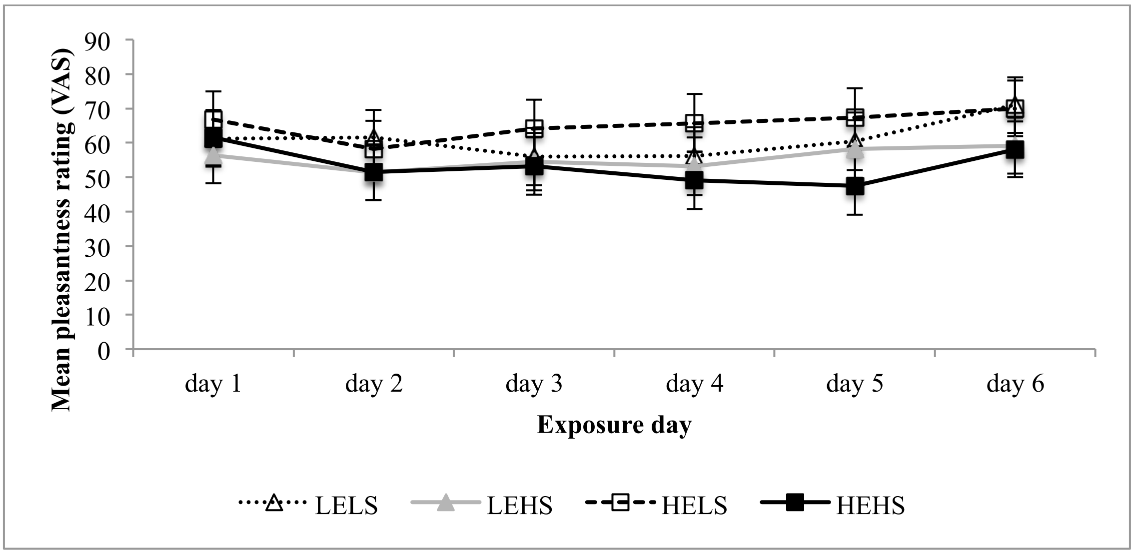


Figure 8.1: Mean (±SEM) pleasantness ratings for each condition across the exposure days in Study Three.

There were no significant differences in pleasantness between energy $F(1,44) = 0.03$, $p = .869$ or sensory $F(1,44) = 1.45$, $p = .236$ versions, and no energy*sensory interaction $F(1, 44) = 0.19$, $p = .665$. There was a significant difference between exposure days, $F(3.66,161.15) = 2.71$, $p = .037$. Bonferroni post hoc tests revealed that pleasantness ratings were significantly higher on day six ($M = 64.6 \pm 4.1$) than both days four ($M = 56.1 \pm 4.2$, $p = .015$) and five ($M = 58.4 \pm 4.2$, $p = .012$). There were no significant interaction effects; day*energy $F(3.66,161.15) = 0.53$, $p = .697$, day*sensory $F(3.66,161.15) = 0.40$, $p = .790$ or day*energy*sensory $F(3.66,161.15) = 1.00$, $p = .407$.

Figure 8.1 shows that pleasantness ratings were relatively stable across the sessions, although those consuming the low sensory versions rated the drinks as slightly more pleasant over time and those consuming the high sensory versions rated the drinks as slightly less pleasant over time.

8.3.1.3 Change from baseline pleasantness

As there were no significant differences in baseline pleasantness between conditions, change from baseline ratings were calculated by subtracting the pleasantness on day one from each subsequent day, and were analysed.

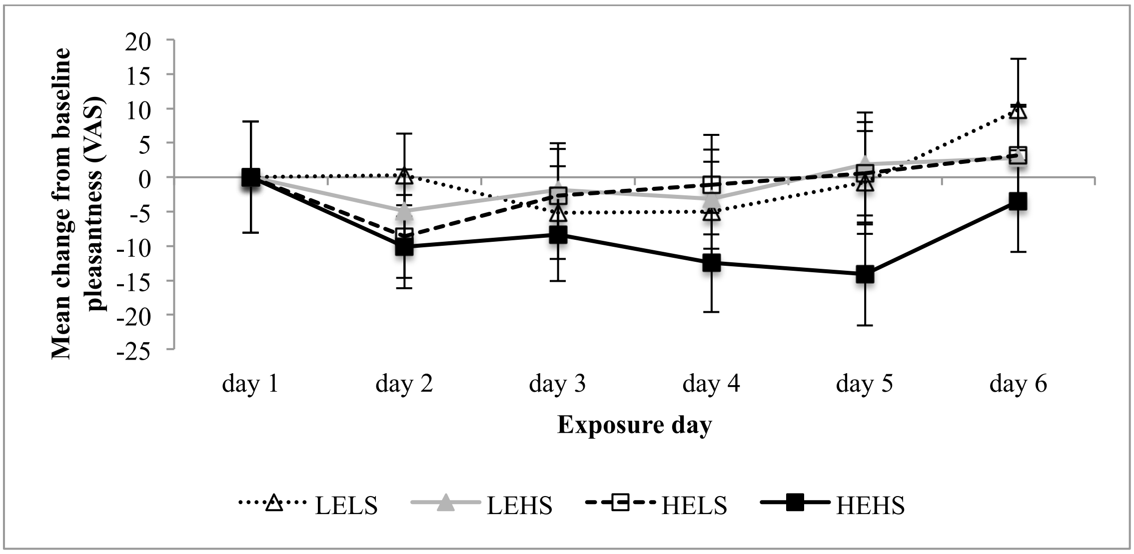


Figure 8.2: Mean (±SEM) change from baseline pleasantness of drinks between conditions in Study Three.

There were no significant differences in change from baseline pleasantness between energy $F(1, 44) = 0.70$, $p = .406$, or sensory $F(1,44) = 0.53$, $p = .471$ versions and no significant energy*sensory interaction $F(1,44) = 0.34$, $p = .564$.

There was a significant difference in change from baseline pleasantness between days $F(3.68,161.83) = 3.56$, $p = .010$, with within subjects linear contrasts also significant $F(1,44) = 6.33$, $p = .016$. Bonferroni post hoc tests revealed that the increase from baseline pleasantness demonstrated on day six ($M = 3.1 \pm 3.7$) was significantly higher than the decreases on days two ($M = -5.8 \pm 3.0$, $p = .044$), four ($M = -5.4 \pm 3.6$, $p = .010$) and five ($M = -3.1 \pm 3.7$, $p = .008$). There were no other significant differences between the days, and there were also no significant interactions; day*energy $F(3.68,161.83) = 0.44$, $p = .763$, day*sensory $F(3.68, 161.83) = 0.34$, $p = .837$ or day*energy*sensory $F(3.68, 161.83) = 1.34$, $p = .260$.

Figure 8.2 indicates that the drinks generally decreased from baseline pleasantness but started to increase again over time. This decreased pleasantness was more evident for the HEHS drink, which continued to decrease in pleasantness until the final session. This contradicted the hypothesis that an acquired liking would be enhanced for the HEHS drink.

8.3.2 Appetite: Hunger ratings

As in the previous studies, before hunger change could be assessed, differences in baseline hunger between conditions on each session were analysed. There were no significant baseline differences in hunger between conditions on any of the six sessions; day one $F(3,44) = 1.28$, $p = .295$, day two $F(3,44) = 0.23$, $p = .873$, day three $F(3,44) = 0.82$, $p = .492$, day four $F(3,44) = 0.32$, $p = .808$, day five $F(3,44) = 1.17$, $p = .331$, day six $F(3,44) = 0.14$, $p = .936$. Table 8.5 shows the mean hunger ratings at the start of each session.

Condition	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
LEHS	62.3 ± 8.5	64.8 ± 9.0	68.2 ± 7.9	64.7 ± 7.1	75.8 ± 7.5	70.5 ± 7.5
LELS	72.1 ± 4.4	59.9 ± 6.9	64.7 ± 6.8	59.5 ± 10.2	76.6 ± 5.4	66.7 ± 20.7
HEHS	51.9 ± 7.5	61.6 ± 6.3	54.5 ± 6.3	66.8 ± 5.8	60.9 ± 5.8	70.7 ± 5.2
HELS	61.5 ± 8.2	67.7 ± 5.9	66.8 ± 6.4	70.0 ± 7.3	71.3 ± 7.6	72.6 ± 7.6

Table 8.5: Mean (±SEM) hunger ratings at the start of each session between conditions in Study Three.

8.3.2.1 Change in hunger immediately after consumption

If the sensory cues were indicating a higher energy intake, immediate reduction in hunger would be predicted in those consuming the thick drinks. Over time, those consuming the HE versions would be expected to show larger reductions in hunger than those consuming the LE versions, but the high sensory versions of the drinks may influence these ratings.

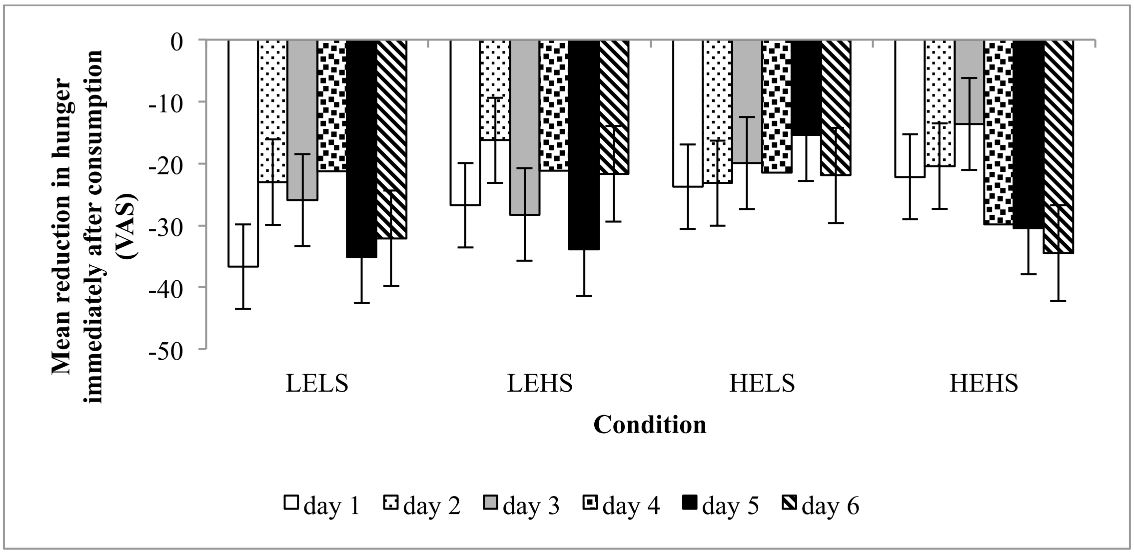


Figure 8.3: Mean (±SEM) hunger change immediately after consumption over days for each condition in Study Three.

There were no significant differences in immediate hunger change between days $F(5,220) = 1.54$, $p = .179$, energy $F(1,44) = 0.45$, $p = .508$ or sensory $F(1,44) = 0.00$, $p = .992$ versions of the drinks. There were also no significant interaction effects; energy*sensory $F(1,44) = 0.57$, $p = .456$, day*energy $F(5,220) = 1.78$, $p = .118$, day*sensory $F(5, 220) = 0.87$, $p = .503$, or day*energy*sensory $F(5,220) = 1.01$, $p = .415$.

From Figure 8.3, it seems those consuming the LE versions of the drink reported similar reductions in hunger immediately after consumption, regardless of whether the drink was thick or thin, except for day five where the reduction was larger in the thin drink. For those consuming the HE drink, it appears that as the sessions progressed, the high sensory (thick) version resulted in a greater reduction in hunger immediately after consumption than the low sensory (thin). This suggests that the sensory characteristics of the drink were influencing hunger, but only for those who consumed the HE version. Hunger change immediately after did not appear to be greater for those consuming the HE than those consuming the LE drinks.

8.3.2.2 Hunger change 30 minute post consumption

Participants remained in the lab for 60 minutes after consuming the drink, and hunger changes were recorded at 30 and 60 minutes. It would be predicted that hunger reduction would remain higher 30 minutes after consumption in those who had consumed the HE version, and that this would be enhanced in the high sensory version, and that this difference would be more pronounced as the sessions continued. Change data were calculated from the initial hunger rating and the hunger rating 30 minutes post consumption.

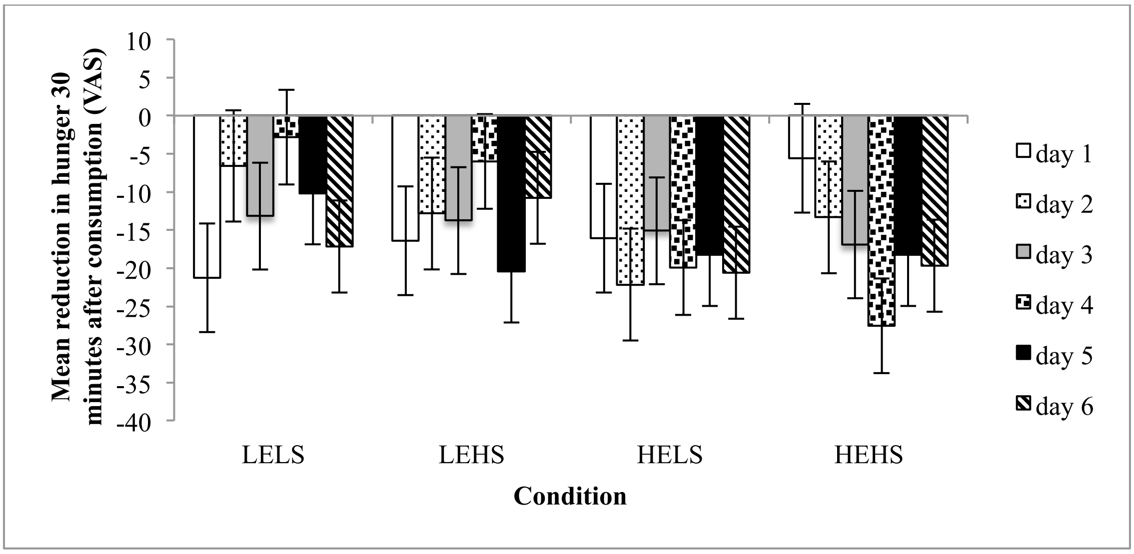


Figure 8.4: Mean (±SEM) hunger reduction 30 minutes post consumption over days for each condition in Study Three.

There were no significant differences in 30 minute post hunger change between days $F(4.57,200.94) = 0.27$, $p = .920$, energy $F(1,44) = 1.25$, $p = .270$ or sensory $F(1,44) = 0.00$, $p = .975$ versions. There was a significant day*energy interaction $F(4.57,200.94) = 2.72$, $p = .025$. Those consuming the HE version of the drink demonstrated a greater reduction in hunger as the sessions progressed compared to those consuming the LE version. A series of Bonferroni corrected one way ANOVA's revealed this difference was only significant on day four $F(1, 46) = 9.93$, $p = .003$ (all other *ps* > .264), with those consuming HE drinks ($M = -23.8 \pm 4.7$) reporting a significantly higher reduction in hunger 30 minutes post consumption than those consuming the LE drinks ($M = -4.4 \pm 3.9$). When analysed separately for each energy condition, there were no significant differences across days for either LE $F(5,115) = 1.43$, $p = .220$ or HE $F(2.92, 67.1) = 1.63$, $p = .191$.

There were no other significant interaction effects; energy*sensory $F(1,44) = 0.13$, $p = .724$, day*sensory $F(4.57,200.94) = 0.90$, $p = .475$, or day*energy*sensory $F(4.57,200.94) = 0.61$, $p = .681$.

Figure 8.4 indicated that at 30 minutes post consumption there was no longer a pattern of sensory effects within the HE conditions as was demonstrated immediately after consumption.

8.3.2.3 Hunger change 60 minutes post consumption

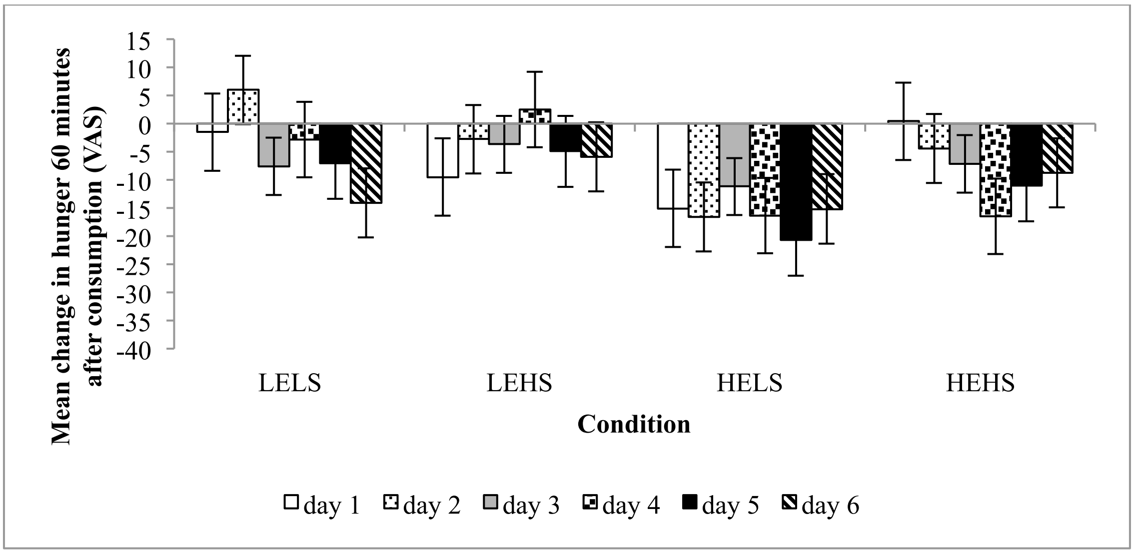


Figure 8.5: Mean (±SEM) changes in hunger 60 minutes post consumption over days for each condition in Study Three.

There were no significant differences in hunger change 60 minutes post consumption of the drinks between days $F(4.70,206.93) = 0.92$, $p = .465$, or sensory $F(1,44) = 1.17$, $p = .286$ versions of the drinks. The difference between energy versions was approaching significance $F(1,44) = 3.82$, $p = .057$, with those who consumed the HE ($M = -11.9 \pm 2.8$) demonstrating a larger reduction in hunger than those who consumed the LE ($M = -4.3 \pm 2.8$). There were no significant interaction effects; energy*sensory $F(1,44) = 0.93$, $p = .342$, day*energy $F(4.70,206.93) = 1.27$, $p = .279$, day*sensory $F(4.70,206.93) = 0.15$, $p = .976$ or day*energy*sensory $F(4.70,206.93) = 1.30$, $p = .266$.

The significant day*energy interaction demonstrated 30 minutes post consumption was not maintained at 60 minutes, but energy did appear to have some effect over hunger changes 60 minutes after consumption. Figure 8.5 reflects these energy differences, and also suggests that the HELS drink actually resulted in greater hunger reduction 60 minutes post consumption than the HEHS drink, which contradicts both the hypothesis and the data from within session.

8.3.3 Appetite: Fullness ratings

It was hypothesised that the reverse pattern to hunger ratings would be observed; a larger increase in fullness was predicted for those consuming the HE drinks, which would be enhanced for the high sensory versions. An initial increase in fullness would be predicted for those consuming the LEHS drink, but over time this increase would be predicted to become in line with the energy rather than sensory information.

8.3.3.1 Baseline ratings

There were significant differences in baseline fullness ratings between conditions on day one $F(3\ 44) = 3.37$, $p = .027$, with Tukey post hoc tests suggesting that those in the HEHS ($M = 42.2 \pm 6.0$) were approaching significantly fuller than those in the LELS ($M = 18.7 \pm 4.2$, $p = .054$). There were no other significant differences in baseline fullness ratings on subsequent days; two $F(3,44) = 2.33$, $p = .088$, three $F(3,44) = 0.86$, $p = .472$, four $F(3,44) = 1.38$, $p = .262$, five $F(3,44) = 2.20$, $p = .102$ and six $F(3,44) = 0.13$, $p = .942$. Mean baseline fullness ratings are reported in Table 8.6. From the table, it can be seen that those in the HEHS condition did consistently report higher fullness ratings across sessions one to five.

Condition	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
LEHS	34.5 ± 8.3	29.1 ± 7.1	27.5 ± 7.7	32.4 ± 8.1	23.8 ± 7.6	25.4 ± 7.6
LELS	18.7 ± 4.2	20.3 ± 4.6	25.7 ± 6.0	22.0 ± 5.8	21.0 ± 3.7	23.8 ± 4.6
HEHS	42.2 ± 6.0	42.5 ± 6.0	36.3 ± 6.1	31.8 ± 5.5	35.6 ± 5.8	24.5 ± 3.8
HELS	19.4 ± 6.1	28.3 ± 6.3	21.5 ± 7.0	17.3 ± 5.6	15.8 ± 4.8	20.4 ± 7.3

Table 8.6: Mean (±SEM) baseline fullness ratings between conditions in Study Three.

8.3.3.2 Change in fullness immediately after consumption

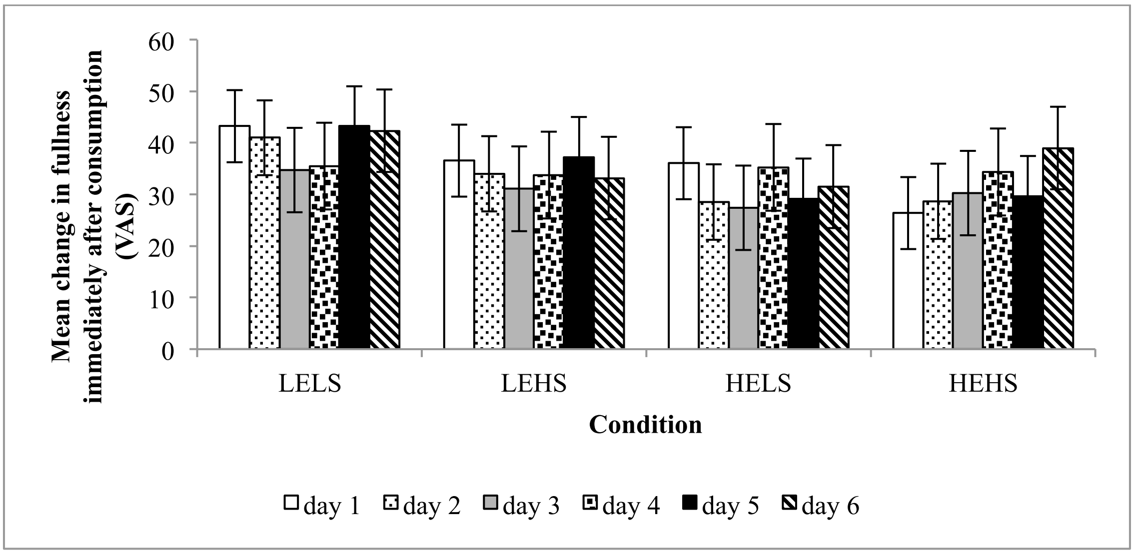


Figure 8.6: Mean (±SEM) fullness change immediately after consumption over days for each condition in Study Three.

There were no significant differences in fullness change between days $F(4.92,216.5) = 0.77$, $p = .572$, energy $F(1,44) = 0.78$, $p = .382$ or sensory $F(1,44) = 0.18$, $p = .670$ versions, and no significant interaction effects; energy*sensory $F(1,44) = 0.19$, $p = .662$, day*energy $F(4.92,216.5) = 0.88$, $p = .495$, day*sensory $F(4.92,216.5) = 0.39$, $p = .854$, or day*energy*sensory $F(4.92,216.5) = 0.53$, $p = .752$.

From Figure 8.6 it can be seen that there is little difference between sensory versions in terms of fullness change, although it appears that by day six, the HEHS drink results in a larger increase in fullness than the HELS, and the opposite pattern was seen in the LE versions.

8.3.3.3 Change in fullness ratings 30 minutes post consumption

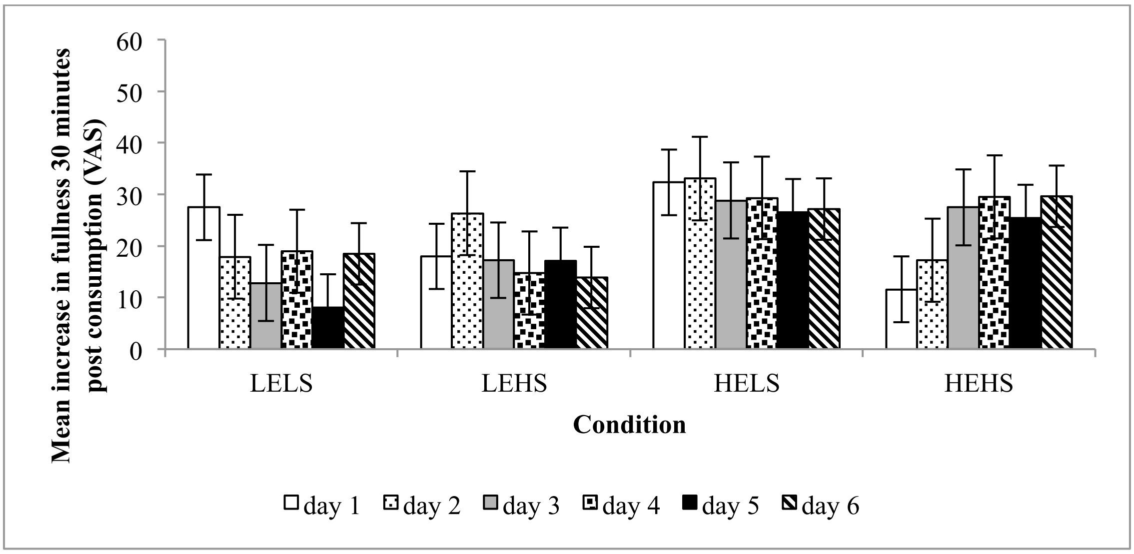


Figure 8.7: Mean (±SEM) change in fullness ratings 30 minutes post consumption across days for each condition in Study Three.

There were no significant differences in fullness change 30 minutes post consumption between days $F(4.52,198.94) = 0.30$, $p = .895$, energy $F(1,44) = 3.24$, $p = .079$ or sensory $F(1,44) = 0.31$, $p = .584$ versions of the drinks, and no significant interaction effects; energy*sensory $F(1,44) = 0.45$, $p =.507$, day*energy $F(4.52,198.94) = 1.22$, $p = .302$, day*sensory $F(4.52,198.94) = 1.42$, $p = .224$ or day*energy*sensory $F(4.52,198.94) = 1.05$, $p = .387$.

Figure 8.7 shows that there were some differences in fullness change 30 minutes post test between the LELS and LEHS drinks, but this was not consistent between days. For the HE versions, initially the low sensory drink resulted in a larger increase in fullness 30 minutes post consumption, but this difference disappeared in the later sessions. There were larger increases in fullness in the HE compared to the LE conditions.

As change across time was predicted, within subjects linear contrasts were examined and found to be significant for day*energy $F(1,44) = 4.34$, $p = .043$, and approached significance for day*sensory $F(1,44) = 3.85$, $p = .056$. Those consuming the HE version reported significantly higher increases in fullness than those consuming the LE versions on days five ($M = 26.0 \pm 4.9$ vs. 12.6 ± 4.1, $p = .043$) and six ($M = 28.4 \pm 4.4$ vs. 16.2 ± 3.9, $p = .043$), whereas the only day where sensory differences were exhibited were day one, with the low sensory ($M = 29.9 \pm 3.9$) showing a larger increase in fullness than high sensory ($M = 14.8 \pm 4.9$, $p = .020$).

8.3.3.4 Change in fullness 60 minutes post consumption

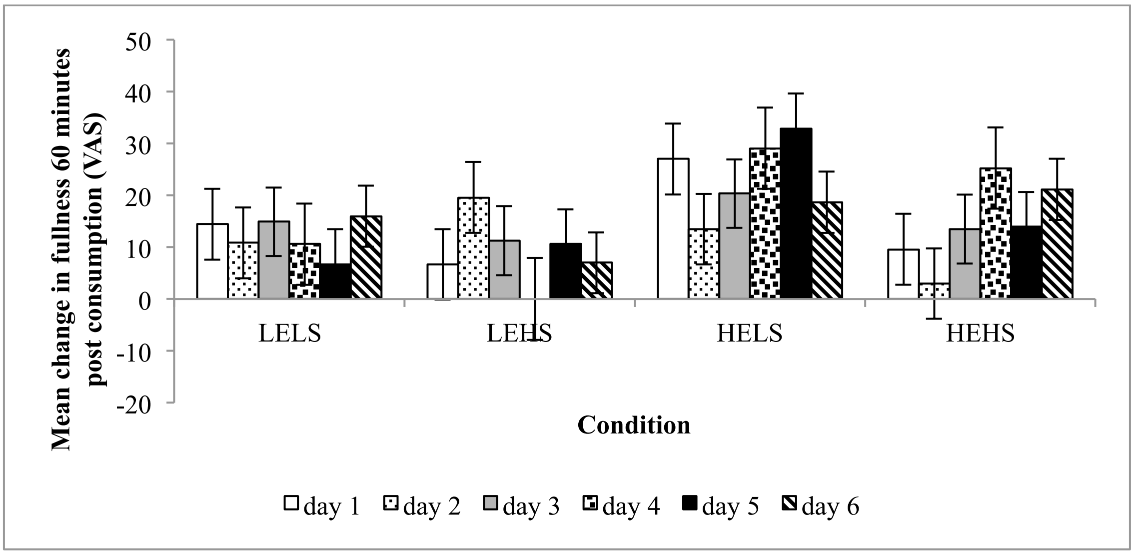


Figure 8.8: Mean (±SEM) fullness changes 60 minutes post consumption across days between conditions in Study Three.

There were no significant differences in fullness change 60 minutes post consumption between days $F(5,220) = 0.36$, $p = .873$, energy $F(1,44) = 3.28$, $p = .077$ or sensory $F(1,44) = 1.79$, $p = .187$ versions. There was a significant day*energy interaction $F(5,220) = 3.12$, $p = .010$, with no other significant interaction effects; energy*sensory $F(1,44) = 0.45$, $p = .508$, day*sensory $F(5,220) = 0.53$, $p = .753$ or day*energy*sensory $F(5,220) = 1.54$, $p = .178$.

A series of Bonferroni corrected one way ANOVAs revealed that differences were only significant on day four $F(1,46) = 7.88$, $p = .007$ (all other $p > .037$), with those who consumed the HE version ($M = 27.2 \pm 6.1$) reporting a significantly larger increase in fullness on this day than those consuming the LE version ($M = 5.3 \pm 4.9$). When analysed separately between energy conditions using Bonferroni corrected repeated measures ANOVAs, there were no significant differences across days for the LE $F(5,115) = 1.06$, $p = .385$ or HE $F(5,115) = 2.13$, $p = .067$.

8.3.3.5 How filling was the drink?

Alongside the appetite ratings taken during each session, participants were also asked how filling the drink was during the taste test. It was predicted that those consuming the high sensory version would rate the drink as more filling than those consuming the low sensory version, and that over time an energy difference would emerge, with the high energy rated as more filling than the low energy.

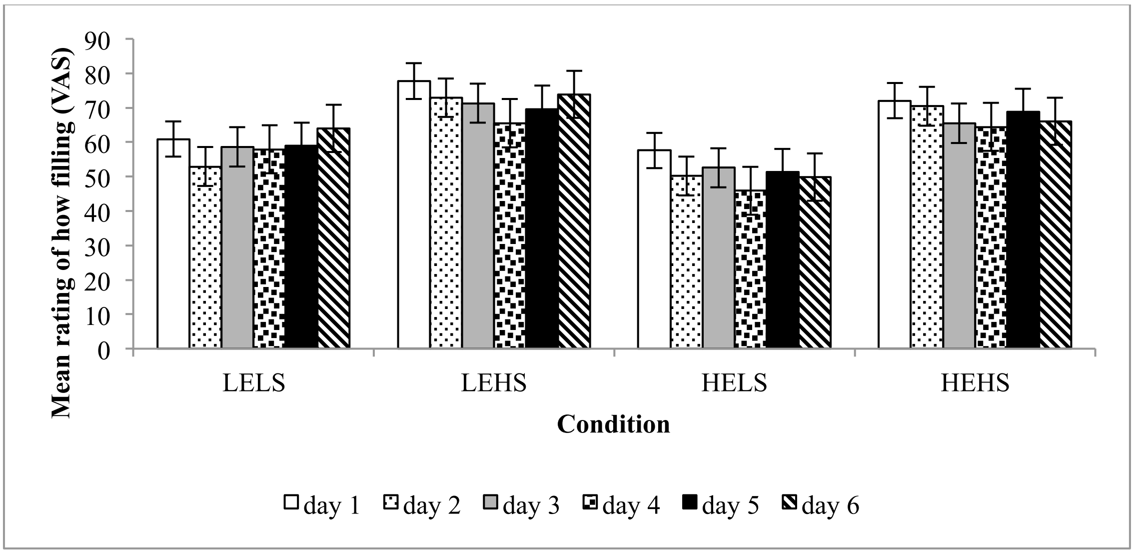


Figure 8.9: Mean (±SEM) rating of how filling the drink was across days between conditions in Study Three.

There was a significant main effect of sensory $F(1,44) = 9.80$, $p = .003$, with those consuming the high sensory version ($M = 69.9 \pm 3.3$) rating the drink as significantly more filling than the low sensory drink ($M = 55.1 \pm 3.3$). There were no significant differences between days $F(2.87,126.25) = 1.61$, $p = .193$ or energy versions $F(1,44) = 1.52$, $p = .225$, and no significant interaction effects; energy*sensory $F(1, 44) = 0.15$, $p = .698$, day*energy $F(2.87,126.25) = 0.42$, $p = .728$, day*sensory $F(2.87,126.25) = 0.40$, $p = .742$ or day*energy*sensory $F(2.87,126.25) = 0.33$, $p = .798$.

Figure 8.9 demonstrates the main effect of sensory; the high sensory versions were consistently rated as more filling than the low sensory versions regardless of energy content and which session. The predicted energy differentiation as time progressed was not demonstrated, although the difference between the HE versions on days five and six did appear to be greater than the difference between the low energy versions.

8.3.4 Desire to eat

Desire to eat ratings were also collected as another measure of appetite, as inclusion of the recommended appetite scales ensures a more sensitive measure, with each influenced in different ways by foods, as discussed in Chapter 2. It was predicted that the desire to eat ratings would follow a similar pattern to the hunger ratings, with a smaller desire to eat after the thick drinks, with energy differences emerging over time.

There were no baseline differences in desire to eat between conditions on each of the test days; day one $F(3,44) = 2.46$, $p = .075$, day two $F(3,44) = 0.70$, $p = .559$, day three $F(3,44) = 0.75$, $p = .526$, day four $F(3,44) = 1.57$, $p = .210$, day five $F(3,44) = 0.82$, $p = .490$ or day six $F(3,44) = 0.78$, $p = .509$. Mean ratings of desire to eat at the start of each day for each condition is shown in Table 8.7.

Condition	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
LEHS	65.0 ± 6.6	58.0 ± 8.6	65.0 ± 6.4	68.8 ± 8.3	78.9 ± 3.6	67.9 ± 4.7
LELS	59.0 ±7.4	68.6 ± 7.8	60.8 ± 9.1	63.2 ± 8.9	68.8 ± 7.8	75.1 ± 9.2
HEHS	78.3 ± 4.7	63.4 ± 6.7	70.4 ± 5.4	77.2 ± 4.3	72.1 ± 7.4	66.5 ± 7.0
HELS	53.3 ± 6.7	54.1 ± 7.0	55.5 ± 7.7	54.9 ± 7.6	64.4 ± 7.3	60.1 ± 6.2

Table 8.7: Mean (±SEM) desire to eat ratings at the start of each session between conditions in Study Three.

8.3.4.1 Immediate changes in desire to eat after consumption

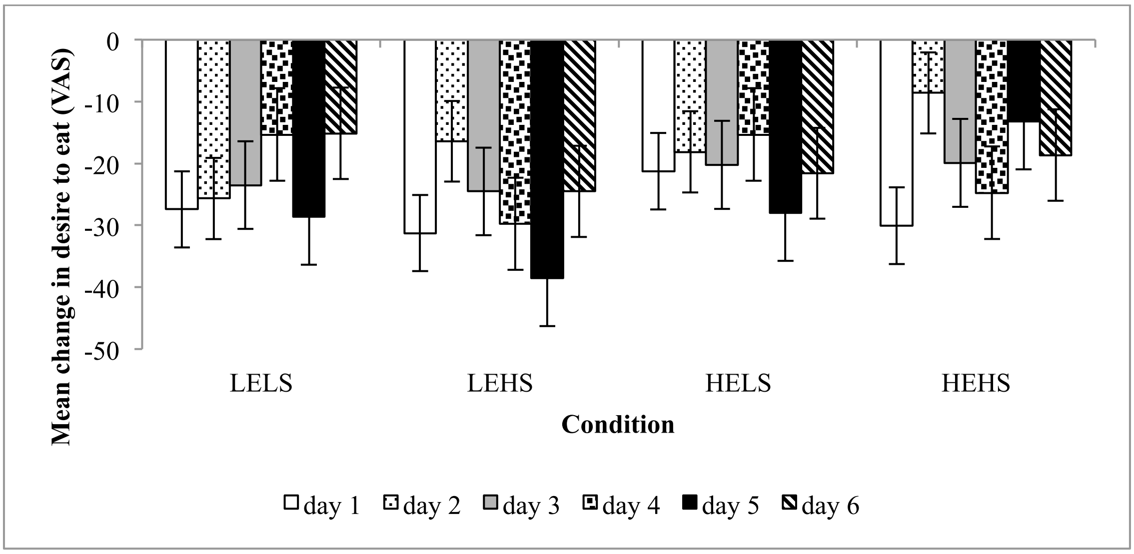


Figure 8.10: Mean (±SEM) changes in desire to eat immediately after consumption over days for each condition in Study Three.

Contrary to the hunger and fullness data, there was a significant difference in change in desire to eat immediately after consumption between days $F(5,220) = 2.54$, $p = .030$, although Bonferroni post hoc tests only hinted at a significant difference between days 5 ($M = -27.1 \pm 3.9$) and 2 ($M = -17.2 \pm 3.3$, p = .097), with all other $p > .119$. There was also a trend for a day*sensory interaction $F(5,220) = 2.10$, $p = .067$. There were no significant differences between energy $F(1,44) = 0.89$, $p = .351$, or sensory $F(1,44) = 0.10$, $p = .760$ conditions, and there were no other significant interaction effects; day*energy $F(5,220) = 0.83$, $p = .527$, energy*sensory F(1,44) = 0.36, p = .552 or day*sensory*energy $F(5,220) = 1.10$, $p = .364$. From Figure 8.10 it can be seen that desire to eat was reduced to the largest extent in the LEHS condition, with day five generally showing the largest reductions across conditions.

8.3.4.2 Changes in desire to eat 30 minutes after consumption

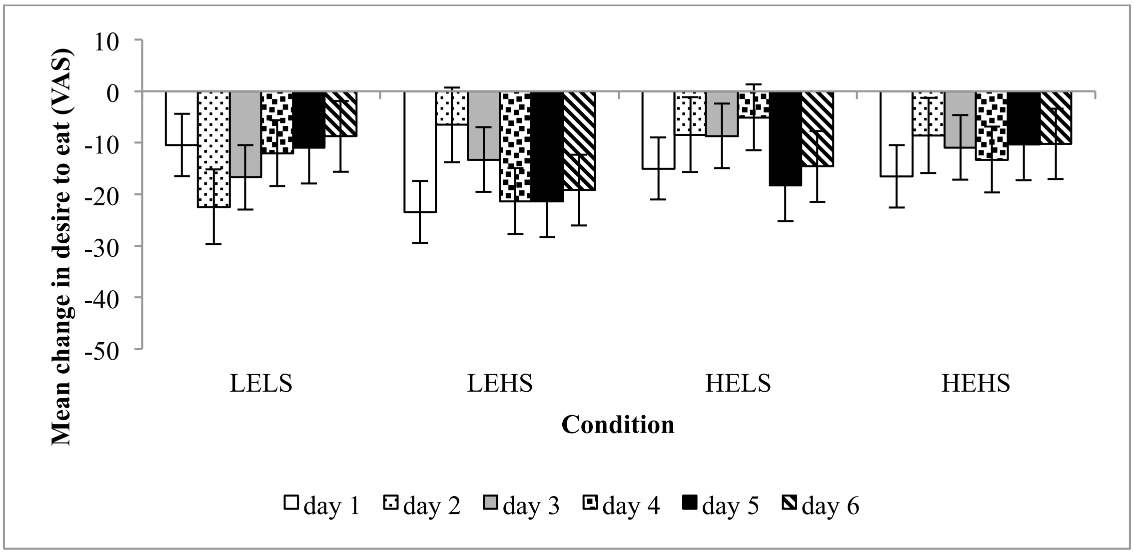


Figure 8.11: Mean (±SEM) changes in desire to eat 30 minutes post consumption over days for each condition in Study Three.

At 30 minutes post consumption the significant differences in changes in desire to eat between days were no longer present $F(5,220) = 0.43$, $p = .826$, with no significant differences between energy $F(1,44) = 0.82$, $p = .371$ or sensory $F(1,44) = 0.21$, $p = .648$ and no significant interaction effects; energy*sensory $F(1,44) = 0.22$, $p = .641$, day*energy $F(5,220) = 0.23$, $p = .948$, day*sensory $F(5,220) = 1.16$, $p = .332$ or day*energy*sensory $F(5,220) = 1.43$, $p = .215$. Figure 8.11 shows that the reduction in desire to eat is much smaller at 30 minutes post consumption compared to immediately afterwards, with relatively similar ratings across time and conditions, with slightly higher reductions remaining after the LEHS drink.

8.3.4.3 Changes in desire to eat 60 minutes post consumption

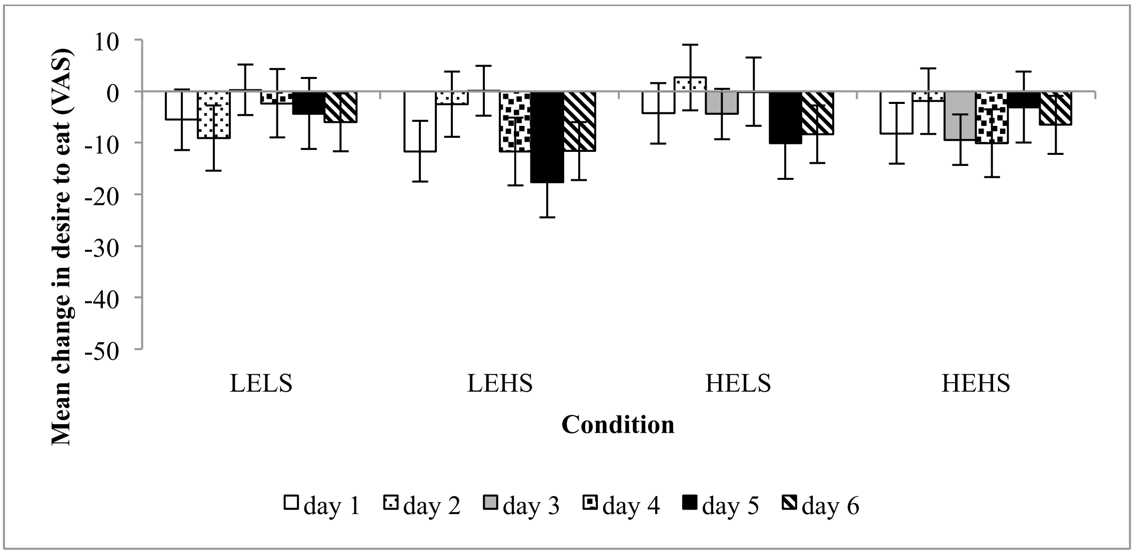


Figure 8.12: Mean (±SEM) changes in desire to eat 60 minutes post consumption over days for each condition in Study Three.

The lack of significant differences in change in desire to eat remained at 60 minutes post test, with no significant differences between days $F(5,220) = 1.01$, $p = .411$, energy $F(1,44) = 0.14$, $p = .706$ or sensory $F(1,44) = 0.78$, $p = .381$ conditions, and no significant interaction effects; energy*sensory $F(1,44) = 0.08$, $p = .784$, day*energy $F(5,220) = 0.84$, $p = .526$, day*sensory $F(5,220) = 0.51$, $p = .767$ or day*energy*sensory $F(5,220) = 1.18$. $p = .322$. As with the other time points, Figure 8.12 shows that the reduction in desire to eat was largest in the LEHS condition.

8.3.5 Prospective consumption

Alongside desire to eat, participants were also asked how much they thought that they could eat right now (prospective consumption), and the change in these ratings was analysed. As with other appetite data, it was predicted that prospective consumption would be reduced for those consuming the high sensory drinks in the initial phases but that over time the energy content would become more salient, and enhanced effects to be demonstrated in the HEHS condition.

There was a significant difference between prospective consumption on day one $F(3,44) = 3.1$, $p = .036$, with those in the HEHS ($M = 78.3 \pm 4.0$) condition recording significantly higher ratings than the HELS ($M = 53.8 \pm 7.2$, $p = .031$) condition. There were no significant baseline differences in prospective consumption for all other days; day two $F(3,44) = 0.19$, $p = .901$, day three $F(3,44) = 0.78$, $p = .515$, day four $F(3,44) = 1.31$, $p = .283$, day five $F(3,44) = 1.94$, $p = .137$ or day six $F(3,44) = 1.38$, $p = .262$. Descriptive statistics are displayed in Table 8.8.

Condition	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
LEHS	60.1 ± 5.5	63.3 ± 6.3	71.1 ± 2.9	71.0 ± 4.8	77.8 ± 2.6	66.3 ± 5.0
LELS	64.1 ± 6.5	66.2 ± 6.6	61.8 ± 8.6	63.8 ± 7.6	62.1 ± 7.3	78.0 ± 7.7
HEHS	78.3 ± 4.0	68.5 ± 5.9	70.3 ± 5.4	74.6 ± 4.3	74.8 ± 5.6	71.3 ± 6.1
HELS	53.8 ± 7.2	62.1 ± 7.5	59.2 ± 8.6	59.4 ± 6.7	64.5 ± 5.5	69.1 ± 3.2

Table 8.8: Mean (±SEM) prospective consumption ratings at the start of each session between conditions in Study Three.

8.3.5.1 Immediate changes in prospective consumption

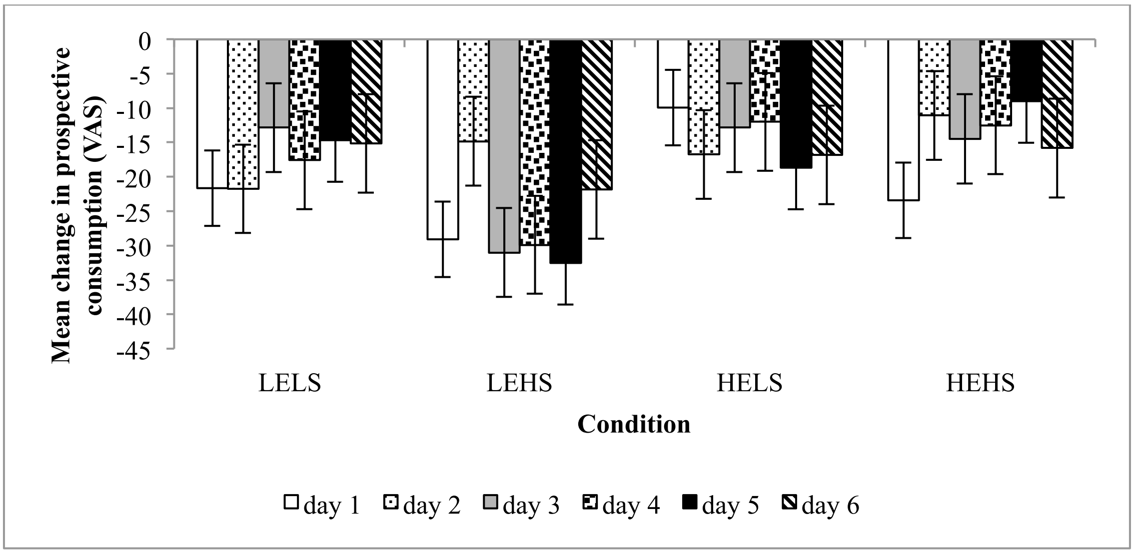


Figure 8.13: Mean (±SEM) change in prospective consumption across days between conditions in Study Three.

There were no significant differences in change in prospective consumption across days $F(5,220) = 0.62$, $p = .686$, energy $F(1,44) = 2.01$, $p = .164$ or sensory $F(1, 44) = 0.75$, $p = .390$ conditions. Two interaction effects were approaching significance; day*sensory $F(5,220) = 2.16$, $p = .059$ and day*energy*sensory $F(5,220) = 2.13$, $p = .063$, with a significant within subject linear contrast for the three way interaction $F(1,44) = 4.56$, $p = .038$. There were no other significant interaction effects; energy*sensory $F(1,44) =$

0.79, *p* = .379 or day*energy *F*(5,220) = 0.71, *p* = .620. As was seen in the desire to eat change data, the largest reduction in prospective consumption was seen in those who consumed the LEHS drink, with all other conditions demonstrating similar patterns of change (Figure 8.13). On day one the change in prospective consumption was lower in the HELS condition, but this became in line with the other conditions as time progressed.

8.3.5.2 Change in prospective consumption 30 minutes after consumption

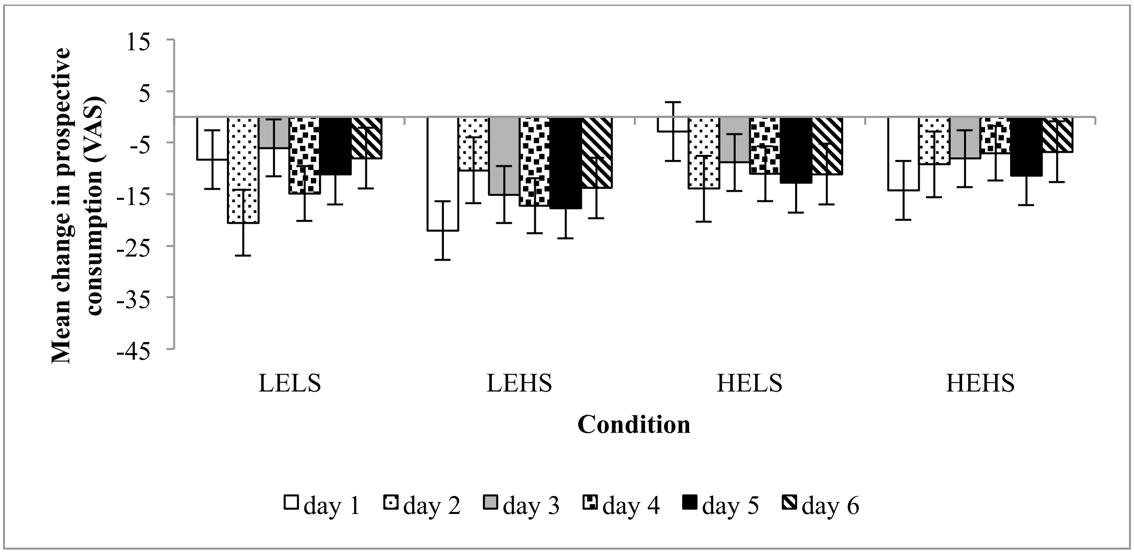


Figure 8.14: Mean (±SEM) change in prospective consumption 30 minutes after drink, across days between conditions in Study Three.

There remained no significant differences in prospective consumption change across days *F*(5,220) = 0.66, *p* = .651 or between energy *F*(1,44) = 0.84, *p* = .365 or sensory *F*(1,44) = 0.20, *p* = .655 conditions. The day*sensory interaction was significant 30 minutes post consumption *F*(5,220) = 2.50, *p* = .032. When data were analysed separately for each day by sensory condition, using Bonferroni corrected independent t-tests, the difference between sensory conditions was non significant on all days (all *p* > .031). When a one way repeated measures ANOVA was conducted for each sensory condition there were no significant differences across days in either the low sensory *F*(3.41, 78.43) = 1.94, *p* = .122 or high sensory *F*(5,115) = 1.25, *p* = .289 conditions.

There were no other significant interaction effects; energy*sensory *F*(1,44) = 0.36, *p* = .554, day*energy *F*(5,220) = 0.30, *p* = .910 or day*sensory*energy *F*(5,220) = 0.51, *p* =

.770. From Figure 8.14 it can be seen that reduction in prospective consumption remained slightly larger for those consuming the LEHS drink.

8.3.5.3 Changes in prospective consumption 60 minutes after consumption of drink

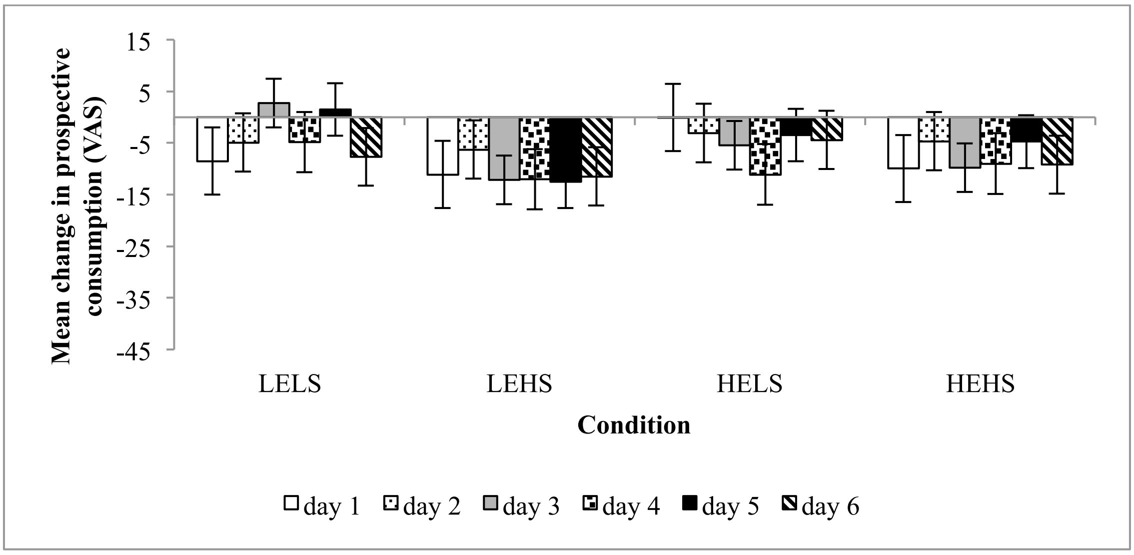


Figure 8.15: Mean (±SEM) change in prospective consumption 60 minutes after drink, across days between conditions in Study Three.

At 60 minutes post consumption of the drink there were no significant differences across days $F(5,220) = 0.73$, $p = .601$, or between energy $F(1,44) = 0.07$, $p = .797$ or sensory $F(1,44) = 1.75$, $p = .192$ conditions. There were also no significant interaction effects; energy*sensory $F(1,44) = 0.25$, $p = .618$, day*energy $F(5,220) = 0.43$, $p = .825$, day*sensory $F(5,220) = 0.52$, $p = .758$ or day*energy*sensory $F(5,220) = 0.85$, $p = .515$. As Figure 8.15 indicates, change in prospective consumption was still slightly larger in the LEHS condition over time compared to the other conditions.

8.3.6 Thirst Ratings

Changes in thirst ratings were analysed in the same way as the hunger and fullness data, and it was predicted that the high sensory versions would lead to a smaller reduction in thirst than the low sensory versions.

Prior to subsequent analysis, baseline thirst ratings for each day were analysed to check there were no differences between conditions. There were no significant differences in thirst ratings for any exposure day: day one $F(3, 44) = 2.00$, $p = .128$, day two $F(3, 44)$

= 1.37, *p* = .266, day three *F*(3, 44) = 0.26, *p* = .855, day four *F*(3, 44) = 0.51, *p* = .680, day five *F*(3, 44) = 1.68, *p* = .184 or day six *F*(3, 44) = 1.24, *p* = .306. Table 8.9 shows the descriptives.

Condition	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
LEHS	61.6 ± 8.7	59.1 ± 7.2	52.2 ± 9.4	52.4 ± 9.8	67.3 ± 8.2	53.5 ± 9.7
LELS	52.4 ± 9.4	50.8 ± 6.5	51.3 ± 7.9	46.4 ± 8.1	56.9 ± 6.9	58.9 ± 7.6
HEHS	67.3 ± 6.0	66.5 ± 4.7	57.6 ± 6.8	61.3 ± 7.2	72.5 ± 6.4	72.3 ± 5.9
HELS	41.3 ± 7.7	51.2 ± 6.9	47.3 ± 8.8	50.3 ± 4.4	53.6 ± 5.4	51.4 ± 9.8

Table 8.9: Mean (±SEM) thirst ratings at the start of each session in Study Three.

8.3.6.1 Change in thirst immediately after consumption

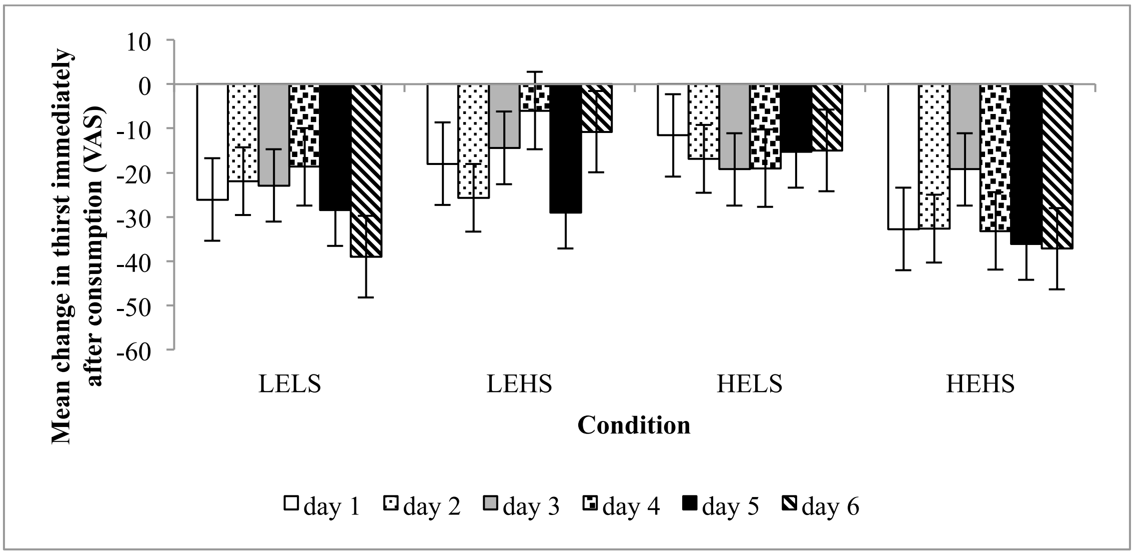


Figure 8.16: Mean (±SEM) thirst changes immediately after consumption across days between conditions in Study Three.

There were no significant differences in thirst changes immediately after consuming the drink between days *F*(5,220) = 1.15, *p* = .337, energy *F*(1,44) = 0.13, *p* = .720 or sensory *F*(1, 44) = 0.29, *p* = .591 versions. There was an energy*sensory interaction approaching significance *F*(1,44) = 3.79, *p* = .058. Consumption of the LE drink resulted in the predicted differences in thirst ratings; those who consumed the low sensory version (*M* = -26.2 ± 6.3) reported larger decreases in thirst than those who consumed the high sensory version (*M* = -17.3 ± 6.3). However, the opposite pattern was demonstrated in the HE drinks; the high sensory version (*M* = -31.9 ± 6.3) resulted in a greater reduction in thirst than the low sensory version (*M* = -16.2 ± 6.3). No other interaction effects were significant; day*energy *F*(5,220) = 0.85, *p* = .517, day*sensory *F*(5,220) = 1.05, *p* = .390 or day*energy*sensory *F*(5,220) = 1.41, *p* = .223.

Figure 8.16 shows that differences between thirst changes in the low and high sensory versions of the LE drink were inconsistent over the exposure days, whereas for the HE drinks, the high sensory consistently resulted in a larger reduction in thirst (apart from on day three).

8.3.6.2 Change in thirst 30 minutes post consumption

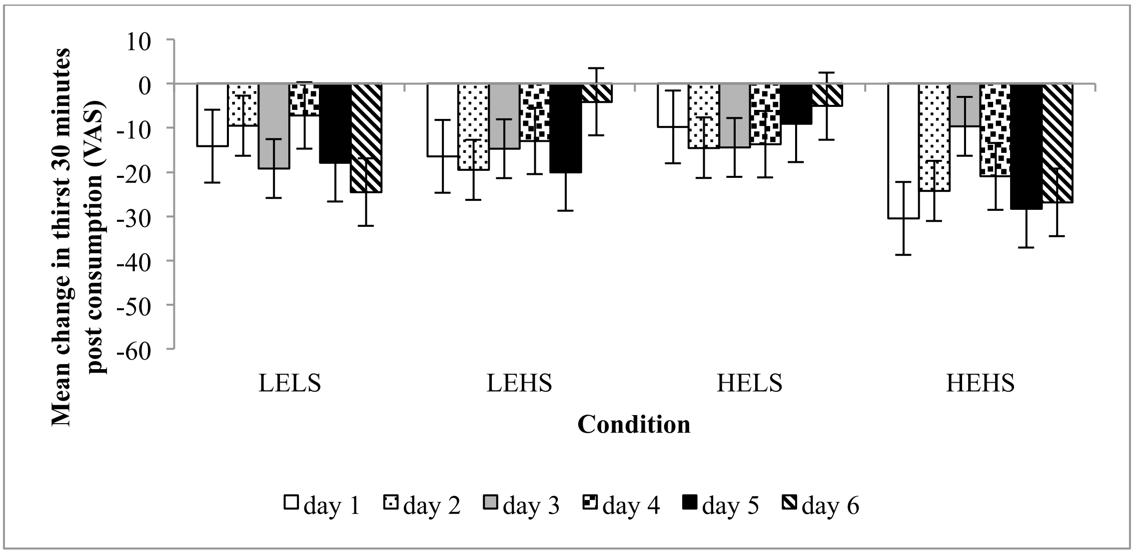


Figure 8.17: Mean (±SEM) changes in thirst 30 minutes post consumption across days between conditions in Study Three.

There were no significant differences in thirst change 30 minutes post consumption between days *F*(5,220) = 0.45, *p* = .810, energy *F*(1,44) = 0.17, *p* = .681 or sensory *F*(1,44) = 1.15, *p* = .290 versions, and no significant interactions; energy*sensory *F*(1,44) = 1.49, *p* = .290, day*energy *F*(5,220) = 0.56, *p* = .733, day*sensory *F*(5,220) = 1.19, *p* = .315 or day*energy*sensory *F*(5,220) = 1.97, *p* = .085.

Figure 8.17 shows a similar pattern to the changes immediately after consumption, the LE drinks resulted in inconsistent differences between sensory versions although by day six the low sensory resulted in greater decreases in thirst. The high sensory version of the HE drink resulted in greater decreases in thirst consistently across exposure days (apart from day three).

8.3.6.3 Changes in thirst 60 minutes post consumption

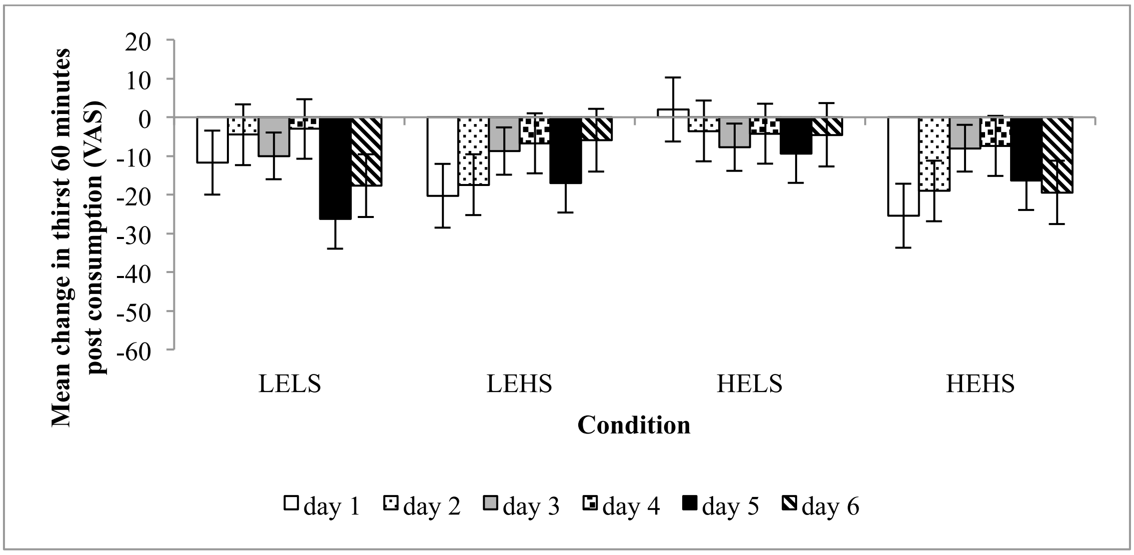


Figure 8.18: Mean (±SEM) changes in thirst 60 minutes post consumption across days between conditions in Study Three.

There were no significant differences in thirst change 30 minutes post consumption between days $F(5,220) = 1.73$, $p = .129$, energy $F(1,44) = 0.18$, $p = .670$ or sensory $F(1,44) = 1.35$, $p = .251$ versions, and no significant interactions; energy*sensory $F(1,44) = 1.14$, $p = .291$, day*energy $F(5,220) = 0.36$, $p = .875$, day*sensory $F(5,220) = 1.69$, $p = .139$ or day*energy*sensory $F(5,220) = 0.81$, $p = .547$.

From Figure 8.18 it can be seen that the thirst changes demonstrated both immediately, and 30 minutes after consumption continued at 60 minutes, with a larger reduction in thirst on day six in the LELS drink than the LEHS version, and the opposite pattern in those who consumed the high energy drink.

8.3.7 Relationship between appetite ratings

The relationship between hunger, fullness, desire to eat and prospective consumption was examined, as each of these measure some dimension of appetite but patterns of change in ratings differed. An average change score was calculated for immediate hunger, fullness desire to eat and prospective consumption ratings, and for these ratings at 30 and 60 minutes post consumption. Pearson correlations were then conducted to examine if the changes in ratings were related.

Table 8.10 shows the Pearson *r* correlation coefficients and their associated significance levels. From Table 8.10 it can be seen that hunger and fullness changes at all time points (immediate, 30 and 60 minutes post consumption) were significantly negatively correlated with each other. Changes in desire to eat and prospective consumption were significantly positively correlated with each other at all time points, but the relationship between the changes in hunger and fullness, was non significant with changes in desire to eat and prospective consumption, suggesting that desire to eat and prospective consumption may be tapping into a different concept to that of hunger and fullness. Ratings of change in thirst were significantly positively correlated with hunger change, and negatively with fullness change, immediately and 60 minutes post consumption. Thirst changes were not significantly correlated with any appetite measures 30 minutes post consumption.

a)

	1	**2**	**3**	**4**
1. Hunger				
2. Full	-.88***			
3. Desire to eat	.02	.14		
4. PC	-.03	.11	.88***	
5. Thirst	.31*	-.32*	-.05	-.05

b)

	1	**2**	**3**	**4**
1. Hunger				
2. Full	-.81***			
3. Desire to eat	-.01	.12		
4. PC	.003	.09	.89***	
5. Thirst	.16	-.28	-.12	-.20

c)

	1	**2**	**3**	**4**
1. Hunger				
2. Full	-.88***			
3. Desire to eat	.02	.14		
4. PC	-.03	.11	.88***	
5. Thirst	.31*	-.32*	-.05	-.05

Table 8.10: Correlation coefficients between change in appetite ratings in Study Three a) immediately after consumption b) 30 minutes post consumption and c) 60 minutes post consumption. * denotes $p < .05$, *** denotes $p < .001$.

8.4 Discussion

The current study aimed to investigate how altering the sensory characteristics of a yoghurt drink could influence learning about the energy content. It was predicted that a high sensory (thick) drink would signal the presence of energy, and that this would enhance FNL for a high energy drink compared to both low energy, and low sensory, versions.

Contrary to hypotheses, there was very little change in pleasantness ratings over time, and the low sensory drinks seemed to show more of an increase in pleasantness over time than the high sensory, which was enhanced in the LE version rather than the HE. This partially supports findings by Mars et al., (2009), who found that pleasantness of a high sensory yoghurt remained unchanged over time, and that in the low sensory versions, the LE was more pleasant than the HE yoghurt. However, generally, these low sensory yoghurts decreased in pleasantness over time, a finding that is not supported by the current study.

Although differences were not significant, sensory characteristics seemed to have more of an effect on immediate hunger changes after the consumption of the HE drinks. By 30 minutes post consumption, differences seemed to be influenced more by the energy content of the drink, with a greater reduction in those consuming the HE drink, although differences were only significant on day four. After 60 minutes, these energy differences were approaching significance, which indicated that, regardless of sensory characteristics, energy differences were perceived at some level. Mattes and Rothacker (2001) found larger and longer lasting reductions in hunger after consumption of a thick drink versus a thin drink, and sensory rather than energy differences have previously been found to be more influential; the more viscous a beverage, the larger the increase in perceived satiety (Lyly et al., 2010). The present findings suggest that when energy differences are incorporated into the design, learning about the post-ingestive effects from the energy overrides the sensory cues, which appear to be more influential in studies not involving repeated exposure.

Similarly, in the fullness changes it was the energy content of the drink that impacted more on the ratings than the sensory characteristics. There were no differences between conditions immediately after consumption, but after 30 minutes there was a larger increase in fullness after the HE drinks over time, with sensory differences only demonstrated on day one. At 60 minutes post consumption the energy differences were only significant on day four, a similar finding to the hunger differences 30 minutes post consumption. These findings support those from the hunger ratings, beverages do have the potential to be satiating, with the energy differences having more of an influence over time than sensory differences. The observed effect of energy primarily influencing appetite ratings partially supports Yeomans and Chambers (2011), where it was shown that sensory manipulations alone were not sufficient to impact upon satiety; these must be relevant to the energy content delivered. However, the enhanced effect on satiety in the HEHS drinks was not observed.

The additional appetite change data of desire to eat and prospective consumption in this study were not significantly correlated with changes in hunger and fullness, but the respective pairs of change were significantly related (this will be discussed in detail in Chapter 11). The data suggested that there were larger decreases in desire to eat and prospective consumption in the LEHS condition with little differences between the other conditions. It appeared that sensory effects were influencing these changes, but only in the LE condition. Liquids are frequently consumed with little compensation for the energy content and are expected to be less satiating (De Graaf, 2011), so perhaps the sensory enhancement was more effective within the LE as drinks are not normally perceived as filling and so this provided a surprising outcome for the participant.

When asked how filling the drink would be, ratings were significantly higher after consuming the high sensory drink than the low sensory drink, with no energy or time differences. It is interesting that the expectation that the drink would be more filling for those consuming the high sensory drink than the low sensory drink did not correspond with the appetite ratings, where energy difference was salient. A recent study by Cassady, Considine and Mattes (2012) demonstrated that expectations generated through cognitive and oro-sensory manipulations resulted in physiological and behavioural differences that may influence satiety levels and dietary compensation. The findings from the present study suggest that expectations were generated but in a much less explicit manner, so perhaps if these manipulations were accompanied by other relevant cues (such as labelling) this would impact upon appetite ratings. Additionally, it would be interesting to investigate whether participants were aware of the expectations that they had formed based on the oro-sensory cues, and whether awareness is actually necessary for these to translate into behavioural changes. Whether learning is implicit has long been a point of discussion within the literature, with some studies suggesting that contingency awareness is necessary, and others suggesting not (see review by Brunstrom, 2004). This will be discussed further in Chapter 12.

Finally, changes in thirst immediately after consumption showed a trend for opposite effects of sensory in the different energy versions; the low sensory LE drink was more thirst quenching than the high sensory LE drink, but the high sensory HE drink was more thirst quenching than the low sensory HE drink. Throughout all time points the high sensory HE drink was more thirst quenching than the low sensory HE drink. It had been predicted that the low sensory drink would be more thirst quenching than the high sensory, which was partly supported but only in the LE drinks. Perhaps the differential effects of the sensory and energy interactions on changes in thirst influenced FNL as the high energy high sensory drinks were the most thirst quenching which may have hindered learning about the energy content in terms of appetite. However, it is surprising that the changes in thirst did not result in increased pleasantness ratings in this condition as it could still be interpreted as an example of FCL, as the flavour could become associated with the alleviation of thirst. Previous research has found increased intake of a drink that had been consumed after a meal of high salt content compared to one that had been consumed after a meal with low salt content, although this could be as a result of conditioned thirst rather than acquired liking for the flavour (Durlach, Elliman, & Rogers, 2002).

In conclusion, this study showed that expectations about a beverage could be influenced by oro-sensory cues, but that the actual energy content impacted more upon actual appetite measures. These expectations appeared not to have much effect on pleasantness ratings, perhaps indicating that satiety related expectations may be more relevant in determining other factors such as meal choice and portion size, rather than enhancing FNL.

Chapter 9: Study 4 - The impact of labelling calorie content in a breakfast context

9.1 Introduction

A variety of sources of information, including packaging information, advertising and opinions of other consumers, all lead to humans having explicit expectations about the likely energy content, sensory qualities, and post-ingestive consequences of new foods and drinks. These expectations may then be confirmed or rejected once a product is consumed, and may be further moderated by previous experiences and associations. However, the role of expectation in acquired liking for flavours has not been studied, with instead the emphasis of research being centred on learning through association.

Manipulation of expectations has been achieved in a number of studies through the labelling of a preload and measuring the consumption of a subsequent test meal. Shide and Rolls (1995) demonstrated that providing information regarding the fat content of a preload had an influence over energy intake, with women consuming more following the ‘low fat’ preload compared to the ‘high fat’, despite similar actual energy content. However, this has not always been demonstrated, for example Yeomans et al., (2001) found that intake at the test meal was dependent upon the actual fat content of the preload, not the labelled content, with lower consumption after the (actual) high fat preload. Labelling did influence the sensory ratings of the soup preload though, with the soups labelled as ‘high fat’ rated as more pleasant and more creamy, than those labelled as ‘low fat’.

Although a number of studies have investigated how beliefs and expectations can influence hedonic, sensory and appetite ratings (discussed in detail in Chapter 7), the present study is unique in focusing upon flavour based learning and the impact upon acquisition of liking.

The present design contrasted measures of liking for, and intake of, two novel yoghurt-based breakfasts over four breakfast test sessions, with one breakfast high in energy and a second low in energy. Different participants consumed and evaluated the breakfasts, either unlabelled, or with either congruent (accurate) or incongruent labels of actual energy content. On days one and four, consumption was *ad libitum* to see whether training with a HE breakfast resulted in an increase in consumption, and on days two and three consumption was fixed at 300g to ensure sufficient exposure to the flavour-energy pairings for each participant. It was predicted that those consuming the HE version of the yoghurt breakfast would demonstrate an acquired liking for the yoghurt, and consume a larger amount, in comparison to those consuming the LE yoghurt. This effect was predicted to be enhanced for those in the congruent labelling condition as expectations of calorie content would match the actual calorie content. Incongruent labelling may result in the opposite pattern of liking, due to the contrast between expected and actual calorie content. In relation to appetite change, HE consumption would be predicted to result in a larger reduction in hunger, and increase in fullness, than LE, with this effect again enhanced by labelling.

9.2 Method

9.2.1 Design

Participants were assigned randomly to one of six conditions, which varied in the energy content and labelling of the breakfast. Participants in three conditions consumed a LE yoghurt (164kcal/300g), with one of these receiving a congruent label (correct energy content), one an incongruent label (incorrect energy content) and one no information (no label control group). The other three conditions consumed a HE version (330kcal/300g), and again each of these three HE conditions received a label from one of the three above.

9.2.2 Participants

Sixty female participants, aged 18-29 (M= 21.5 ± 0.4) with a mean BMI of 22.3 ± 0.4 were recruited, mainly from the Psychology subject pool and course credits database. All participants scored less than seven on the restraint scale of Three Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985). There were a number of additional exclusion criteria as outlined in Section 2.4.1. The University of Sussex ethics committee approved the experimental design and protocol. The demographic information for each condition is presented in Table 9.1. There were no significant differences between conditions in age $F(5, 54) = 0.10$, $p = .992$, BMI $F(5, 54) = 0.51$, $p = .766$ or restraint score $F(5,54) = 2.29$, $p = .059$.

Condition	Age	BMI	TFEQ-R
LE Congruent	21.7 ± 1.0	22.5 ± 1.2	2.1 ± 0.5
LE Incongruent	21.4 ± 1.1	21.6 ± 1.0	3.8 ± 0.6
LE No label	21.5 ± 1.0	23.5 ± 1.1	2.2 ± 0.5
HE Congruent	21.5 ± 1.1	21.7 ± 0.7	4.0 ± 0.6
HE Incongruent	20.9 ± 0.6	22.5 ± 1.3	3.4 ± 0.6
HE No label	21.7 ± 0.8	21.8 ± 0.5	3.6 ± 0.5

Table 9.1: Mean (± SEM) age, BMI and TFEQ-R score for each condition of Study Four.

9.2.3 Test foods

The yoghurt-based breakfast was produced in house using fat free natural yoghurt (Yeo Valley, UK), each portion flavoured with 16 drops of almond extract (Supercook, UK), 2g ground nutmeg (Schwartz, UK), 2 drops banana flavouring (International Flavours and Fragrances) and 2 (HE) or 3 (LE) drops of yellow food colouring (Supercook UK). Drops were added using pipettes. Cold stewed apple was mixed in with the yoghurt to provide a novel texture. Maltodextrin (Cargill) was added to the yoghurt for the HE breakfast, and aspartame provided some sweetness.

HE	(g)	kcal
Yoghurt	206	119.5
Maltodextrin	51	193.8
Aspartame	0.02	n/a
Apple	43	15.5
TOTAL	**300**	**328.8**
LE		
Yoghurt	257	149.1
Aspartame	0.05	n/a
Apple	43	15.5
TOTAL	**300**	**164.5**

Table 9.2: Composition of each portion of yoghurt breakfast in Study Four.

9.2.4 Labels

A fictitious brand name was created (Black Cap Dairy), with two versions of label used to manipulate expectations about the yoghurt. One was named 'Natural flavoured yoghurt- a natural high energy breakfast" labelled as 330kcal (the correct calorie content of the high energy yoghurt). The second was named 'Natural low fat flavoured yoghurt – a natural low energy breakfast' labelled as 164kcal (the correct calorie content of the low energy yoghurt). Figure 9.1 shows the labels presented with the breakfasts.

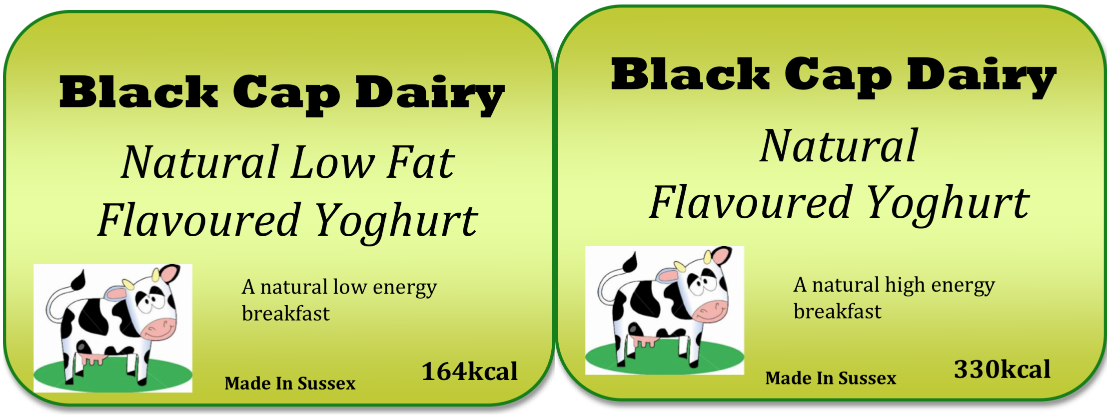


Figure 9.1: Breakfast labels used in Study Four.

9.2.5 Expected Satiety

A measure of expected satiety, using a method of constant stimuli (Brunstrom, Shakeshaft & Scott-Samuel, 2008) was presented on test days 1 and 4. Booklets were produced containing packshots of two alternate breakfasts; Crunchy Nut Cornflakes and porridge. Examples of stimuli are shown in Figure 9.2. Based on the Brunstrom et al., (2008) methodology, image 10 was used as the standard, and was equivalent to the median point between the low and high energy versions of the yoghurt breakfast (246.7 kcal). The first image therefore needed to be 0.1 times the calorie content of this standard (24.67 kcal), image 2 was 0.2 times the calorie content, and so on. The final image (number 40) of Crunchy Nut cornflakes was 986.8 kcal (4 times the calorie content of the median) and the final image of porridge (number 30) was 740.1 kcal (3 times the calorie content of the median). The bowls used in the images were the same bowls as those that the yoghurt was served in.

Figure 9.2: Examples of images used for method of constant stimuli (images shown are the standard) in Study Four.

9.2.6 Procedure

Participants were required to report to the laboratory on four mornings, at a time between 08.15 and 10.00, over a period of one to two weeks. Only water had been consumed from 23.00 the night before each session. On arrival, participants completed a set of computerised mood and appetite ratings, (hungry, thirsty, full, lively, clear-headed, tired, nauseous, energetic, headachy, drowsy, calm) using the SIPM software. On all sessions, the yoghurt was presented along with the label (except in the control conditions, where there was no label) and a taste test (pleasant, creamy, novel, bitter, sour, sweet, fruity, familiar) was completed, along with an explicit question asking how many calories were in the serving. On days one and four, participants were presented with the two expected satiety booklets and were asked to select the picture that they would expect to fill them up to the same extent as the portion of yoghurt they had received. *Ad libitum* eating then followed this, with a refill provided once 250g were consumed. On days two and three, a fixed amount (300g) of the yoghurt breakfast was consumed. After consumption, participants completed another set of computerised mood and appetite ratings, and then completed the same ratings using a paper version one hour after leaving the laboratory (having refrained from eating and drinking except for water). On the final session, participants were debriefed, height and weight recorded, and reimbursed for their time. See Appendix 6 for materials.

9.2.7 Data analysis

9.2.7.1 Preliminary analysis

Data were examined for normality using K-S tests, histograms and boxplots. The majority of variables were normally distributed, with a few exceptions which were only non-normal in one instance (i.e. by energy or by label). Fullness ratings at the beginning of each session were generally non-normal, but as change data were normal, group sizes were equal, homogeneity of variance was met, and transforming did not resolve normality the original data were retained, approaching interpretation with some caution.

9.2.7.2 Main analysis

One way independent ANOVAs were conducted to explore any baseline differences between groups. Pleasantness, intake, appetite ratings and expected satiety values were analysed using a series of three way mixed ANOVAs (day*energy*label), with two way independent ANOVAs conducted on change in intake and day one analyses for one hour post test appetite changes. Significant interactions were broken down using Bonferroni corrected t-tests or ANOVAs. As restraint scores were slightly lower in some conditions (see Table 9.1), this was also added as a covariate to examine any influence on results. Restraint was a non-significant covariate in all analyses (all p > .158) except for expected satiety with porridge images (p = .027). Therefore, ANOVAs were conducted and reported for all analyses except for expected satiety with the porridge images, where restraint was included as a covariate in a three way mixed ANCOVA (Section 9.5.3.1).

9.3 Results

The aim of this study was to investigate the influence of explicit information on flavour nutrient learning, by determining whether an acquired liking occurred for the HE yoghurt, and if this pattern was altered by the presence of information.

9.3.1. Pleasantness

In order to assess whether an acquired liking was demonstrated, pleasantness ratings across time were analysed. Initially, it was established that there were no significant differences in baseline pleasantness between conditions, $F(5,54) = 0.76$, $p = .583$. Table 9.3 shows the baseline pleasantness ratings for the six conditions. Whilst the baseline differences were not significant between conditions information may have influenced the initial rating of the yoghurt breakfast, as there was a larger difference in pleasantness between the two energy versions in the control conditions than in the other information conditions.

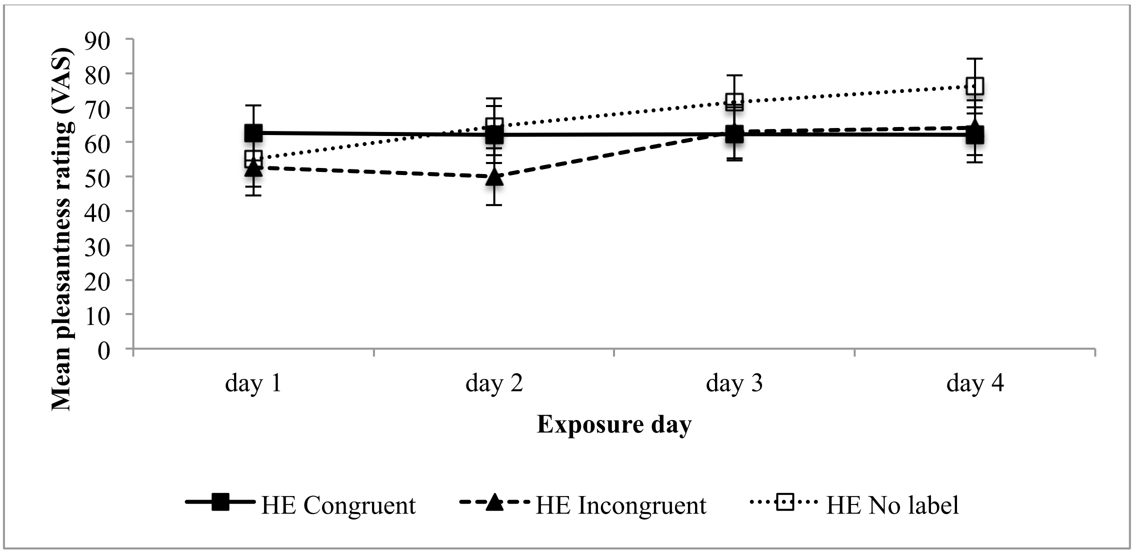
Condition	**Baseline pleasantness (±SEM)**	**Intake on day one (±SEM)**
LE Congruent	61.3 ± 8.9	209.5 ± 31.1
LE Incongruent	60.8 ± 7.8	186.8 ± 44.2
LE No label	72.8 ± 9.4	201.5 ± 45.4
HE Congruent	62.7 ± 7.0	254.3 ± 40.1
HE Incongruent	52.6 ± 8.8	137.1 ± 27.5
HE No label	55.1 ± 6.0	315.7 ± 40.1

Table 9.3: Mean (±SEM) baseline pleasantness ratings and intake between conditions in Study Four.

9.3.1.1 Pleasantness ratings across days

A conditioned flavour preference after consumption of the HE version of the breakfast was predicted, but a key point of interest in this study was the effect of manipulating the cognitive information, i.e. the label. The congruent information was predicted to strengthen the flavour preference learning, so those in the congruent HE condition would show an enhanced acquired liking for the breakfast.

a)



b)

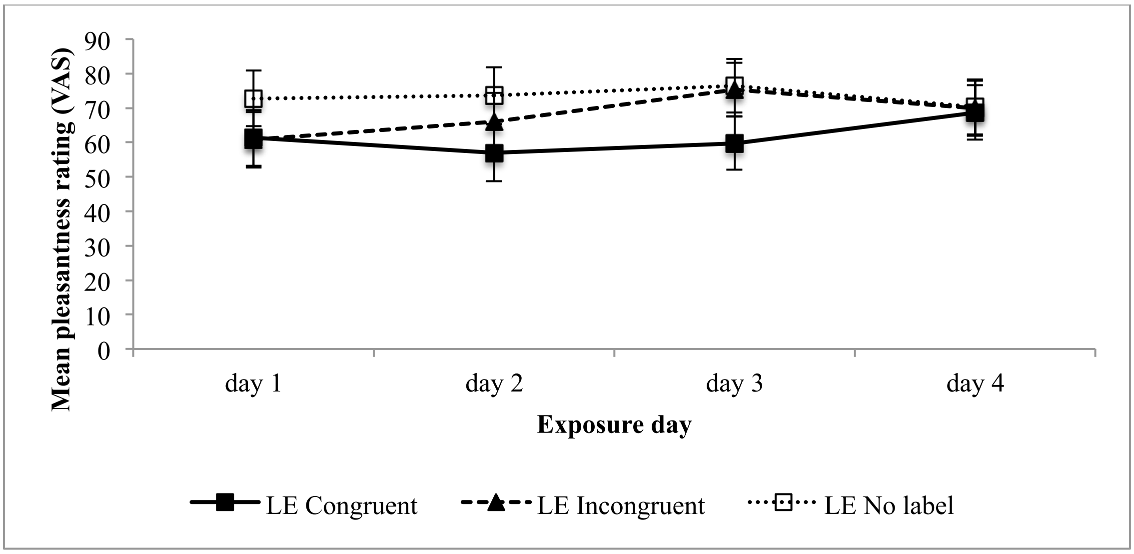


Figure 9.3: Mean (±SEM) pleasantness ratings across exposure days between label conditions for a) HE breakfast and b) LE breakfast in Study Four.

As can be seen in Figure 9.3, different patterns in liking were evident across the conditions. For those consuming the HE breakfast, the congruent label resulted in a higher baseline rating, but very little change in pleasantness across the exposure days, which was in contrast to the other HE conditions which demonstrated increasing pleasantness. For the LE conditions, labelling appeared to result in lower baseline ratings, which increased to the same level as the control condition across exposure days.

Analysis of pleasantness ratings across the four days found a significant main effect of day $F(3,162) = 4.86$, $p = .003$ with liking increasing in a linear pattern. Bonferroni post hoc tests revealed that pleasantness was significantly lower on day one ($M = 60.9 \pm 3.3$) than both days three ($M = 68.1 \pm 3.2$, $p = .011$) and four ($M = 68.6 \pm 3.2$, $p = .041$), with no difference between the other days (all $p > .091$). There was no significant effect of energy $F(1,54) = 0.90$, $p = .348$, label $F(2,54) = 0.80$, $p = .455$ or energy*label interaction $F(2,54) = 0.32$, $p = .725$.

Since the prediction was for liking to change as a function of time, the critical tests were for within-subjects linear contrasts. These revealed no significant day*energy $F(1,54) = 1.49$, $p = .227$ or day*label $F(2,54) = 1.22$, $p = .303$ interactions, but did reveal a significant day*energy*label interaction $F(2,54) = 3.30$, $p = .045$. To interpret this interaction, regression slopes were calculated for each participant, and Bonferroni corrected t-tests conducted between energy versions for each label condition.

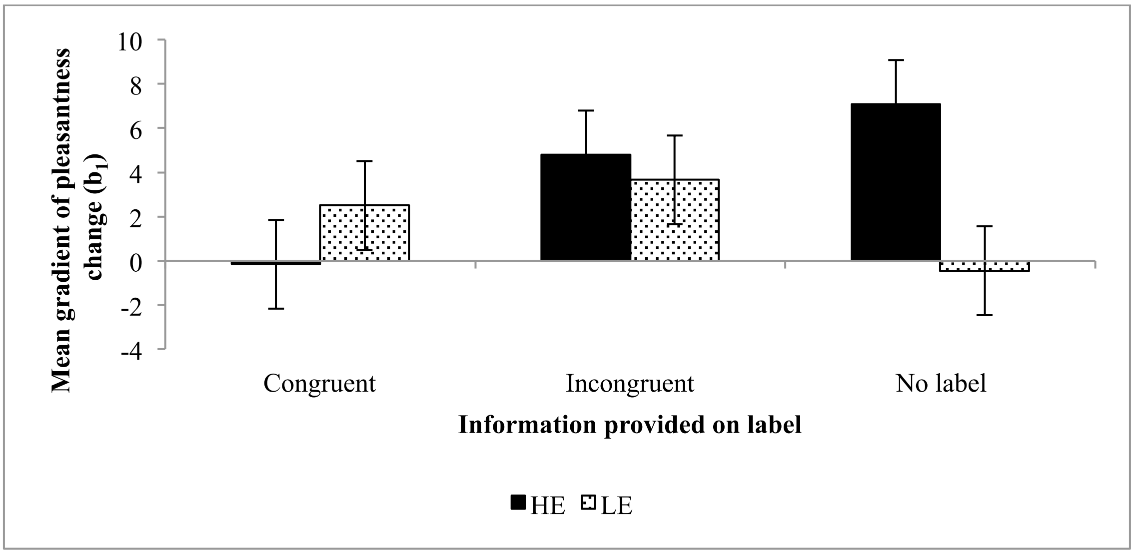


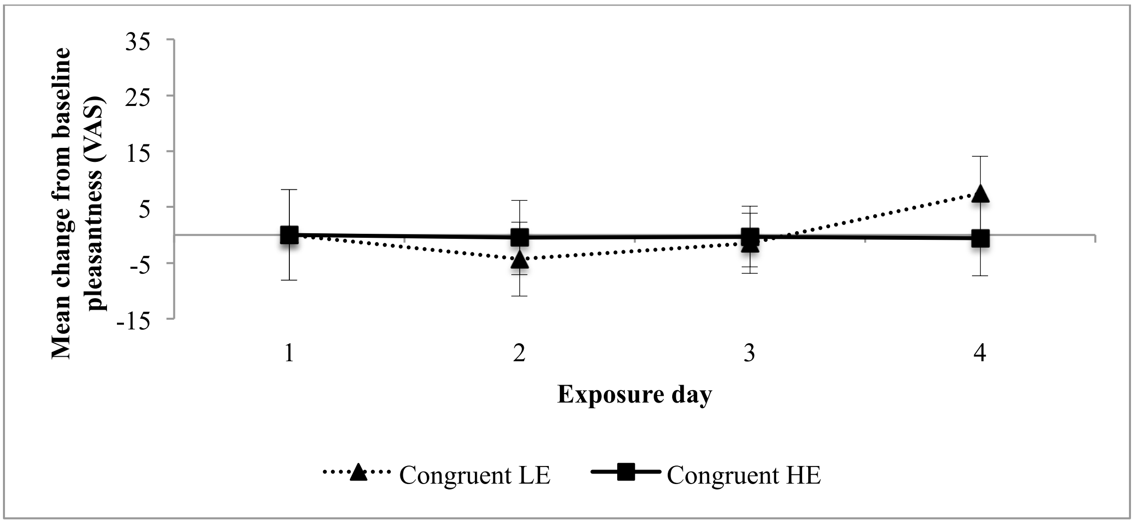
Figure 9.4: Mean (±SEM) gradient of pleasantness change between label and condition in Study Four.

As shown in Figure 9.4, when the congruent information was provided there was very little change in the HE (*M* = -0.2 ± 2.0) and a small increase in the LE (*M* = 2.5 ± 2.0). These differences were not significantly different $t(18) = 0.76$, $p = .457$. When incongruent information was provided, there is little difference in change between energy versions (LE; *M* = 3.7 ± 2.0, HE; *M* = 4.8±2.0), reflected in a non-significant t-test $t(18) = 0.50$, $p = .625$. When no information was provided, there was an increase in pleasantness for the HE (*M*= 7.1 ± 2.0) but little change for the LE condition (*M* = -0.5 ± 2.0), and this difference was significant $t(18) = -2.90$, $p = .010$.

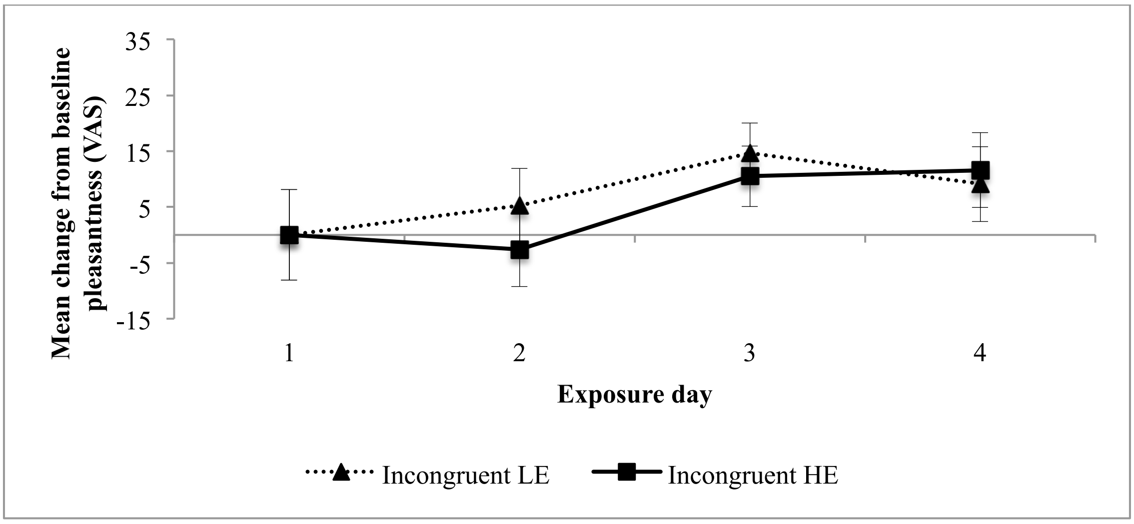
9.3.1.2 Change from baseline pleasantness

Alongside analysing the regression slopes, change from baseline pleasantness was also analysed, to investigate changes at each exposure day, by subtracting pleasantness on day one from pleasantness on each subsequent test day. Data are presented in Figure 9.5.

a) Congruent label conditions



b) Incongruent label conditions



c) No label conditions

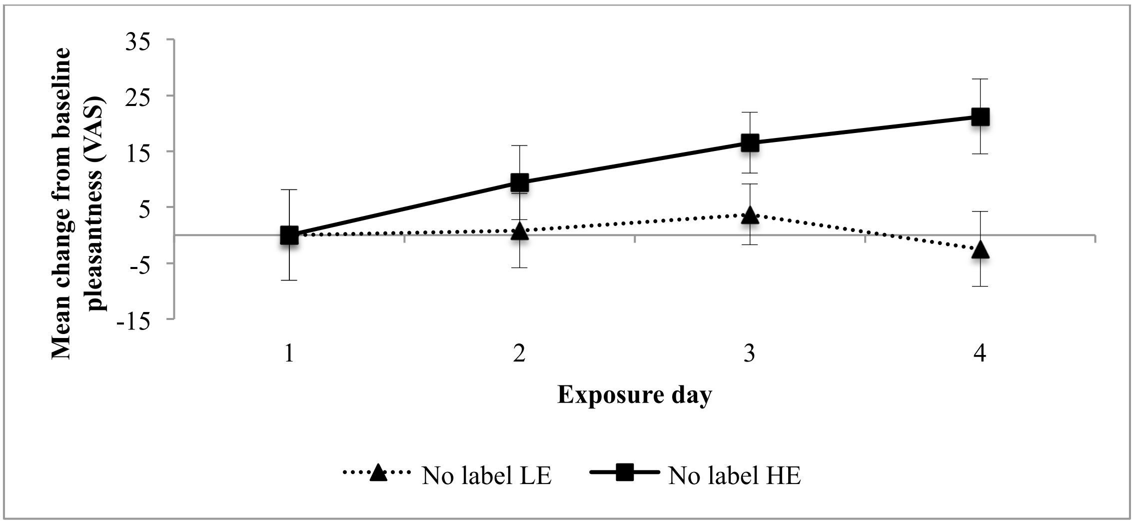


Figure 9.5: Mean (±SEM) change from baseline pleasantness across exposure sessions in Study Four.

As can be seen from Figure 9.5, when a congruent label was presented alongside the yoghurt breakfast, a small increase in pleasantness occurred for the LE version but contrary to prediction, there was no change in liking for the HE. When incongruent information was provided, liking patterns became confused initially, with those consuming the LE showing an increase in pleasantness in line with what would be expected from consuming a HE version. Those consuming the HE version showed an initial decrease in pleasantness but this started to increase after the second exposure (in line with the control condition). Finally, when no information was provided, the predicted acquired liking was demonstrated: those consuming the HE yoghurt showed an increase in pleasantness whereas those consuming the LE yoghurt showed a slight decrease in pleasantness.

ANOVA results showed a significant difference only between days $F(2,108) = 3.93$, $p = .023$, with no significant differences between energy $F(1,54) = 0.74$, $p = .394$ or label $F(2,54) = 1.64$, $p = .203$ conditions, and no interaction effects; day*energy $F(2,108) = 0.41$, $p = .662$, day*label $F(4,108) = 0.72$, $p = .578$, energy*label $F(2,54) = 1.86$, $p = .166$ and day*energy*label $F(4,108) = 1.45$, $p = .224$. Bonferroni post hoc tests showed that pleasantness changes were only significantly different between days two ($M = 1.4 \pm 2.7$) and four ($M = 7.7 \pm 2.7$, $p = .048$), although also approaching significantly different between days two and three ($M = 7.3 \pm 2.2$, $p = .075$). Within subject linear contrasts were also approaching significance for the day*energy*label interaction $F(2,54) = 2.68$, $p = .077$.

From the pleasantness results, it is evident that the presence of information influenced patterns of liking for the breakfast, as the changes observed were different to those that occurred when no information was provided. The predicted enhancement of pleasantness for the HE breakfast with a congruent label was not observed, with very little change occurring after an initial higher pleasantness than in the other conditions.

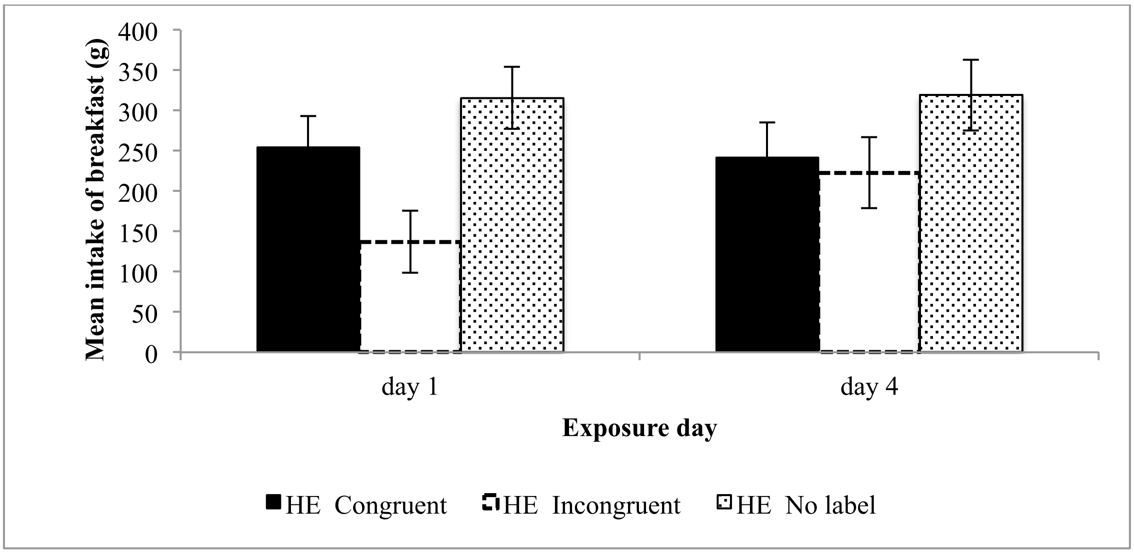
9.3.2 Intake

It was predicted that *ad libitum* intake would increase between days one and four for those trained with the HE breakfast compared to those trained with the LE breakfast, and that the congruent labelling would once again enhance this effect. Before investigating change data, baseline intake on day 1 was analysed (see Table 9.3).

At baseline there was a significant difference in intake between conditions $F(5,54) = 2.51$, $p = .041$. Bonferroni post hoc tests revealed that those consuming the HE breakfast with no label consumed significantly more ($M = 315.7 \pm 38.6$) than those consuming the HE breakfast with the incongruent label ($M = 137.1 \pm 38.6$, $p = .028$). No other conditions significantly differed from each other (all $p > .329$).

In order to take these baseline differences into account, a 3 way mixed ANOVA including day was initally used to analyse intake data before change data were calculated. There was a significant effect of day $F(1,54) = 8.55$, $p = .005$, with significantly higher consumption on day four ($M = 266.3 \pm 17.9$) than day one ($M = 217.5 \pm 15.8$). There was no significant difference in intake between energy $F(1,54) = 0.20$, $p = .656$ or label conditions $F(2,54) = 2.03$, $p = .141$ and no significant interaction effects: energy*label $F(2,54) = 1.63$, $p = .205$, day*label $F(2,54) = 1.41$. $p = .254$, day*energy $F(1,54) = 1.95$, $p = .168$, day*energy*label $F(2,54) = 0.44$, $p = .650$. This contradicted the hypothesis that those consuming the HE version would consume more breakfast on day four than those consuming the LE, and that this effect would be enhanced in the congruent labelling condition. Also, the increased pleasantness shown in the control HE group did not result in an increased intake.

a)



b)

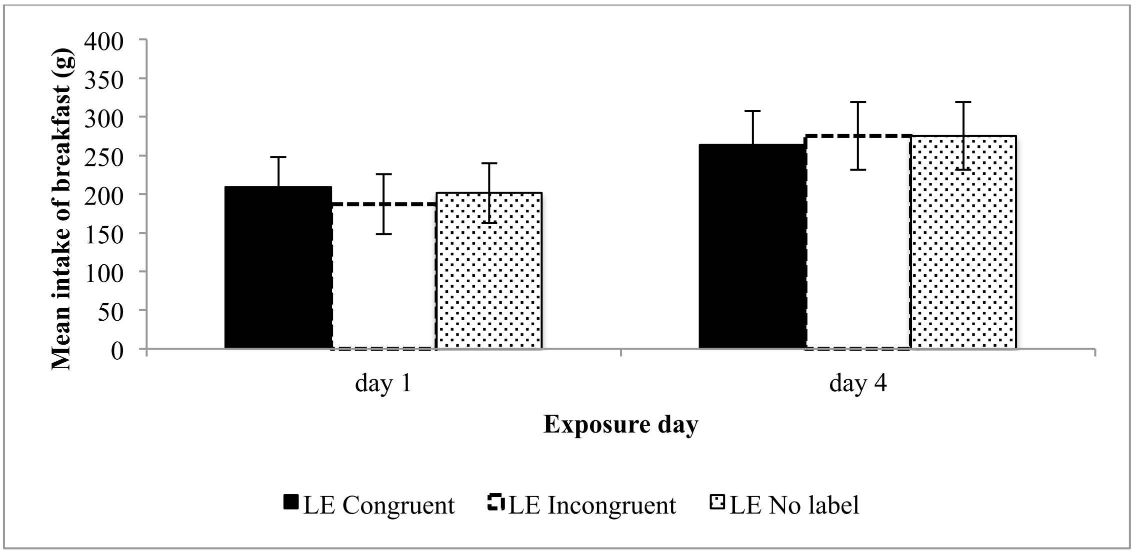


Figure 9.6: Mean (±SEM) intake of breakfast pre and post training after a) HE or b) LE versions in Study Four.

Although no significant interaction effects were found, it is clear from Figure 9.6 that the intake pattern for those consuming the LE breakfast was very similar regardless of labelling, with all increasing their intake between days 1 and 4. For those consuming the HE breakfast, this pattern differed depending on the label. Those with no information showed a stable intake, although this was higher than all other conditions. Those with incongruent information consumed less than other conditions on day 1 (as reflected in the baseline differences) but this increased on day 4, whereas those with congruent information consumed less on day 4. Patterns of intake were therefore not as predicted.

When change from baseline data were analysed, there were no significant differences in intake change between energy $F(1,54) = 1.96$, $p = .168$ or label conditions $F(2,54) = 1.41$, $p = .254$, and no energy*label interaction $F(2,54) = 0.44$, $p = .650$.

9.3.3 Appetite ratings: Hunger

Condition	Day 1	Day 2	Day 3	Day 4
LE Congruent	61.6 ± 5.1	56.1 ± 8.4	61.8 ± 8.0	68.3 ± 7.2
LE Incongruent	65.8 ± 5.4	78.2 ± 3.5	82.8 ± 4.4	77.6 ± 5.1
LE No label	73.2 ± 6.1	84.8 ± 3.9	73.6 ± 8.5	73.4 ± 7.8
HE Congruent	68.4 ± 6.1	68.3 ± 8.5	79.2 ± 6.1	69.1 ± 7.6
HE Incongruent	71.3 ± 9.7	77.6 ± 6.0	74.8 ± 7.5	74.3 ± 7.1
HE No label	74.5 ± 5.4	72.4 ± 3.8	69.2 ± 6.2	82.4 ± 3.9

Table 9.4: Mean (±SEM) hunger ratings at the start of each session in Study Four.

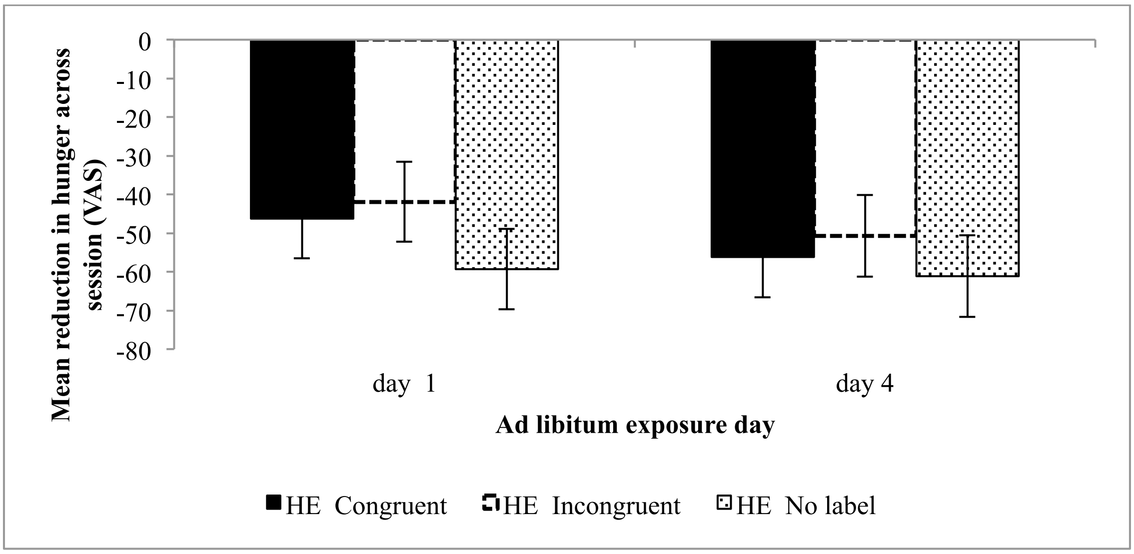
As with other data, the initial focus was on ratings prior to intake to test for confounding baseline differences. As homogeneity of variance was violated on days one and two, the Welch and Brown-Forsythe statistics were reported for these days. There were no significant baseline hunger differences on day one; Welch $F(5,25.10) = 0.72$, $p = .614$, Brown-Forsythe $F(5,41.18) = 0.56$, $p = .728$, or days three $F(5,54) = 1.15$, $p = .344$ and four $F(5,54) = 0.64$, $p = .669$. On day two there were no significant differences in hunger according to the Welch statistic, $F(5,24.84) = 2.30$, $p = .075$, but there were significant differences according to the Brown-Forsythe statistic, $F(5,36.52) = 2.70$, $p = .036$. Games-Howell post hoc tests revealed the only difference approaching significance was those in the LE no label condition rating lower hunger ($M = 56.1 \pm 8.4$) than those in the LE incongruent information ($M = 84.8 \pm 3.9$, $p = .072$).

9.3.3.1 Hunger change across each session

Hunger change for each session was calculated, from the ratings taken upon arrival at the laboratory and those taken after consuming the breakfast. It was predicted that those consuming the HE breakfast would show a larger reduction in hunger across the session compared to those consuming the LE breakfast, with the congruent labels enhancing this difference.

9.3.3.1.1 Ad libitum sessions (days one and four)

a)



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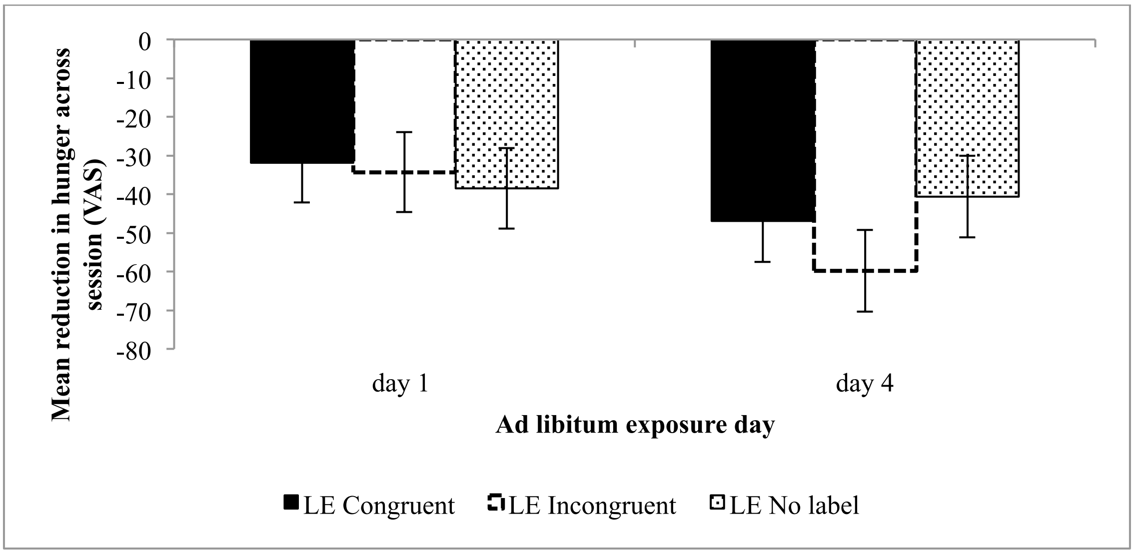


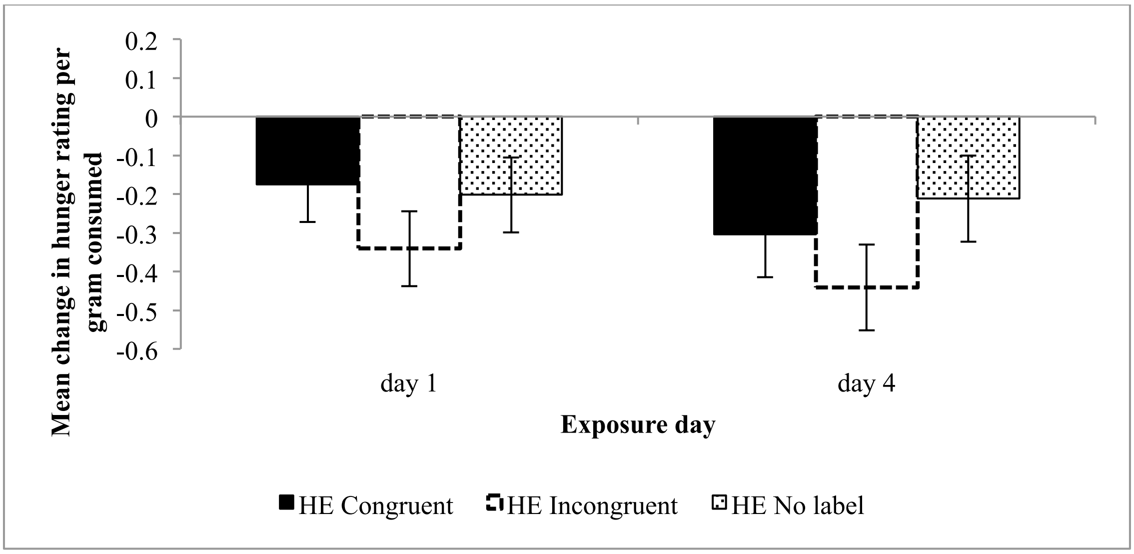
Figure 9.7: Mean (±SEM) reduction in hunger across *ad libitum* sessions after a) HE or b) LE breakfasts in Study Four.

As half of the sessions allowed *ad libitum* consumption and half required a fixed consumption, these days were analysed separately. There was a significant difference in hunger reduction between the *ad libitum* sessions $F(1, 4) = 6.35$, $p = .015$, with a larger reduction on day four ($M = -52.6 \pm 4.3$) than day one ($M = -42.0 \pm 4.2$). There were no significant differences between energy $F(1,54) = 2.01$, $p = .162$ or label $F(2,54) = 0.13$, $p = .875$ conditions and no significant interaction effects; energy*label $F(2,54) = 0.69$, $p = .504$, day*label $F(2,54) = 1.16$, $p = .322$, day*energy $F(1,54) = 0.79$, $p = .379$ or day*energy*label $F(2,54) = 0.34$, $p = .716$. Therefore, contrary to prediction, neither label nor energy content made a significant difference to hunger reduction in the *ad libitum* sessions (Figure 9.7).

9.3.3.1.2 Hunger change per gram of breakfast consumed

As consumption on days one and four was *ad libitum*, actual intake of breakfast was likely to influence hunger ratings on these days. Therefore, the relative satiating effect of the breakfast was analysed, by calculating hunger change per gram.

a)



b)

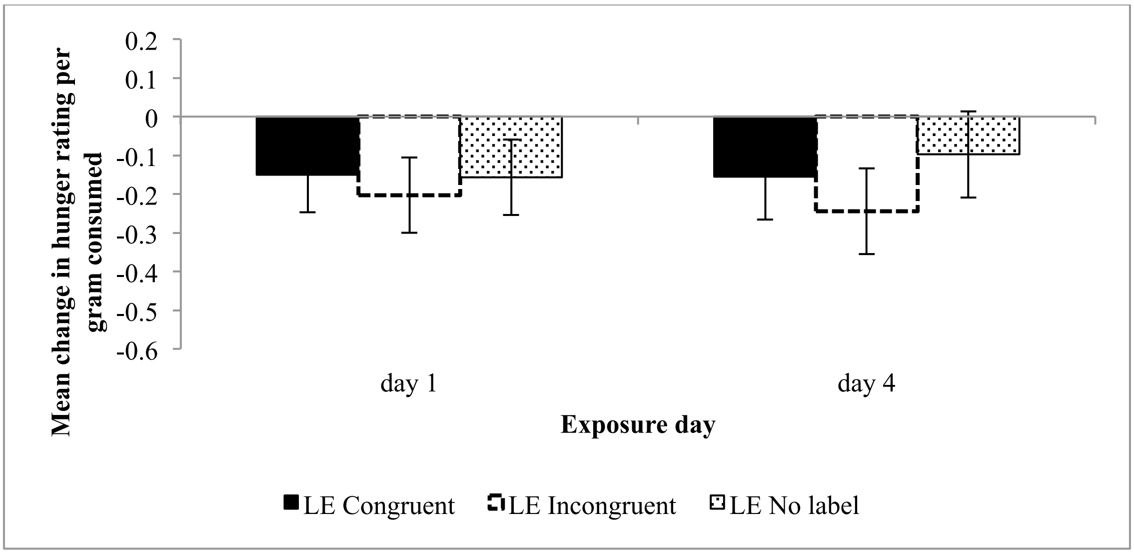
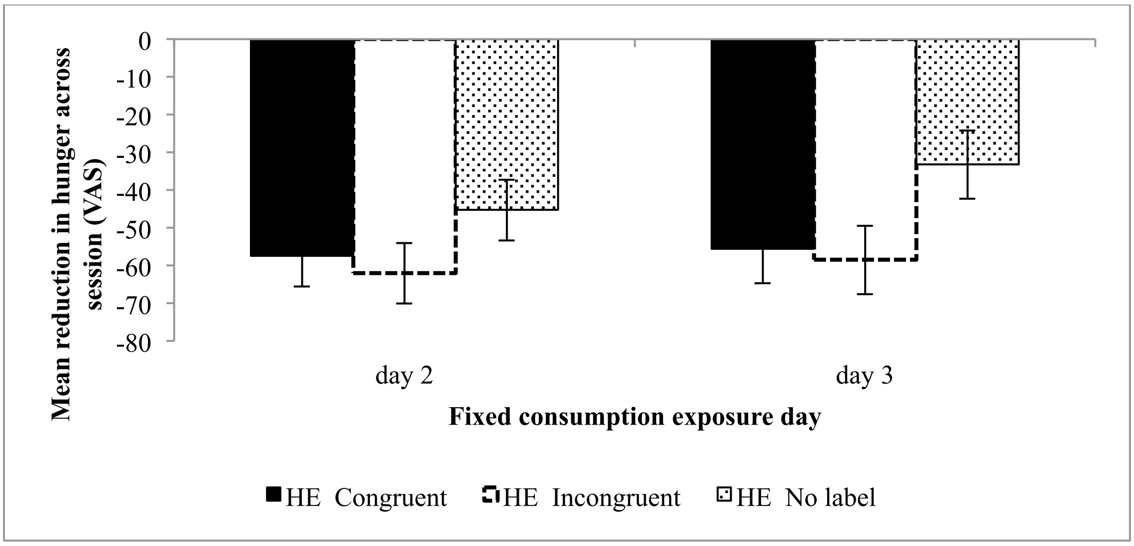


Figure 9.8: Mean (±SEM) change in hunger per gram of a) HE or b) LE breakfast consumed in Study Four.

There was no significant difference in hunger change per gram between exposure days $F(1,54) = 1.02$, $p = .317$, energy $F(1,54) = 2.11$, $p = .153$ or label $F(2,54) = 1.24$, $p = .297$ conditions. There were also no significant interaction effects; energy*label $F(2,54) = 0.13$, $p = .875$, day*energy $F(1,54) = 1.23$, $p = .272$, day*label $F(2,54) = 0.69$, $p = .506$ or day*energy*label $F(2,54) = 0.07$, $p = .929$. Figure 33 shows that there was very little difference in hunger reduction per gram consumed of the LE breakfasts across label conditions or between days. Those who consumed the HE breakfasts showed larger reductions per gram than the LE, with both label conditions showing a slight increase in hunger reduction on day four.

9.3.3.1.3 Fixed consumption sessions (days two and three)

a)



b)

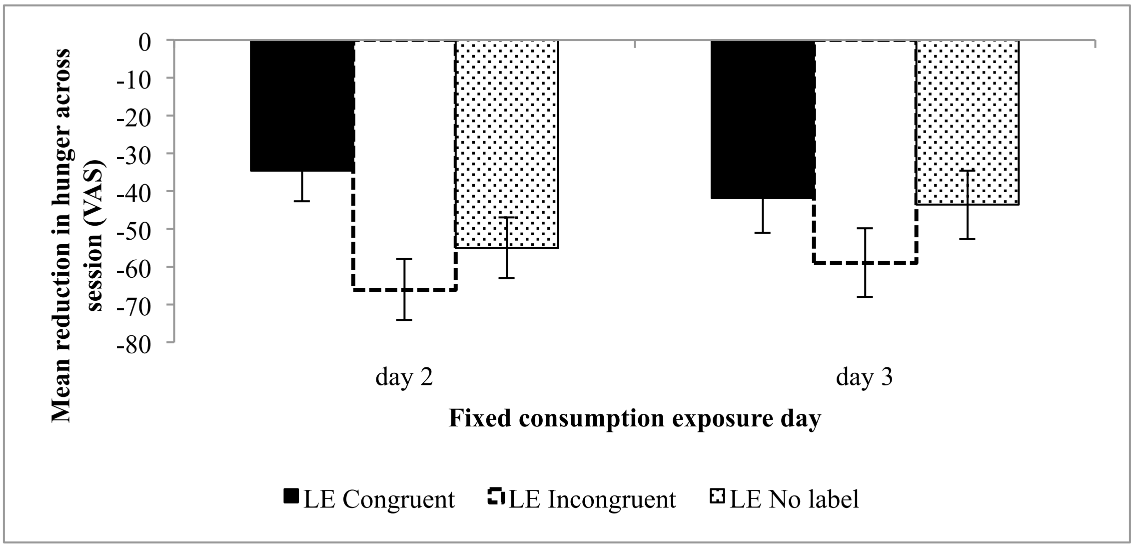


Figure 9.9: Mean (±SEM) reduction in hunger across fixed consumption sessions for a) HE or b) LE breakfasts in Study Four.

Sessions two and three required consumption of a fixed amount. There were no significant differences in hunger reduction between these training sessions $F(1,54) = 1.81$, $p = .184$, or between energy conditions $F(1,54) = 0.11$, $p = .739$. The difference between label conditions was approaching significance $F(2,54) = 3.03$, $p = .057$. There was a larger reduction in hunger in the incongruent labelling condition ($M = -61.4 \pm 5.2$) compared to the congruent ($M = -47.4 \pm 5.2$) and no label ($M = -44.3 \pm 5.2$).

There were no significant interaction effects; energy*label $F(2,54) = 1.96$, $p = .150$, day*energy $F(1,54) = 0.09$, $p = .769$, day* label $F(2,54) = 1.38$, $p = .260$ or day*energy*label $F(2,54) = 0.28$, $p = .757$. Once again, energy content of the breakfast did not appear to influence hunger changes across the sessions (as demonstrated in Figure 9.9).

9.3.3.2 Hunger change one hour post test

Although no differences in hunger change between label and energy conditions immediately after consumption, perhaps one hour post test differences would emerge. Therefore, change from start of session to one hour post test was calculated and analysed. It was hypothesised that differences between energy conditions could emerge during this time, and that the labelling may influence these differences.

A two way independent ANOVA was conducted on hunger change one hour post test for day one, as on this session consumption was *ad libitum*. A three way mixed ANOVA was conducted for days two and three when consumption was fixed to 300g. Figure 9.10 shows hunger reduction across day one.

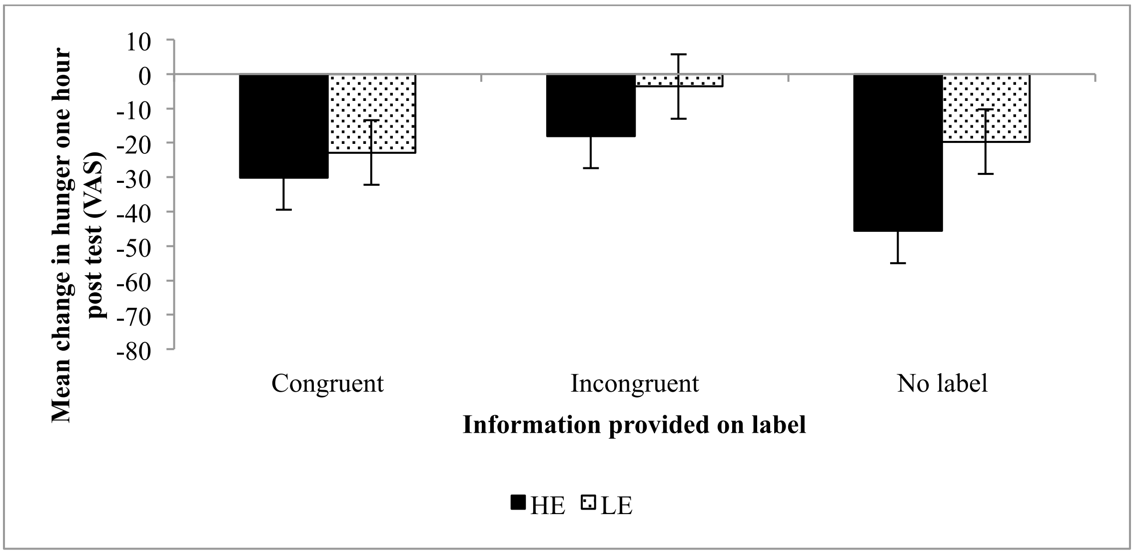


Figure 9.10: Mean (± SEM) reduction in hunger one hour post test across day one between labels and energy content in Study Four.

9.3.3.2.1 Ad libitum session (day one)

Hunger change one hour post test was significantly different between energy conditions $F(1,54) = 4.29$, $p = .043$, with twice the reduction after the HE ($M = -31.2 \pm 5.4$) than the LE ($M = -15.4 \pm 5.4$) breakfast. The difference in one hour post hunger change was approaching significance between labels $F(2,54) = 2.88$, $p = .065$, where the incongruent label ($M = -10.8 \pm 6.6$) resulted in a smaller reduction one hour post test than the congruent ($M = -26.5 \pm 6.6$) and no label ($M = 32.7 \pm 6.6$). There was no significant energy*label interaction $F(2,54) = 0.50$, $p = .609$. Figure 9.10 plots this information, but once again, these changes may have been influenced by intake and therefore, hunger change per gram of breakfast consumed were calculated and analysed for day one.

9.3.3.2.2 Hunger change per gram of breakfast consumed

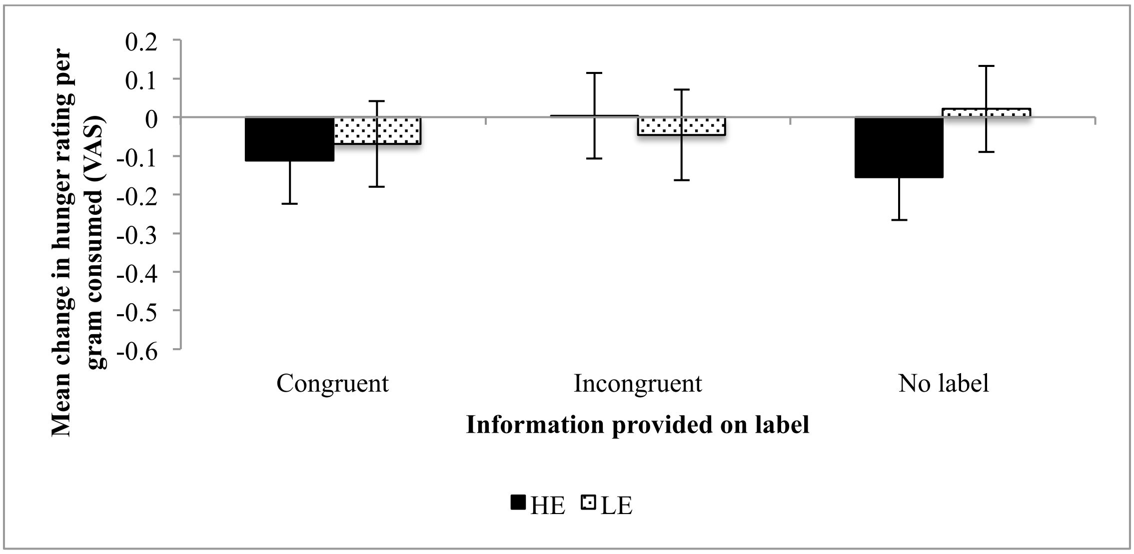
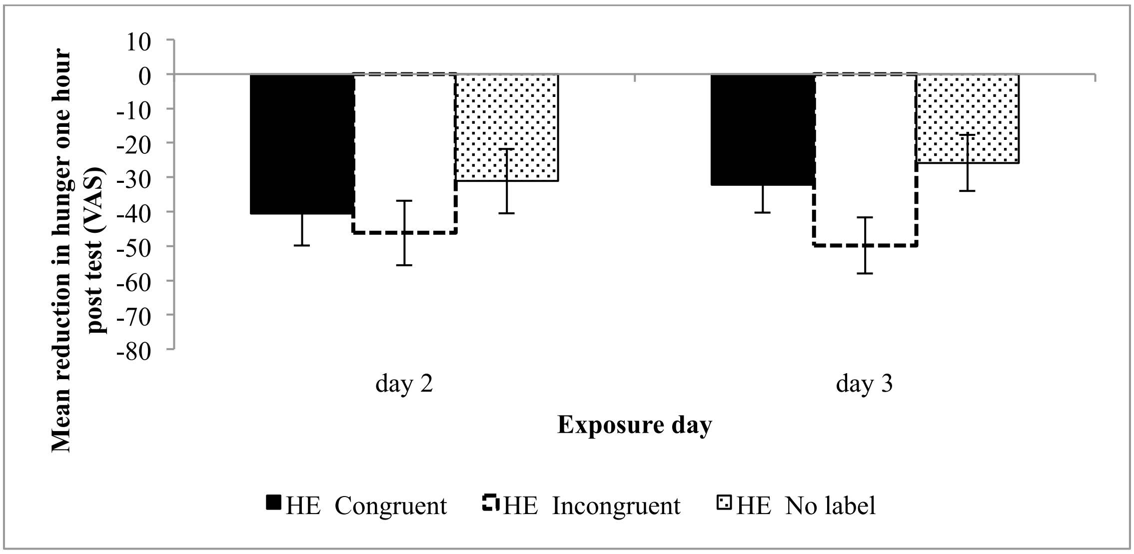


Figure 9.11: Mean (±SEM) hunger change at one hour post test per gram consumed on day one in Study Four.

Once *ad libitum* intake was taken into account in the hunger change one hour post test on day one, there were no significant differences between energy $F(1,53) = 0.38$, $p = .541$, label $F(2,53) = 0.20$, $p = .820$ and no energy*label interaction $F(2,53) = 1.01$, $p = .604$. Figure 9.11 shows that there was very little difference in change in hunger per gram between conditions.

9.3.3.2.3 Days two and three

a)



b)

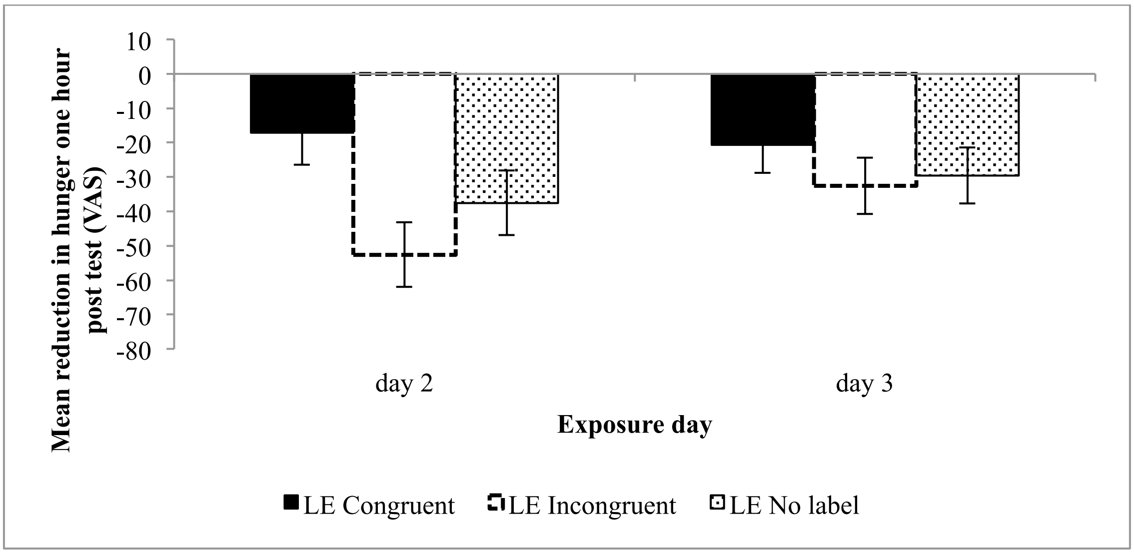


Figure 9.12: Mean (±SEM) reduction in hunger one hour post test on days two and three between labels for a) HE and b) LE breakfasts in Study Four.

On the fixed consumption days, the difference between label conditions was significant $F(2,54) = 4.2$, $p = .020$, but during this period, the incongruent label ($M = -45.3 \pm 4.6$) resulted in a significantly larger decrease in hunger one hour post test than the congruent label ($M = -27.6 \pm 4.6$, $p = .026$), and a larger decrease than no label $M = -31.0 \pm 4.6$), but not significant ($p = .095$). The difference between congruent and no label was not significantly different ($p > .999$).

There was no longer a significant difference between energy conditions on days two and three $F(1, 54) = 1.25$, $p = .268$ and no energy*label interaction $F(2,54) = 1.50$, $p = .231$. There were also no significant differences in one hour post test hunger reduction across the sessions $F(1,54) = 1.50$, $p = .225$ or interaction effects; day*energy $F(1,54) = 0.26$, $p = .612$, day*label $F(2,54) = 0.13$, $p = .875$ and day*energy*label $F(2,54) = 1.20$, $p = .309$. Although not significant, hunger reduction did appear to be larger in general after the HE breakfast compared to the LE as seen in Figure 9.12. However, it is interesting to note that label appeared to have more of an influence over the post hunger changes than the actual energy content on the days where consumption was fixed.

9.3.4 Appetite ratings: Fullness

Once again, baseline fullness ratings were explored before any subsequent analyses were conducted. There were no significant differences in baseline fullness between conditions on days one $F(5, 54) = 2.21$, $p = .067$ or four $F(5, 54) = 1.07$, $p = .387$. As homogeneity of variance was violated on days two and three the Welch and Brown-Forsythe statistics were reported for these days. There were no significant differences in baseline fullness ratings between conditions on day three; Welch $F(5, 24.82) = 1.39$, $p = .261$, Brown-Forsythe $F(5, 40.55) = 0.99$, $p = .435$. On day two, the Welch statistic revealed no significant differences between conditions, $F(5, 24.88) = 2.25$, $p = .081$, but the Brown-Forsythe statistic did reveal a significant difference, $F(5, 38.47) = 2.71$, $p = .034$. Games-Howell post hoc test showed no significant differences between conditions on day two, but from the means in Table 9.5 it can be seen that those in the LE congruent condition gave considerably higher fullness ratings ($M = 33.3 \pm 8.3$) compared to those in the LE incongruent condition ($M = 7.4 \pm 3.4$).

Condition	**Day 1**	**Day 2**	**Day 3**	**Day 4**
LE Congruent	29.3 ± 7.3	33.3 ± 8.3	18.1 ± 4.5	21.8 ± 6.1
LE Incongruent	13.3 ± 4.4	7.4 ± 3.4	6.9 ± 3.2	7.3 ± 2.6
LE No label	8.1 ± 3.3	10.4 ± 3.9	21.7 ± 7.1	21.9 ± 7.6
HE Congruent	17.4 ± 4.1	22.7 ± 6.5	13.7 ± 3.7	19.2 ± 5.5
HE Incongruent	15.0 ± 6.4	15.5 ± 6.1	14.9 ± 5.9	19.5 ± 6.5
HE No label	9.7 ± 3.7	17.1 ± 4.0	21.3 ± 7.5	13.2 ± 3.4

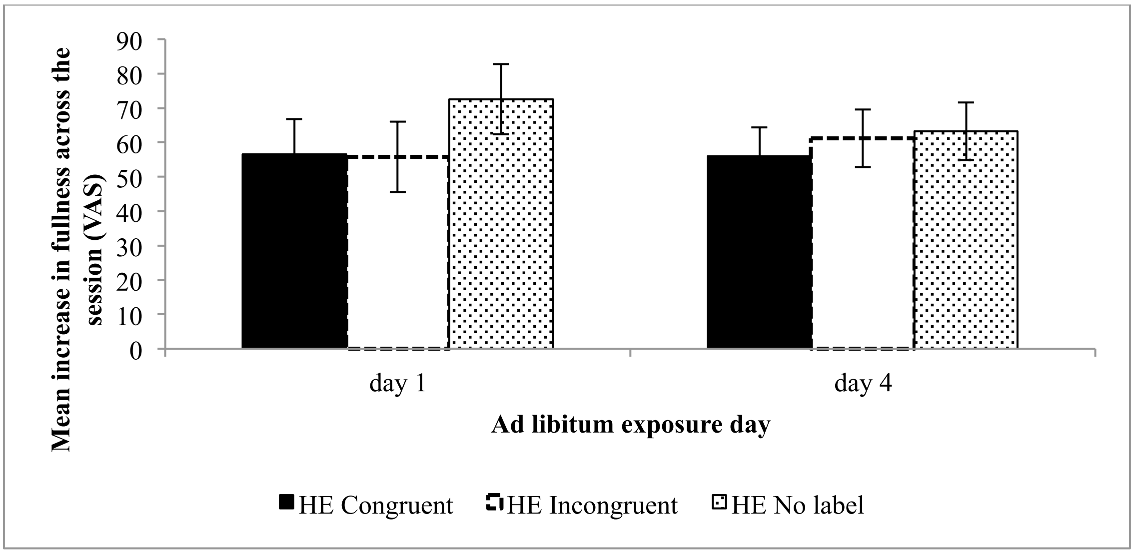
Table 9.5: Mean (±SEM) fullness ratings at the start of each session in Study Four.

9.3.4.1 Fullness changes across session

Change in fullness across each session and one hour post test were calculated. It was predicted that those consuming the HE versions of the breakfast would demonstrate a greater increase in fullness, and once again, that those in the congruent label condition would show an enhanced effect. Once again, these were analysed separately for the *ad libitum* and fixed consumption days, and changes per gram of breakfast consumed were also analysed.

9.3.4.1.1 Ad libitum sessions (days one and four)

a)



b)

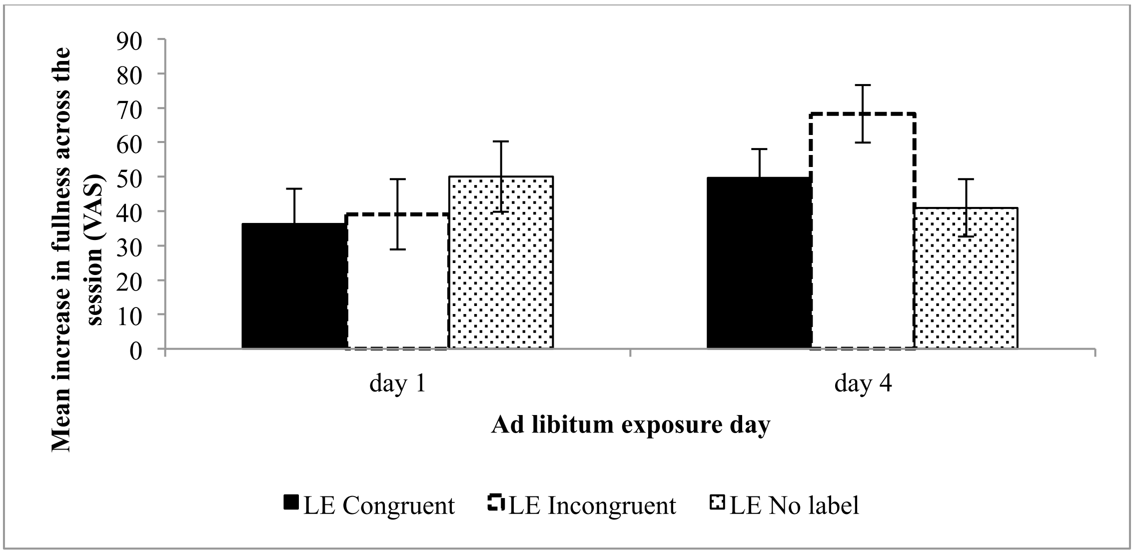


Figure 9.13: Mean (±SEM) increase in fullness ratings across the session between labels after a) HE or b) LE breakfast in Study Four.

There was no significant effect of day $F(1, 54) = 1.39$, $p = .243$ or label $F(2, 54) = 0.49$, $p = .614$ on change in fullness. There was a significant difference between energy conditions $F(1, 54) = 4.41$, $p = .041$, and as predicted, those consuming the HE breakfast ($M = 60.9 \pm 4.55$) reported a larger increase in fullness than those consuming the LE breakfast ($M = 47.4 \pm 4.55$). There was also a significant day*label interaction $F(2, 54) = 3.53$, $p = .036$.

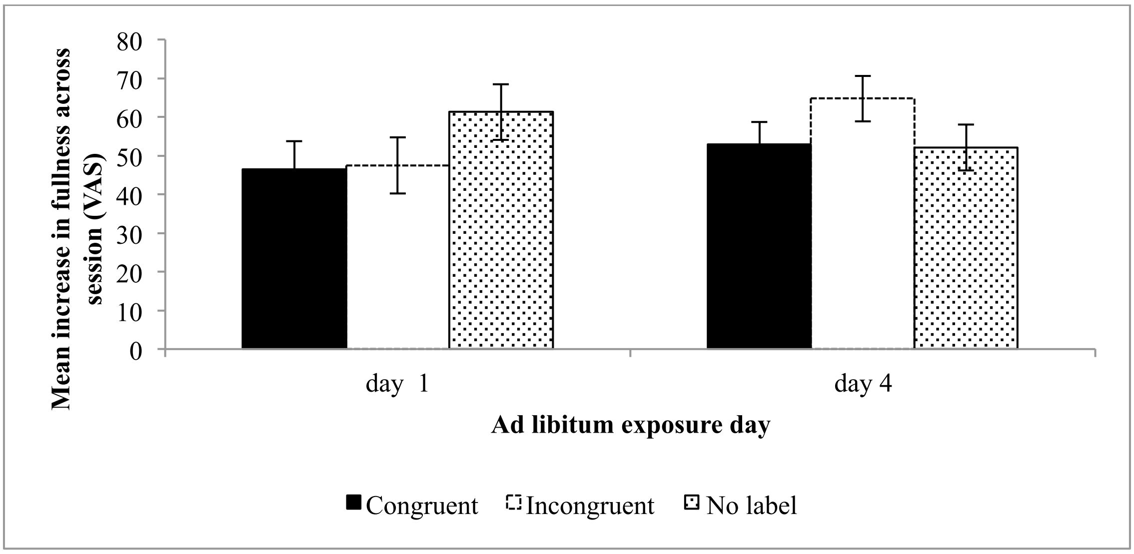


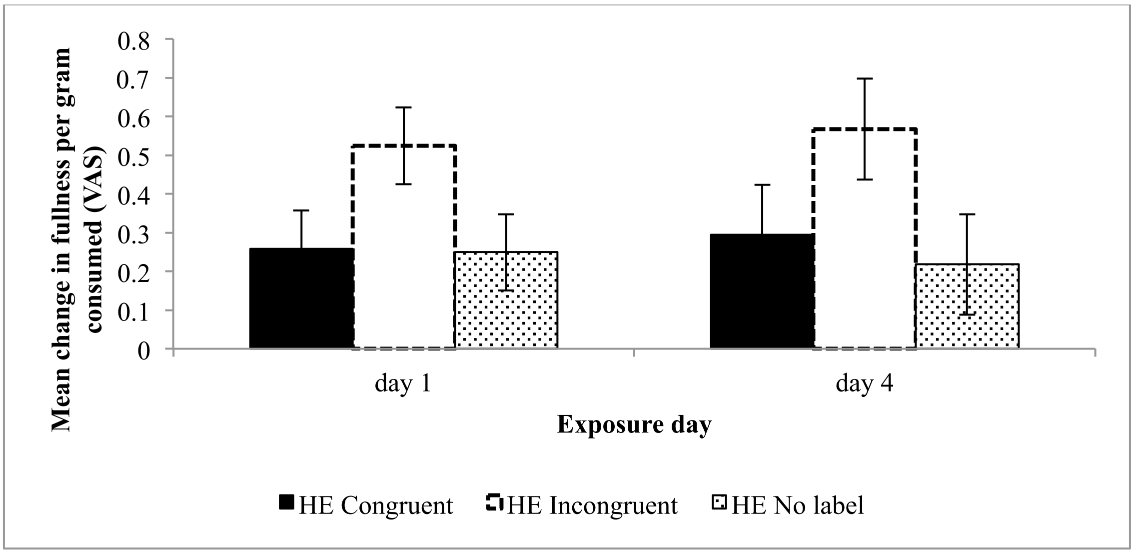
Figure 9.14: Mean (±SEM) increase in fullness on *ad libitum* intake days between label conditions in Study Four.

As can be seen in Figure 9.14, those with no label showed a larger increase in fullness on day one than did those in the label conditions, but on day four this increase was lower, and those in the incongruent label conditions reported the largest increase in fullness ratings. Bonferroni corrected one way ANOVAs revealed these differences were not significant between label conditions on either day one $F(2, 57) = 1.26$, $p = .292$ or day four $F(2, 57) = 1.41$, $p = .253$. When analysed using a paired samples t-test for each label condition, fullness changes were significantly different in the incongruent label only $t(19) = -2.75$, $p = .013$, with a larger increase in fullness on day four ($M = 64.8 \pm 5.4$) than day one ($M = 47.5 \pm 6.5$). Fullness change was similar for days one and four for congruent, $t(19) = -0.87$, $p = .395$ and no label $t(19) = 1.19$, $p = .248$ conditions.

There were no other significant interaction effects on change from baseline fullness ratings; energy*label $F(2, 54) = 0.62$, $p = .540$, day*energy $F(1, 54) = 2.42$, $p = .126$, or day*energy*label $F(2, 54) = 0.69$, $p = .508$.

9.3.4.1.2 Fullness change per gram of breakfast consumed

a)



b)

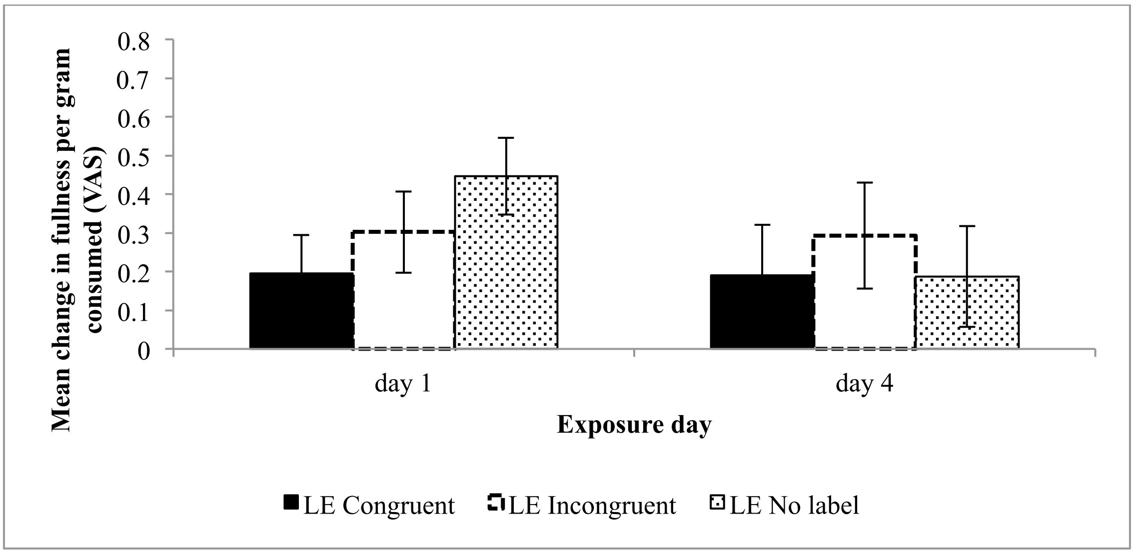


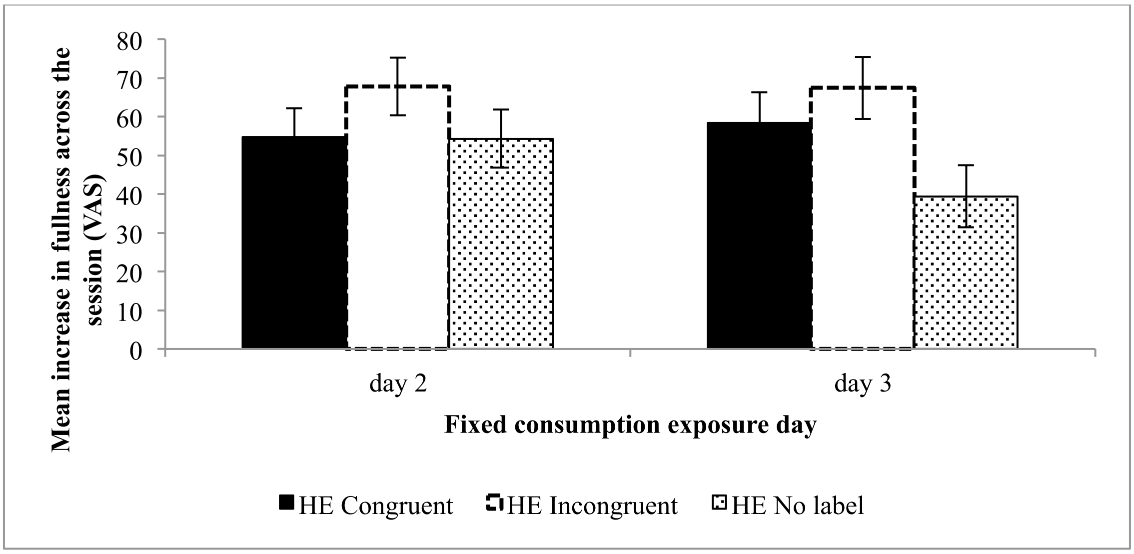
Figure 9.15: Mean (±SEM) fullness change per gram consumed of a) HE or b) LE breakfast in Study Four.

When analysed as change per gram consumed, there were no significant differences in fullness change within the days $F(1,53) = 0.58$, $p = .450$, between energy $F(1,53) = 1.03$, $p = .314$ or label $F(2,53) = 1.90$, $p = .159$ conditions. There were also no significant interaction effects; energy*label $F(2,53) = 1.36$, $p = .265$, day*energy

$F(1,53) = 1.16$, $p = .286$, day*label $F(2,53) = 1.18$, $p = .315$ or day*energy*label $F(2,53) = 0.38$, $p = .687$. As can be seen in Figure 9.15, fullness change per gram consumed was larger in the HE incongruent condition with no difference across days, and changes were generally lower after the LE breakfasts, particularly on day four.

9.3.4.1.3 Fixed consumption sessions (days two and three)

a)



b)

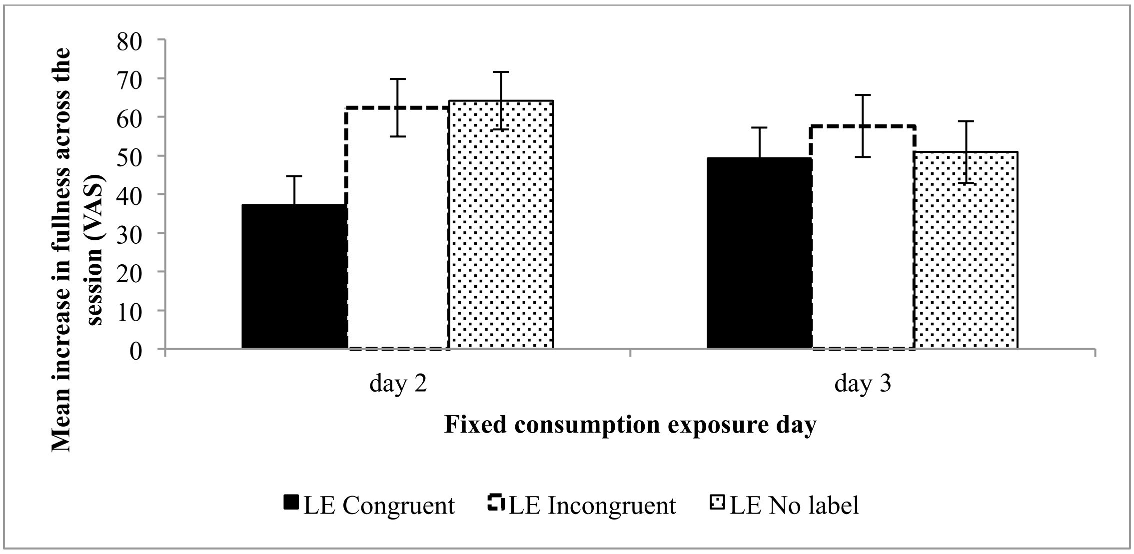


Figure 9.16: Mean (±SEM) increase in fullness across fixed consumption sessions between labels after a) HE or b) LE breakfasts in Study Four.

As with the analysis of hunger ratings, the fixed consumption exposure days were analysed separately to the *ad libitum* consumption days to investigate change in fullness ratings.

There were no significant differences between days *F*(1, 54) = 0.79, *p* = .377, label *F*(2, 54) = 2.56, *p* = .087 or energy *F*(1, 54) = 0.40, *p* = .528. As seen on the *ad libitum* exposure days, there was a significant day*label interaction *F*(2, 54) = 3.65, *p* = .033.

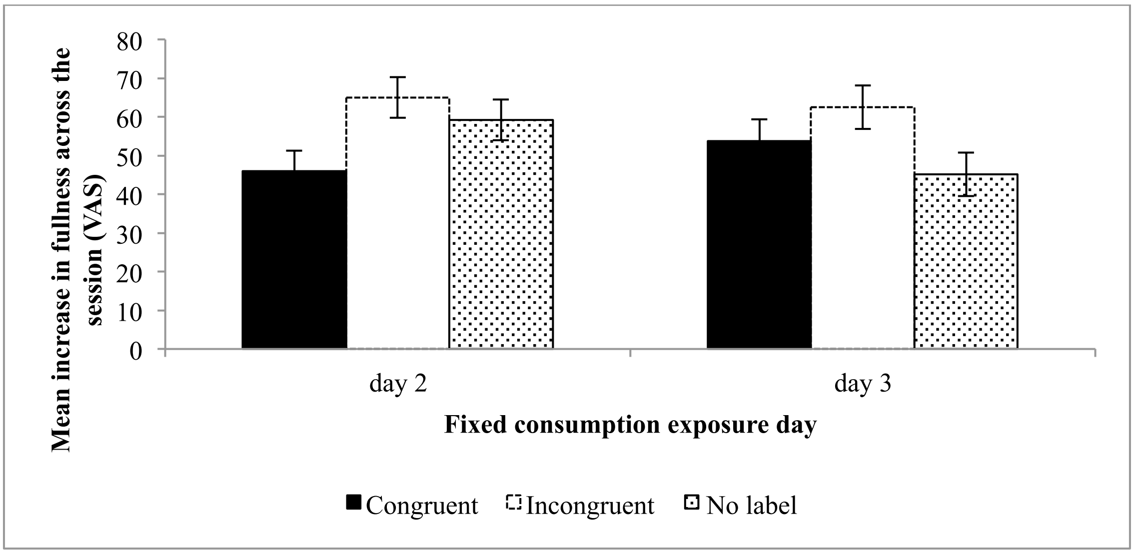


Figure 9.17: Mean (±SEM) increase in fullness across fixed consumption sessions between label conditions in Study Four.

As shown in Figure 9.17, those given the incongruent label remained consistent in the increase in fullness on both fixed consumption sessions. Those in the no label condition show a lower increase in fullness on day three compared to day two, with the opposite pattern displayed for those given the congruent information. One way ANOVAs with Bonferroni corrections revealed no significant differences on day two, *F*(2, 57) = 3.39, *p* = .041 or day three *F*(2, 57) = 2.38, *p* = .102. When analysed using t tests for each label condition, differences were only significant between the no label condition *t*(19) = 2.57, *p* = .019, with a larger increase in fullness on day two (*M* = 59.3 ± 5.5) than day three (*M* = 45.2 ±6.0), with no differences for congruent *t*(19) = -1.27, *p* = .221 or incongruent *t*(19) = 0.49, *p* = .627 label conditions.

There were no other significant interaction effects; day*energy *F*(1, 54) = 0.08, *p* = .775, energy*label *F*(2, 54) = 1.81, *p* = .173, or day*energy*label *F*(2, 54) = 0.31, *p* = .737.

As previously demonstrated in hunger changes, energy content of the breakfast consumed did not impact upon change in fullness across the session.

9.3.4.2 Fullness change one hour post test

As with the hunger ratings, fullness change one hour post test was also analysed. Again, differences in fullness were calculated between start of session and one hour post test to investigate any influences of energy or label for this period of time. A two way independent ANOVA was conducted for day one, as on this session consumption was *ad libitum*. A three way mixed ANOVA was conducted for days two and three when consumption was fixed to 300g.

9.3.4.2.1 Ad libitum session (day one)

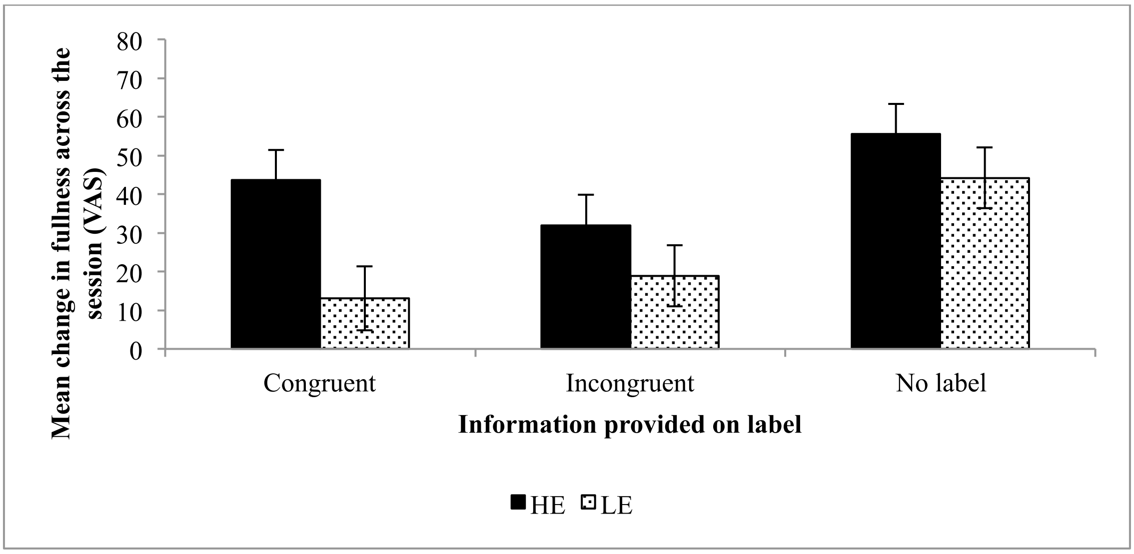


Figure 9.18: Mean (±SEM) fullness change one hour post test on day one of Study Four.

There were significant differences in fullness change one hour post test on day one between both energy $F(1,53) = 7.89$, $p = .007$ and label $F(2,53) = 5.69$, $p = .006$ conditions but with no significant energy*label interaction $F(2,53) = 0.87$, $p = .423$. As predicted, those who consumed the HE breakfast ($M = 43.7 \pm 4.6$) reported a significantly larger increase in fullness than those who consumed the LE version ($M = 25.4 \pm 4.6$). In terms of labels, those who received no information ($M = 49.9 \pm 5.6$) demonstrated a significantly larger increase in fullness than both the congruent ($M = 28.4 \pm 5.7$, $p = .029$) and incongruent ($M = 25.4 \pm 5.6$, $p = .009$) conditions, with no significant differences between these conditions ($p > .999$).

9.3.4.2.2 Fullness change per gram consumed

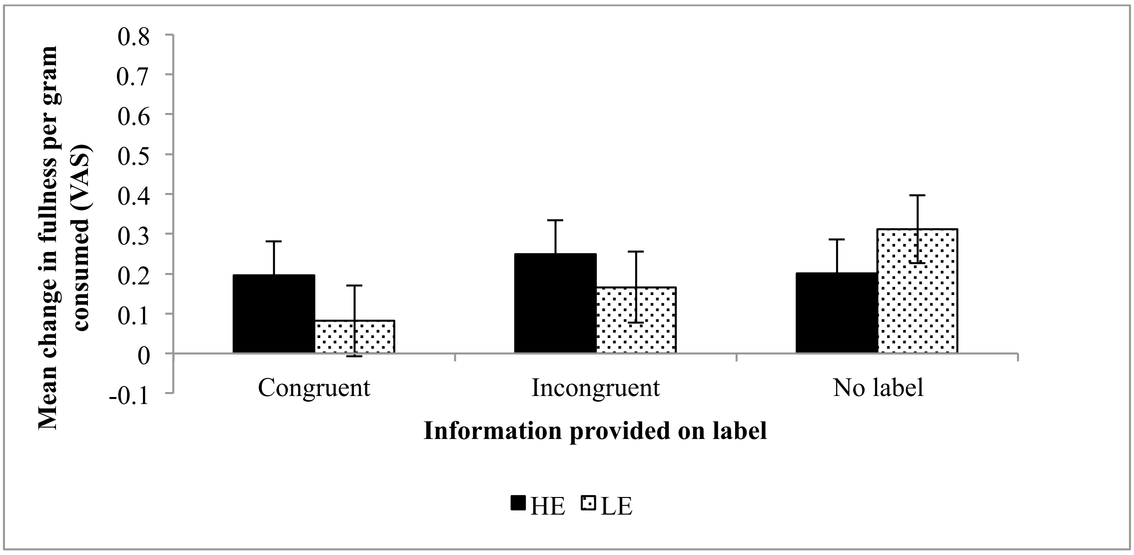
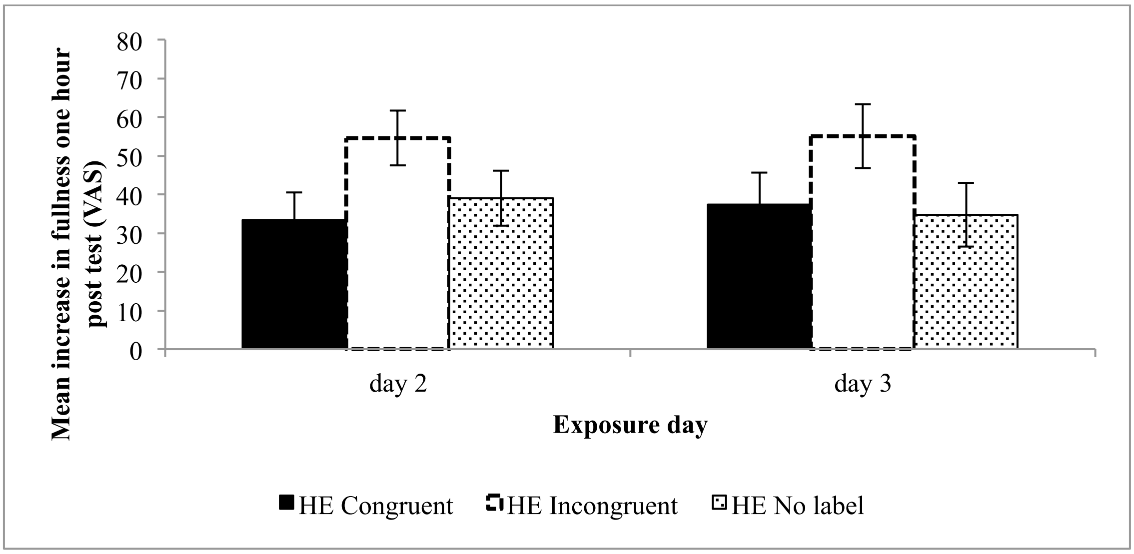


Figure 9.19: Mean (±SEM) fullness change at one hour post test per gram consumed on day one in Study Four.

Once *ad libitum* intake was taken into account in the fullness change one hour post test on day one, there were no significant differences between energy $F(1,52) = 0.17$, $p = .680$, label $F(2,52) = 0.93$, $p = .399$ and no energy*label interaction $F(2,52) = 1.01$, $p = .371$. As Figure 9.19 shows, fullness change per gram at one hour post test was similar after the HE breakfast regardless of label, whereas the largest increase in fullness one hour post test in LE was when no information was provided.

9.3.4.2.3 Fixed consumption (days two and three)

a)



b)

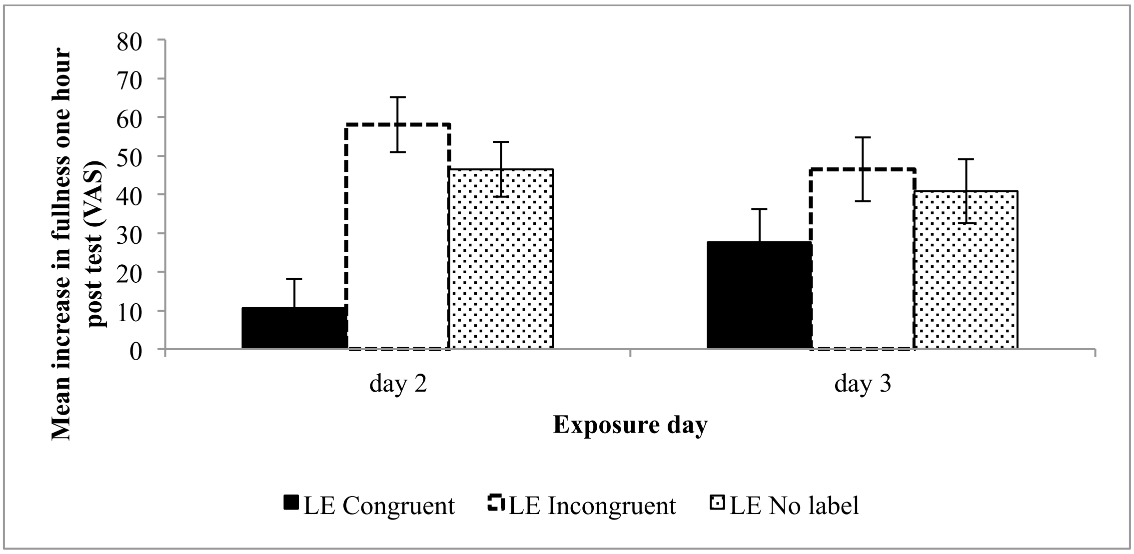


Figure 9.20: Mean (±SEM) increase in fullness one hour post test between label conditions after a) HE or b) LE breakfasts in Study Four.

In the same pattern as was demonstrated in the hunger ratings one hour post test, there remained a significant difference between labels in fullness changes one hour post test $F(2,54) = 7.28$, p = 002, but no difference between energy versions $F(1, 54) = 0.24$, $p = .627$. The incongruent label ($M = 53.6 \pm 4.5$) resulted in a significantly larger increase in fullness one hour post test than the congruent label ($M = 29.5 \pm 4.5$, $p = .001$) but was not significantly different to no label ($M = 40.3 \pm 4.5$, $p = .120$). There was also no significant difference between the congruent and no label conditions ($p = .281$).

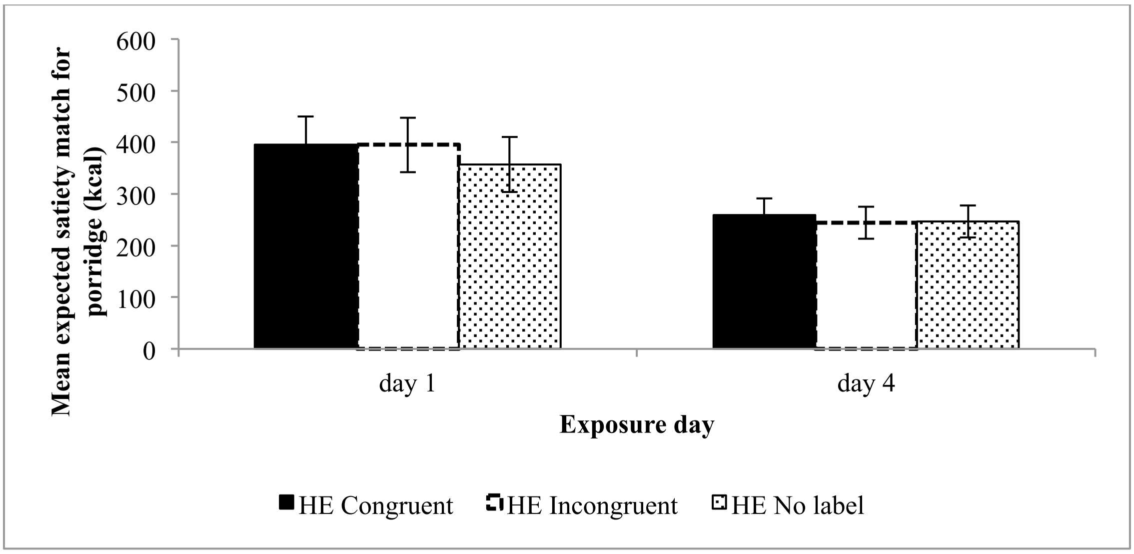
There were no significant differences in fullness change one hour post test across test days $F(1,54) = 0.002$, $p = .962$, and no significant interaction effects; energy*label, day*energy $F(1,54) = 0.004$, $p = .948$, day*label $F(2,54) = 1.75$, $p = .184$ or day*energy*label $F(2,54) = 0.83$, $p = .443$.

9.3.5 Expected satiety measures

As expectations were manipulated by the information provided, satiety expectations were measured at the first and last sessions of the study. It was of interest to see whether this expectation would change after consumption of the breakfast, and if the information provided influenced these expectations. The two comparison breakfasts (porridge and Crunchy Nut Cornflakes) were analysed separately.

9.3.5.1 Porridge

a)



b)

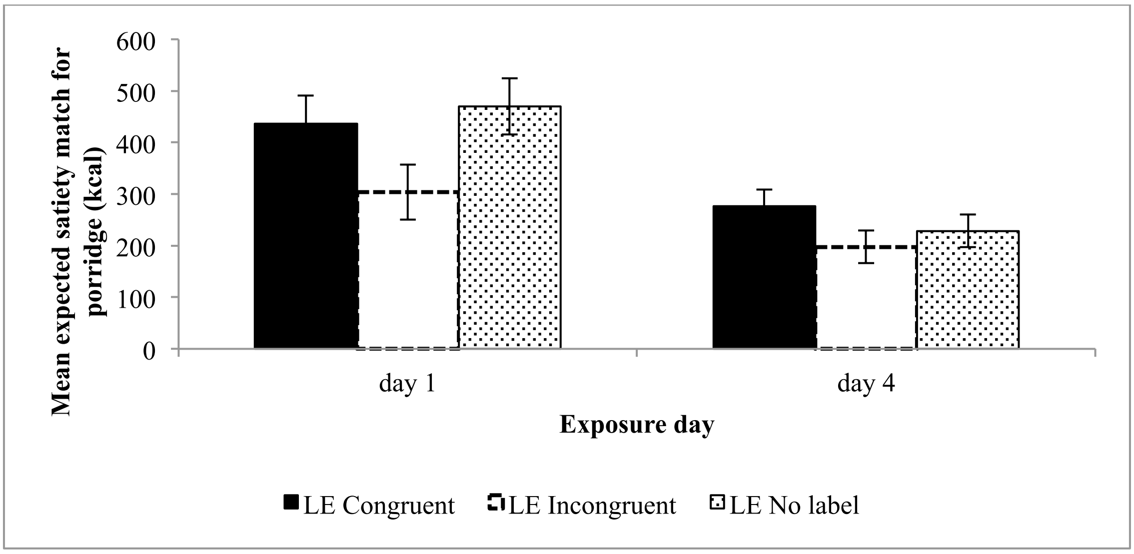


Figure 9.21: Mean (±SEM) calorie match for the porridge images after a) HE or b) LE breakfast in Study Four, with covariate of restraint included.

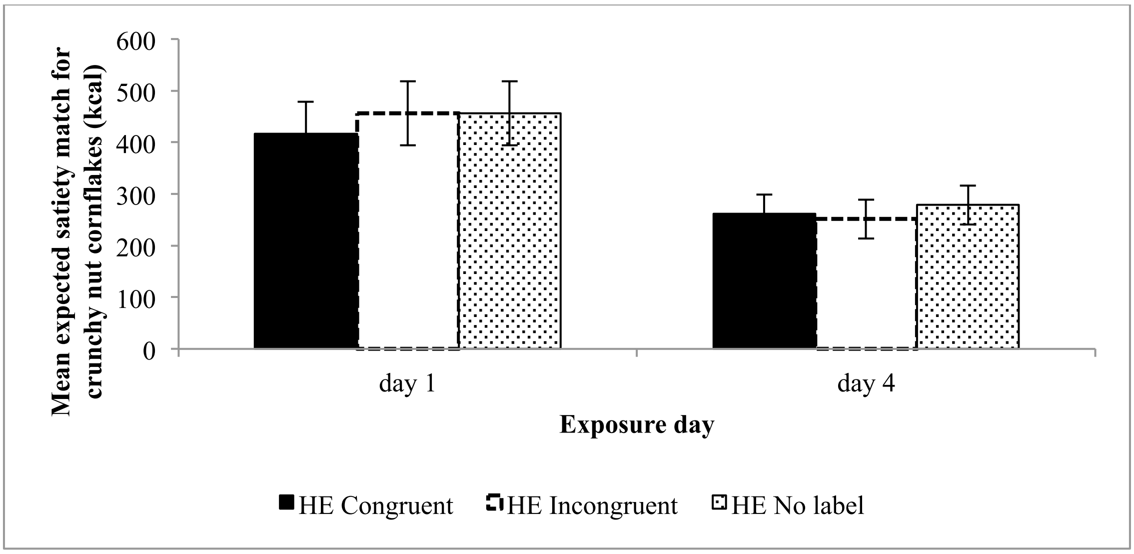
As discussed in Section 9.2.7.2, restraint was a significant covariate, $F(1,53) = 5.16$, $p = .027$, in this analysis, therefore ANCOVA statistics were reported. After controlling for restraint, there was a significant difference between days one and four on the number of calories matched for expected satiety, $F(1, 53) = 5.85$, $p = .019$. As shown in Figure 9.21, regardless of energy content or label, all conditions significantly reduced the amount of porridge that would be considered to be as satiating as the breakfast presented to them between day one ($M = 393.1 \pm 21.6$) and day four ($M = 242.2 \pm 12.7$).

There was a day*restraint interaction which was approaching significance $F(1,53) = 3.79$, $p = .057$. As restraint score increased, the number of calories used as match between porridge and breakfast also increased but this was only significant on day one ($\beta = 31.60$, $t\ (53) = 2.39$, $p = .020$) and not day four ($\beta = 11.82$, $t\ (53) = 1.52$, $p = .134$).

As is also indicated in Figure 9.21, after controlling for the effects of restraint, there were no significant effects of energy $F(1, 53) = 0.01$, $p = .940$ or label $F(2, 53) = 1.14$, $p = .328$ on the number of calories expected to match the breakfast, and no significant interaction effects: day*energy $F(1, 53) = 1.10$, $p = .299$, day*label, $F(2, 53) = 0.66$, $p = .522$, energy*label $F(2, 53) = 1.24$, $p = .298$, or day*energy*label $F(2, 53) = 2.30$, $p = .111$.

9.3.5.2 Crunchy Nut Cornflakes

a)



b)

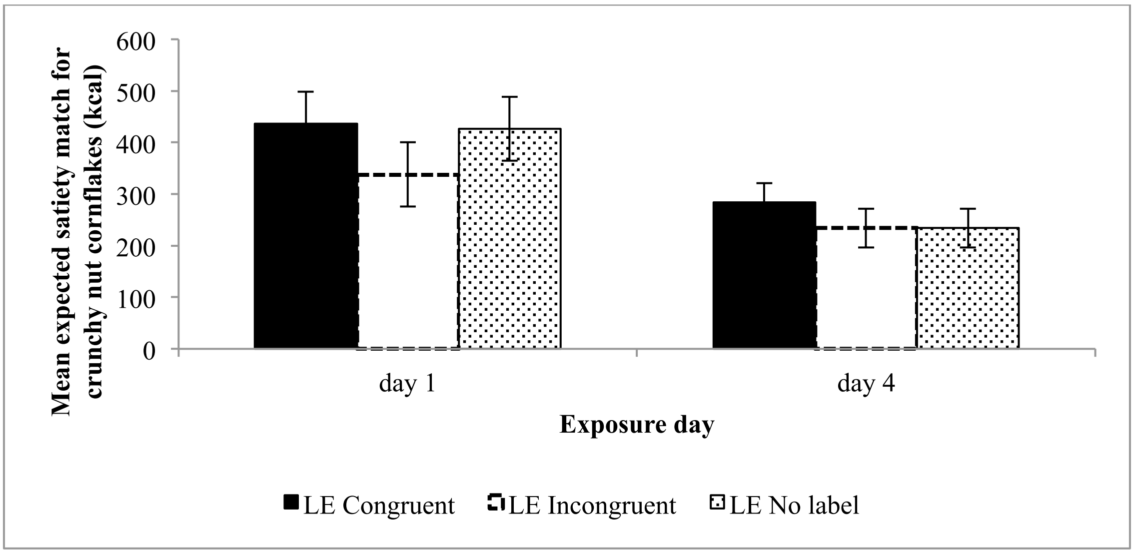


Figure 9.22: Mean (±SEM) calorie match for the crunchy nut cornflake images after a) HE or b) LE breakfast in Study Four.

The same pattern was demonstrated for the Crunchy Nut Cornflakes as with the porridge; the number of calories represented by the image to be equally as satiating as the breakfast was significantly lower on day four (M = 257.4±15.4) than on day one (M = 421.9±25.3), $F(1, 54) = 83.0, p < .001$.

There were no significant effects of energy $F(1, 54) = 0.55, p = .463$ or label $F(2, 54) = 0.27, p = .767$, and no interaction effects; energy*label $F(2, 54) = 0.47, p = .626$, day*energy $F(1, 54) = 0.67$, $p = .416$, day*label $F(2, 54) = 0.32$, $p = .724$ or day*energy*label $F(2, 54) = 1.00$, $p = .374$.

9.4 Discussion

The present study set out to explore whether providing explicit information about the energy content of yoghurt-based breakfast influenced FNL. From the literature it was predicted that consumption of the HE breakfast would result in acquired liking and increased intake, with subsequent effects on appetite ratings. It was hypothesised that providing congruent information about the breakfast would enhance these effects.

As predicted, consumption of the HE breakfast led to an acquired liking in comparison to the LE breakfast, supporting numerous studies in this area (as reviewed by Brunstrom, 2007; Yeomans, 2006b; Yeomans, 2008). This pattern was modified by information, although not in line with the predictions. Congruent information about the HE breakfast did result in a higher pleasantness at baseline, but further learning regarding the positive post-ingestive consequence did not occur; pleasantness remained stable over time. Incongruent information appeared to confuse initially, with pleasantness change appearing to reflect the labelled rather than actual calorie content, but over time this became in line with the pattern predicted by flavour nutrient learning.

Differences in intake were not significant, supporting findings from some previous research (e.g., Hogenkamp, Brunstrom, Stafleu, Mars, & De Graaf, 2012; Rolls, et al., 1988; Rolls, et al., 1989). Although differences in intake were not significant, labelling did appear to have some influence when consuming the HE breakfast; when no information was provided, intake remained higher and stable across *ad libitum* days, whereas congruent information led to a small decrease in intake and incongruent information resulted in increased intake. Intake and pleasantness changes appeared to occur independently to each other, with those in the HE no label condition demonstrating a clear increase in pleasantness but no differences in *ad libitum* intake, and those in the LE no label condition showed increased intake but no such increase in pleasantness. This finding supports some research from both animal (Mehiel & Bolles, 1988) and human (Capaldi & Privitera, 2007; Yeomans, et al., 2008b) studies. Intake on day four may also have been influenced by the expectation that had been set up by the portions during the training days as mean intake was between 240 and 320g across all conditions. It has been shown that visual cues can influence intake, often by indicating how much should be consumed, and setting up a consumption norm (Wansink, Painter, & North, 2005a) which may be relevant here. In a previous study (Wilkinson & Brunstrom, 2009), mean intake on test day was found to be nearer to typical portion sizes for chilled dairy desserts in the UK rather than at the larger amount from the fixed training sessions, and authors suggested that further exposures may be necessary for expected satiation to influence intake. It is interesting that individuals in the present study continued to consume a similar amount to the fixed consumption days rather than adjusting back to social norms, although there were no differences between energy versions. In addition, people have a tendency to eat until they clear their plate, and have often made a decision as to how much they will consume on initial presentation of a portion of food (Wansink, et al., 2005a) which could also explain the intake values on day four, as previous sessions may have helped determine the decision about how much to consume, and potentially created a barrier to asking for a refill.

In terms of appetite, hunger ratings were not sensitive to the energy differences, with all conditions reporting larger reductions in hunger on day four than day one. Fullness ratings appeared to be sensitive to the energy differences with those consuming the HE breakfast reporting a larger increase in fullness than those consuming the LE breakfast, although only on the *ad libitum* sessions. Changes per gram consumed were analysed for these sessions to provide a more theoretically meaningful interpretation and avoid the likely confound of intake on appetite changes. When these change data were explored, incongruent labelling appeared to be the most influential, and in general appeared to influence appetite ratings regardless of the energy content, with higher fullness ratings and larger reduction in hunger across session and one hour post test. Wooley and Wooley (1972) reported that appetite ratings were in line with the initial judgement made by participants regarding calorie content rather than the actual calorie content. Perhaps those consuming the LE version but informed it was HE were rating their appetite in line with the expected calorie content, whereas those consuming the HE but informed it was LE found the breakfast exceeded their expectations in terms of appetite.

Finally, with regard to expected satiety, images chosen to be as satiating as the breakfast presented were significantly lower in calories on day four compared to day one in all conditions. This suggests that as familiarity with the stimuli increased, the expected satiety was brought in line with the actual calories that were consumed. Brunstrom, Shakeshaft and Alexander (2010b) found that familiarity with a food and its expected satiety were related, with familiar foods expected to be more satiating than novel foods. A shift in expected satiety was observed in a food that was consumed less than once a year but not for a food consumed more regularly. In the present study, although novel in flavour, yoghurt itself is a familiar food experienced by individuals regularly, but perhaps not in the context of a breakfast. It is also commonly consumed in smaller portions, so this may have led to expected satiety being high before consumption. After four days of exposure to the novel flavour, learning about the relative satiating properties of the breakfast appeared to have occurred, although this was not influenced by the energy content of the breakfast consumed. The lack of energy effect over time was also shown in a recent study where expected satiation of a LE or HE soup was not adjusted after repeated consumption, although the HE was rated higher than the LE on the initial day, suggesting that sensory attributes may be playing a role (Hogenkamp, et al., 2012).

This study was the first within a FNL paradigm to investigate the influence of labelling calorie information and from these findings it is evident that explicit information about the calorie content of a yoghurt based breakfast did influence the acquired liking that would be predicted from flavour nutrient learning. Of particular interest was the effect of congruent labelling on liking for the HE yoghurt; pleasantness ratings were initially higher in this condition than in the other conditions that consumed the HE yoghurt, but as no further change was demonstrated this suggested that no further learning occurred. The initial association made between calories on the label and the flavour of the breakfast may have prevented any subsequent post-ingestive associations between flavour and energy received, and as expectations regarding the calorie content were met no further learning was necessary. Further research is needed to explore this mechanism.

An important consideration that was not addressed in the present study was the expectations that were actually generated by the labels; what expectation did the term ‘high energy’ generate, and were there differences between this and the low energy label? In future studies, a pilot study would be conducted to investigate the expectations generated by the specific labels, and also more explicit questioning at debrief to attempt to assess individuals' evaluations of the labels and expectations about the breakfast. More explicit measures of expected satiety and satiation could also be included, to assess if these expectations changed over repeated consumption, although recent research has indicated that this may not be the case (Hogenkamp, et al., 2012).

In conclusion, this study has demonstrated that manipulating expectations about the calorie content of a breakfast does influence acquired liking, with information about the correct calorie content of a HE breakfast actually blocking the predicted acquired liking over time.

Chapter 10: Study 5 - The impact of labelling calorie content and hedonic factors within a breakfast context

10.1 Introduction

Labelling can highlight hedonic, nutritional or sensory characteristics of a food and the information provided can influence an individual's expectations and perceptions of a product, as shown in Study Four and discussed in detail in Chapter 6. Study Four concentrated on nutritional labelling in terms of calorie content, and how providing this information, and whether this was accurate would impact upon acquired liking, with interesting findings regarding congruent information in a HE version. The present study aimed to expand upon this information, whilst introducing a condition that highlighted the hedonic information rather than caloric content.

Labelling can affect both sensory and hedonic evaluations of foods. Wansink, van Ittersum and Painter (2005b) found that sensory perceptions were biased by descriptive names given on a food menu; foods were rated as 'tastier', more appealing and to contain more calories, and also received more positive comments than an identical, regularly named food. On a similar note, Wansink and Park (2002) found that suggesting a product contained soy had a negative impact upon taste of a food product, although this was particularly relevant in a taste-conscious group compared to a health conscious group. Descriptive labelling therefore appears to influence how a food is perceived, and this can also affect physiological responses. In a study by Crum et al., (2011) merely labelling a preload as either 'indulgent' or 'sensible' resulted in a different ghrelin release response despite identical nutrient content, with the 'sensible' milkshake causing very little response compared to the 'indulgent'.

Information about the fat content of a food can influence both the expected and actual ratings with reduced fat products often expected to be less pleasant than regular foods. In a study by Kähkönen and Tuorila (1998), when provided with information, reduced fat sausages were expected to be less pleasant, juicy, salty and fatty than regular sausages, and in reality were rated as less salty and fatty, but with no differences in pleasantness or juiciness. Both types of sausage were expected to be more pleasant than when no information was provided. In terms of actual ratings, no differences between the sausages were observed when tasting 'blind' whereas the reduced fat were rated as less salty and less fatty than the regular sausages when information was provided. Similar findings were reported by Tuorila, Cardello and Lesher (1994), with fat free products expected to be less pleasant than regular versions, with this being reflected only in the actual ratings of cheese. This research also supported the assimilation model (based upon the principles of cognitive dissonance, Festinger, 1957), discussed in detail earlier (Section 7.5), as hedonic and sensory ratings of labelled foods were brought in line with expectations compared to ratings at baseline. It appears that the suggestion that something is reduced in fat leads to an expectation that this will not taste as good as the regular food product.

10.2 Part a)

It is clear that labelling does affect expectations and perceptions about a food, and before this could be further explored in a learning context it was important to establish what expectations were generated by the labels used in this study, and to determine if these expectations differed according to the information provided. Participants were exposed to the proposed label stimuli in the absence of food in order to investigate what expectations each label elicited. It was predicted that the HE label would be expected to be higher in calories, creamier and more filling than the LE label. The hedonic label was predicted to generate expectations of higher pleasantness and creaminess compared to the other labels.

10.2.1 Method

10.2.1.1 Participants

Ten female participants (aged 19-26, $M = 20.7 \pm 0.8$) who scored less than 7 on the TFEQ were recruited for a pilot study to rate expectations generated by three yoghurt labels. As in previous studies, participants were excluded if they had taken part in Study Four.

10.2.1.2 Labels

Two of labels that were rated in this study were identical to those used in Study Four, with one describing a high energy breakfast with the calorie content shown (330kcal), one describing a low energy breakfast with the associated calorie content (164kcal) and a new label using a hedonic description (Luxury) rather than providing information about calories. The energy labels can be seen in Figure 9.1 and the additional hedonic label is in Figure 10.1.

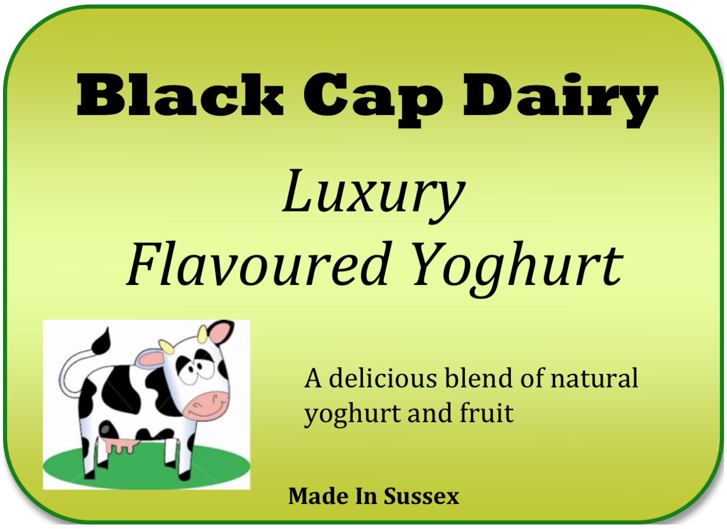


Figure 10.1: Additional hedonic label used in Study Five.

10.2.1.3 Procedure

Participants reported to the laboratory for a single 15 minute session. The labels were presented face down, with the hedonic label as ‘A’, the LE label as ‘B’ and the HE label as ‘C’. Participants were informed that they should turn over each label when asked, and that the information provided was for a 300g portion of yoghurt. The first set of questions asked ‘how many calories would you expect to consume in a 300g portion of yoghurt’ (A, B or C, order was randomised). Participants were instructed to place the label face down after answering each question. A ‘taste test’ then followed for each label, asking how (creamy, thick, filling, pleasant, fruity, sweet) they would expect the yoghurt to taste based upon the label, using the SIPM software. Finally, again randomised, participants were given a multiple choice question regarding the number of calories they would expect to consume, with the following options: 82, 164, 247, 330, 412. Upon completion, participants were reimbursed and debriefed as to the purpose of the pilot.

10.2.2 Results

Data were analysed using repeated measures ANOVA. Where sphericity could not be assumed the appropriate corrected statistics were reported (if $\varepsilon < .75$, Greenhouse Geisser, if $\varepsilon > .75$, Huynh-Feldt).

10.2.2.1 Calorie content

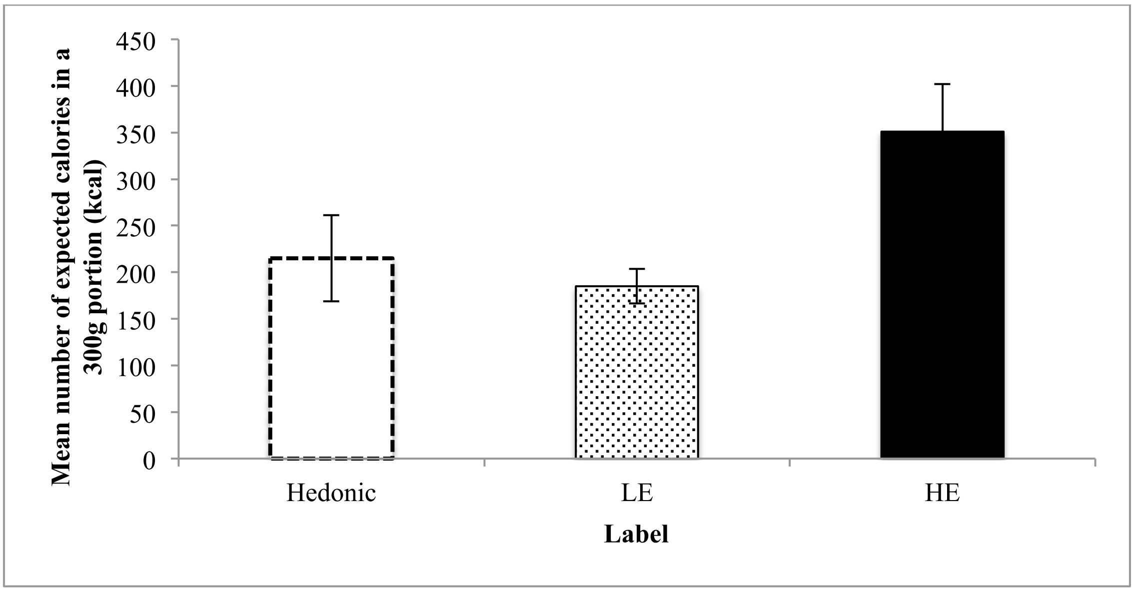


Figure 10.2: Mean (±SEM) expected calorie content generated by the labels in Part a).

When given an open question regarding calorie content, there was a significant difference in expectation between the labels $F(2,18) = 4.96$, $p = .019$, with the HE label ($M = 351.0 \pm 51.1$) expected to contain significantly more calories than the LE label ($M = 185.0 \pm 18.6$, $p = .034$). The hedonic label ($M = 215.0 \pm 46.2$) was not expected to be significantly different to either the LE ($p > .999$) or HE ($p = .251$) label. A similar pattern was observed when given multiple choice answers $F(1.21,10.90) = 19.32$, $p = .001$, with the hedonic label considered to be in the middle of the other labels. The LE label ($M = 155.8 \pm 8.2$) was expected to be significantly lower in calories than both the HE ($M = 329.9 \pm 12.3$, $p < .001$) and hedonic labels ($M = 288.2 \pm 33.2$, $p = .006$), but no significant differences between latter two labels ($p = .884$). This implied that participants were attending to the calorie information provided on the energy label, and that the hedonic label suggested a calorie content somewhere between the two.

10.2.2.2 Pleasantness

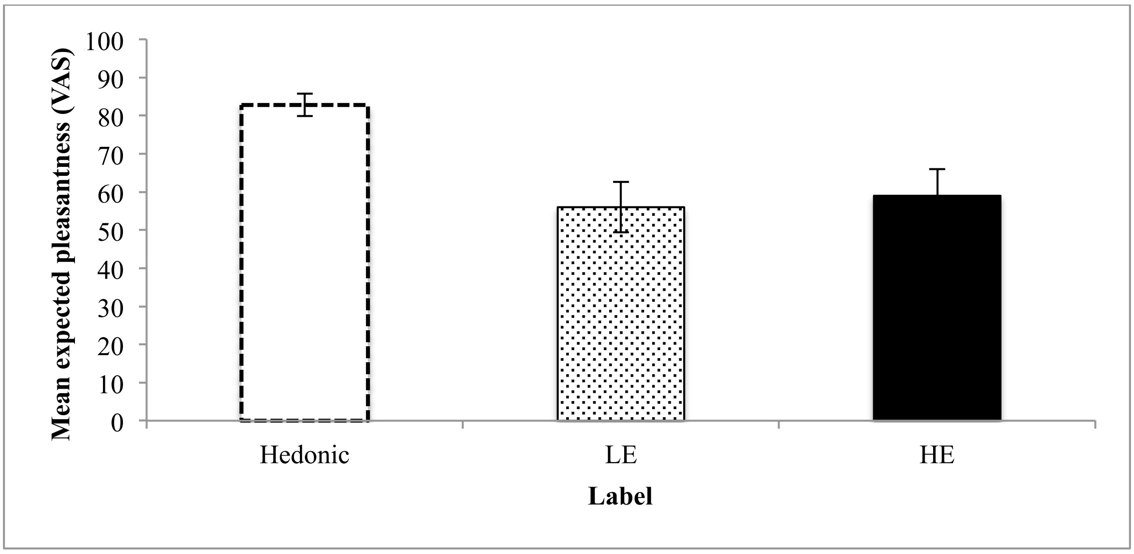


Figure 10.3: Mean (±SEM) expected pleasantness ratings based on labels in Part a).

There was a significant difference in the expected pleasantness based upon the labels $F(1.18,10.64) = 5.30$, $p = .038$. As predicted, the hedonic label ($M = 82.8 \pm 3.0$) was expected to be significantly more pleasant than both the HE ($M = 59.0 \pm 7.0$, $p = .005$) and LE ($M = 56.0 \pm 6.6$, $p = .033$) labels, whereas the energy labels were not expected to differ significantly ($p > .999$).

10.2.2.3 Creaminess

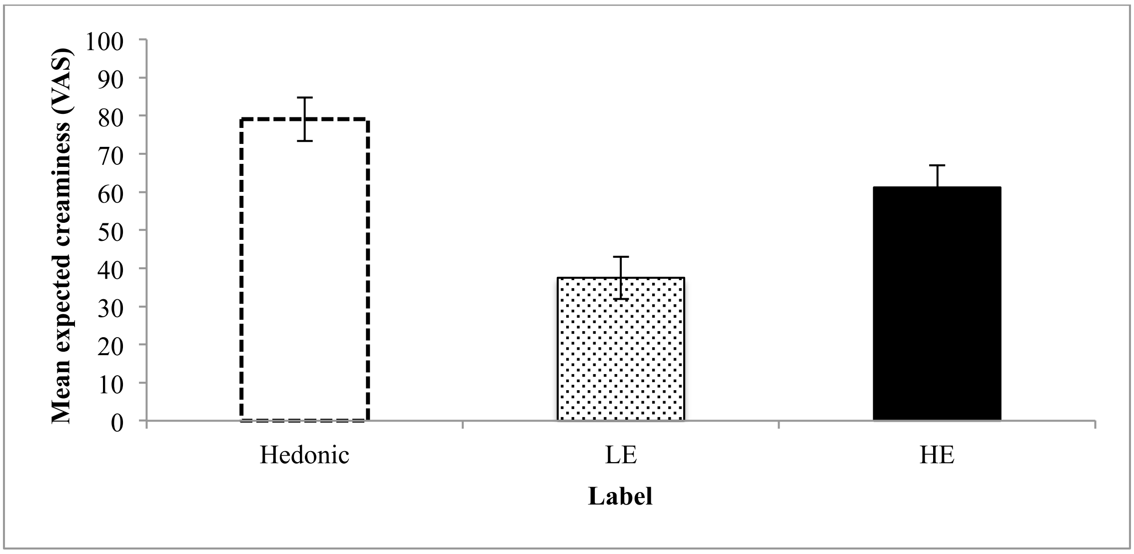


Figure 10.4: Mean (±SEM) expected creaminess generated by each label in Part a).

There was a significant difference between labels as to how creamy participants expected the yoghurt to taste $F(1.25,11.29) = 15.55$, $p = .001$. The hedonic ($M = 79.1 \pm$

5.7) label generated an expectation that the yoghurt would be significantly creamier than the LE (*M* = 37.5 ± 5.6, *p* = .003), but not the HE (*M* = 61.2 ± 5.7, *p* = .233). The HE was expected to be significantly creamier than the LE (*p* < .001).

10.2.2.4 Filling

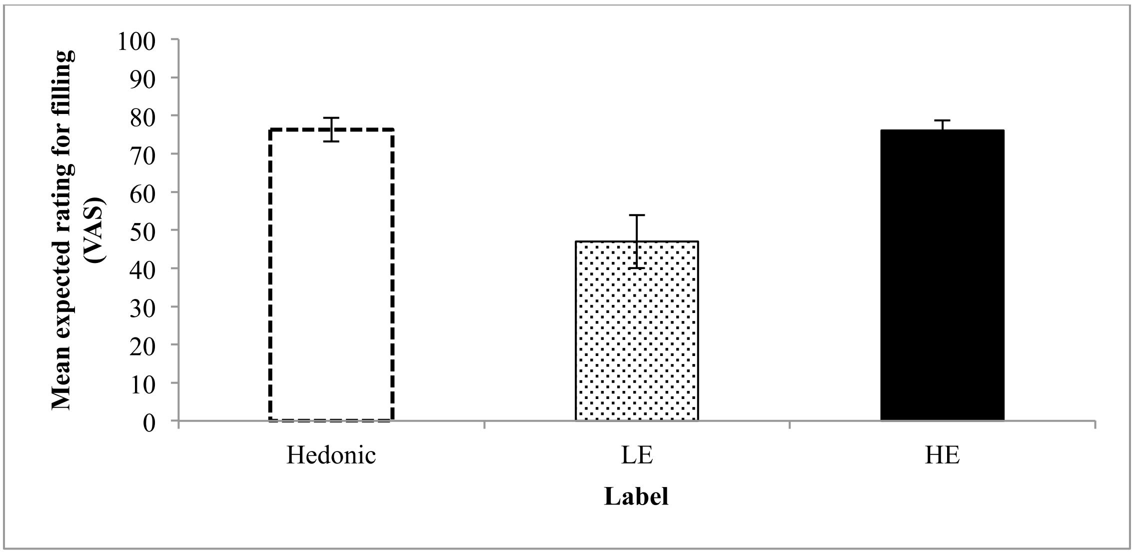


Figure 10.5: Mean (±SEM) expectation of how filling the yoghurt would be based upon label in Part a).

There was a significant difference in how filling the yoghurts were expected to be based upon the labels $F(2,18) = 11.39$, $p = .001$. The LE (*M* = 46.0 ± 7.0) was expected to be significantly less filling than both the HE (*M* = 76.1 ± 2.6, *p* = .014) and hedonic (*M* = 76.3 ± 3.1, *p* = .020) labelled yoghurts, with no differences between these two (*p* > .999).

10.2.2.5 Thickness

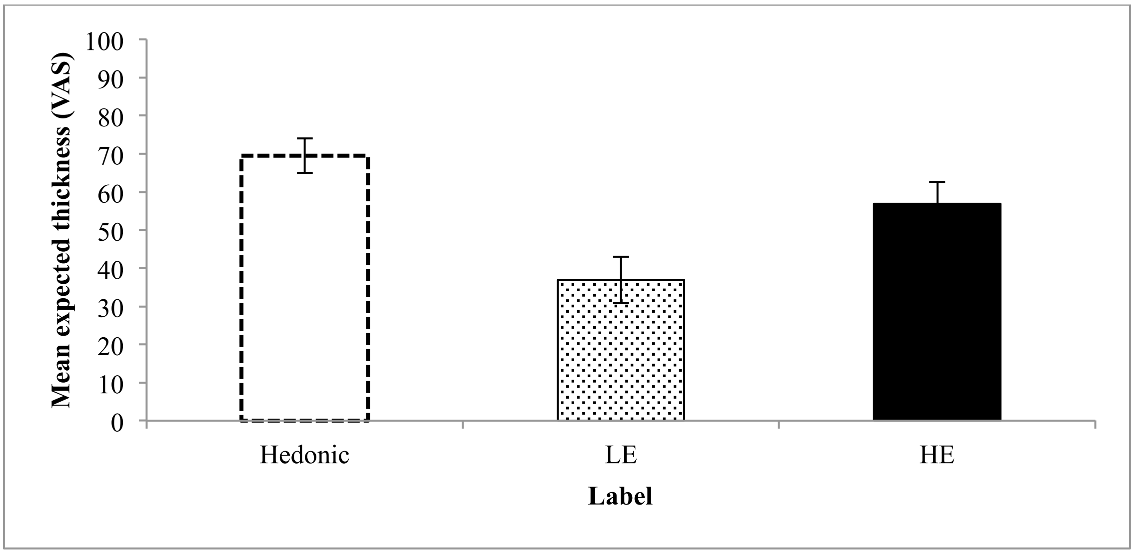


Figure 10.6: Mean (±SEM) expected thickness of the yoghurt based upon label in Part a).

There was a significant difference in expected thickness of the yoghurt $F(2,18) = 17.05$, $p < .001$. All labels differed significantly from each other, with LE ($M = 36.9 \pm 6.1$) expected to be thinner than the HE ($M = 56.9 \pm 5.8$, $p = .026$) and hedonic ($M = 69.5 \pm 4.5$, $p = .002$) labelled yoghurts, and the HE expected to be thinner than the hedonic ($p = .042$). The hedonic label successfully generated expectations of thickness.

10.2.2.6 Fruitiness

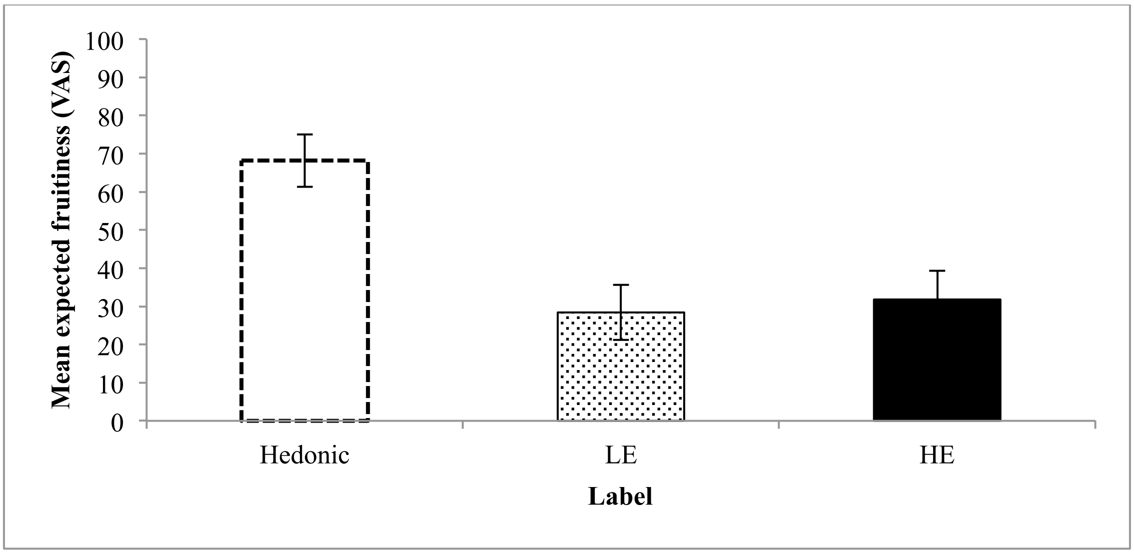


Figure 10.7: Mean (±SEM) expected fruitiness of yoghurts based upon label in Part a).

There was a significant difference in how fruity the yoghurt was expected to be based upon the label $F(2,18) = 10.06$, $p < .001$. The hedonic label ($M = 68.2 \pm 6.9$) generated higher expectations of how fruity the yoghurt would taste than both the LE ($M = 28.4 \pm 7.2$, $p = .002$) and HE ($M = 31.8 \pm 7.5$, $p = .045$) labels. There were no significant differences between the energy labelled conditions ($p > .999$).

10.2.2.7 Sweetness

Label	**Expected sweetness**
Hedonic	60.2 ± 6.3
LE	42.7 ± 8.0
HE	53.4 ± 8.2

Table 10.1: Mean (±SEM) expected sweetness rating for each label in Part a).

Finally, there were no significant differences in expected sweetness between the labels $F(2,18) = 2.16$, $p = .145$, see Table 10.1.

10.2.3 Discussion

Part a) of Study Five aimed to establish whether labels providing information about energy content elicited the expectations predicted, and what expectations were generated when a label focused upon hedonic rather than caloric factors. As predicted, the HE label did generate an expectation of higher calorie content than both other labels, and creamier, thicker and more filling than expectations from the LE label. A yoghurt with the hedonic label was expected to be more filling than the LE, and creamier, thicker, fruitier and more pleasant than a yoghurt where information about energy content was provided. In terms of calories, the hedonic label was midway between the LE and HE.

These findings show that the information provided on a label influences expectations about how a food will taste, which supports previous research in this area (Kähkönen & Tuorila, 1998; Tuorila, et al., 1994). The main part of this study will explore whether these labels influence the actual taste of the yoghurt (as seen in the study by Wansink, et al., 2005b), how this impacts upon FNL, and how expectations change as learning takes place.

10.3 Part b)

As two flavoured yoghurts would be experienced in the main study (Part c) it was important to establish that these were matched in sensory and hedonic characteristics, and that the different energy versions of each flavour were also matched.

10.3.1 Method

10.3.1.1 Participants

Nine female participants (aged 19-28, *M* =21.7 ± 0.9) scoring less than seven on the TFEQ were recruited to taste and rate eight samples of yoghurt. Participants were not allowed to have participated in Study Four, or Part a) of the present study.

10.3.1.2 Test foods

All test foods consisted of natural fat free yoghurt (Yeo Valley, UK) with different flavourings added. Flavour A consisted of mandarin flavouring (International Flavours and Fragrances), rhubarb flavouring (International Flavours and Fragrances) and vanilla extract (Nielsen-Massey Vanillas Int. NL), and Flavour B was identical to that described in Study Four: almond extract (Supercook, UK), banana flavouring (International Flavours and Fragrances) and ground nutmeg (Schwartz, UK). Yellow food colouring (Supercook, UK) was added to both flavours.

10.3.1.3 Procedure

Participants reported to the laboratory for a 15 minute session. After completing a consent form, they were presented with eight 25g samples of yoghurt labelled A-G. Each flavour and energy combination was presented twice. Computerised sensory and hedonic ratings (bitter, creamy, familiar, fruity, novel, pleasant, sour, sweet) were completed, with samples rated in a randomised order. They were instructed to take a mouthful of the sample, keep it in their mouth for five seconds and then swallow. Water was consumed between each sample. After completion of all eight samples, participants were reimbursed for their time and debriefed as to the purpose of the pilot.

10.3.2 Results

As each sample was tasted and rated twice, a mean was taken for each rating of each sample. One way repeated measures ANOVAs were conducted for each of the hedonic and sensory ratings.

	Flavour A	Flavour B	
Pleasant			$F(3,24) = 1.32, p = .261$
LE	42.1 ± 3.2	54.6 ± 6.3	
HE	49.5 ± 9.2	61.6 ± 6.2	
Novel			$F(3,24) = 0.19, p = .903$
LE	54.9 ± 4.6	59.1 ± 6.4	
HE	54.0 ± 5.9	56.2 ± 4.0	
Sweet			$F(3,24) = 1.54, p = .231$
LE	44.1 ± 5.7	50.5 ± 4.2	
HE	54.9 ± 8.3	54.9 ± 4.7	
Familiar			$F(3,24) = 0.93, p = .443$
LE	41.6 ± 4.3	38.4 ± 6.5	
HE	42.7 ± 5.2	49.1 ± 4.5	
Creamy			$F(3,24) = 0.31, p = .820$
LE	56.4 ± 4.6	63.6 ± 4.4	
HE	58.2 ± 5.6	57.9 ± 5.5	
Fruity			$F(3,24) = 2.50, p = .084$
LE	42.9 ± 4.5	52.4 ± 4.7	
HE	62.4 ± 4.8	52.1 ± 6.8	

Table 10.2: Mean (±SEM) sensory and hedonic ratings of the two flavour and energy combinations of yoghurt tasted in Part b).

10.3.3 Conclusion

As seen in Table 10.2, the yoghurts were appropriately matched for use in the main study. As there were no differences between the LE and HE versions, the LE version could be used for the taste test for all participants.

10.4 Part c)

In Parts a) and b) it was established that the labels developed as stimuli for this study generated the expectations that they were designed to. Also, samples were sufficiently matched between flavours and energy versions to be used in the main study. Part c) aimed to further investigate the findings from Study Four; particularly the unexpected finding that congruent labelling of a HE breakfast appeared to prevent the predicted acquired liking. In order to explore this finding, a hedonic label condition for HE was introduced to manipulate expectations using semantic content rather than focusing on energy content. As shown in Part a), this label generated an expectation that the yoghurt would be creamier, thicker, fruitier and more pleasant than the other labels, but was midway in terms of calories. Therefore, a comparison between HE labelled with the calories, and HE labelled in descriptive terms could be made, as perhaps the expectation was that the calorie labelled HE breakfast would be over-satiating, preventing an increase in pleasantness. It would be interesting to see if any form of positive labelling would also result in this effect, or whether this was specific to energy labelling.

Using a mixed design study, participants completed an initial taste test that established their eligibility to continue into the main study, and which also served to determine which stimuli would be used for the exposure breakfast. The initial session, and subsequent three sessions, continued with fixed consumption of a yoghurt breakfast, with participants consuming either a HE or LE version; three HE conditions received either no label, a congruent calorie label or a hedonic label, and two LE conditions either no label or a congruent calorie label. A final session repeated the original taste test to see if ratings of exposure and non-exposure flavours differed. It was predicted that those consuming the HE versions would demonstrate an acquired liking for the breakfast over time in comparison to those consuming the LE version, which would be enhanced for the hedonic condition. However, due to the findings from Study Four, those in the HE congruent condition were predicted to show relatively stable pleasantness ratings with effects of FNL prevented by the flavour expectations generated by labelling. In terms of the taste test, ratings were predicted to increase for the exposure versus non-exposure flavour. Labels were predicted to generate expectations in the same direction as demonstrated in the pilot study; with those in the hedonic label condition predicted to expect the breakfast to be more pleasant than the other conditions, and the LE to be less filling. These ratings would be predicted to change over time in line with their actual energy content.

10.4.1 Method

10.4.1.1 Design

Participants were randomly assigned to one of five conditions, which differed in energy content of the breakfast and the label presented. Participants in two conditions consumed a LE yoghurt breakfast (164kcal), with those in one condition receiving no label and the other a label with the correct calorie content of the breakfast. Those in the other three conditions consumed a HE version of the breakfast (330kcal), with one condition receiving no label, one a label with correct calorie content and the final one with a hedonic (luxury) label.

10.4.1.2 Methodological alterations compared to Study Four

Incongruent labelling was not included in this study as the focus of this study was to explore the blocking effect demonstrated in Study Four and it was decided that the addition of the hedonic label condition allowed exploration of this finding whilst remaining within a reasonable scope regarding recruitment and design. Additionally, a taste test was introduced into this study to ensure that participants were within an optimal range for acquired liking or disliking and to provide a comparison flavour to the exposure flavour, so some form of preference could be explored. Expected satiety measures were not included, as in Study Four it was merely found that there was a general decrease in calorie match, but this was across all conditions. Instead, explicit questions about the expectations of how filling the breakfast itself would be were included, to see if these expectations changed as a result of repeated consumption. Finally, as there were only small patterns of change in intake regarding the label and energy content of the breakfast, it was decided that all sessions would involve fixed consumption. This also allowed a clearer picture in terms of exposure to the breakfast, with sufficient consumption across all test days to maximise exposure to flavour and nutrient associations for those consuming the HE breakfast.

10.4.1.3 Participants

Sixty female participants, aged 18-26 (M = 20.7 ± 0.3) with a mean BMI of 22.2 ± 0.4 were recruited, mainly from the Psychology subject pool and course credits database. As with the previous studies, all participants scored less than 7 on the TFEQ restraint scale (Stunkard & Messick, 1985) and met the eligibility criteria outlined in Section 2.4.1. Additionally, all participants completed an initial taste test, which determined their eligibility for the full study (see Section 10.4.1.6.1). The demographic information for each condition is displayed in Table 10.3. There were no significant differences between conditions in BMI, $F(4,55) = 0.66$, $p = .622$ or restraint score, $F(4, 55) = 0.98$, $p = .426$. Homogeneity of variance was violated for age, therefore Brown-Forsythe and Welch statistics were reported; there were no significant differences between conditions in age $F(4, 47.02) = 0.72$, $p = .584$ and $F(4, 27.2) = 0.67$, $p = .620$.

Condition	Age	BMI	TFEQ-R
LE Control	20.3 ± 0.6	21.2 ± 1.2	3.3 ± 0.5
HE Control	21.3 ± 0.9	21.9 ± 1.3	3.8 ± 0.5
LE Congruent	20.0 ± 0.5	23.5 ± 0.9	4.1 ± 0.5
HE Congruent	20.6 ± 0.7	22.2 ± 0.7	2.8 ± 0.5
HE Hedonic	21.5 ± 0.9	22.3 ± 0.4	3.1 ± 0.6

Table 10.3: Mean (±SEM) age, BMI and TFEQ-R score for each condition in Study Five.

10.4.1.4 Test foods

Two different flavoured yoghurts were developed for the taste test (days one and five), and a pilot study was conducted to confirm that the flavours were matched in sensory characteristics and pleasantness, and between high and low energy version (see Part b). Table 10.5 shows the different flavours used and 20g servings were presented in the taste tests on days one and five. Bramley apples were stewed in large batches in a small amount of water, blended and frozen. Each day, samples were defrosted, and mixed into the yoghurt portion. Maltodextrin (Cargill) was added in the HE versions, which added some sweetness, therefore more aspartame was added to the LE version to match it. The composition of the yoghurt portions were the same as in Study Four and reported in Table 10.4.

HE	(g)	**KCALS**
Yoghurt	206	119.5
Maltodextrin	51	193.8
Aspartame	0.02	n/a
Apple	43	15.5
TOTAL	**300**	**328.8**
LE		
Yoghurt	257	149.1
Aspartame	0.05	n/a
Apple	43	15.5
TOTAL	**300**	**164.5**

Table 10.4: composition of each portion of yoghurt used in Study Five.

Flavour A	Flavour B
1 drop mandarin	2g nutmeg
2 drops rhubarb	16 drops almond extract
3 drops vanilla extract	2 drops banana
2 (HE) or 3(LE) drops yellow colouring	2 (HE) or 3(LE) drops yellow colouring

Table 10.5: Flavour composition of each portion of yoghurt in Study Five.

10.4.1.5 Labels

The labels used in this study are discussed in Section 10.2.1.2. One label provided a hedonic description of the yoghurt (luxury) and the other two provided caloric information about the LE and HE versions. The labels are shown in the previous Figures 9.1 and 10.1.

10.4.1.6 Procedure

Participants reported to the laboratory at a time between 08.15 and 10.00 on five mornings (two or three a week), having consumed only water from 23.00 the night before. Sessions one to four involved a fixed consumption (300g) of breakfast.

10.4.1.6.1 Day one

Upon arrival at the laboratory, participants completed a computerised set of mood and appetite ratings using the SIPM software (hungry, thirsty, full, lively, clear-headed, tired, nauseous, energetic, headachy, drowsy, calm, desire to eat, amount they could eat) and were then presented with a 20g portion of each flavour of the LE versions of the yoghurt, labelled A and B. Flavours were tasted in a random order, and computerised ratings were completed (pleasant, creamy, novel, sweet, fruity, filling, familiar, thick) for each. At this point, the experimenter reviewed the pleasantness ratings of each sample; at least one flavour had to have been rated pleasantness between 30 and 70 on the 100 point pleasantness scale in order to progress onto the next part of the study. This then determined which flavour was used for that participant for the duration of the study, and if both flavours met this criterion, the one rated closest to 50 was selected. If neither flavour met this criterion, participants could not participate further and were reimbursed for their time and debriefed as to the nature of the taste test. This criterion of pleasantness was put into practice in order to control for ceiling and floor effects, which could limit the extent to which effects of learning would be detected, and thereby to allow a substantial change in pleasantness in either direction to be detected across the full study.

Those who progressed to the next part of the study were then asked to complete the set of mood and appetite ratings again, and were then presented with a 300g serving of the yoghurt breakfast (in the selected flavour), along with the appropriate label (or no label, depending on condition). At this stage, participants were asked to rate how many calories they could expect to consume in the portion presented, and how filling and pleasant they expected the breakfast to be. They then consumed the full portion of yoghurt and completed the mood ratings again. A paper version of the mood scales was given to participants to be completed one hour after leaving the laboratory, with intake restricted to only water during this time (see Appendix 7 for materials).

10.4.1.6.2 Days two-four

Upon arrival at the laboratory, the computerised mood and appetite ratings were completed, and the yoghurt and label presented. Again, participants completed the expectation ratings, and then completed a taste test before consuming the entire portion of yoghurt. In the same procedure as day one, mood and appetite ratings were completed after consumption and one hour post test.

10.4.1.6.3 Day five

The final day consisted of a taste test with the same procedure as day one. A set of debrief questions were then completed and weight and height recorded. Participants were debriefed and reimbursed for their time.

10.4.1.7 Data analysis

10.4.1.7.1 Preliminary analysis

Normality was examined using KS tests, boxplots and histograms. The majority of variables were normally distributed within conditions, with non-significant KS tests, and no variable was non-normal in multiple conditions. Boxplots revealed two significant outliers for pleasantness on day three, and a few outliers throughout appetite ratings but none that were repeatedly shown to be significant. Therefore, all data were included in the analyses.

10.4.1.7.2 Analysis

One way independent ANOVAs were conducted to explore baseline group, actual and expected pleasantness, and appetite ratings, and expectation data. Two way mixed ANOVAs were used to analyse absolute, expected and change from baseline pleasantness ratings over time, discrepancies between expected and actual pleasantness, and changes in appetite ratings and expectations. A three way mixed ANOVA was conducted on exposure vs non-exposure flavour samples between conditions and over time. Where sphericity could not be assumed, the appropriate statistics were reported ($\varepsilon < .75$ Greenhouse Geisser, $\varepsilon > .75$ Huynh-Feldt).

10.4.2 Results

10.4.2.1 Pleasantness

As in previous studies, in order to assess acquired liking, pleasantness changes over time were analysed. First, it was established that there were no baseline differences in pleasantness between conditions; as homogeneity of variance was violated, Brown-Forsythe $F(4,46.91) = 0.71$, $p = .592$ and Welch $F(4,27.02) = 0.77$, $p = .555$ statistics were reported. Means are shown in Table 10.6.

Condition	Baseline pleasantness rating
LE Control	55.8 ± 2.0
HE Control	51.0 ± 3.7
LE Congruent	51.8 ± 3.4
HE Congruent	55.3 ± 2.5
HE Hedonic	49.8 ± 3.9

Table 10.6: Mean (±SEM) baseline pleasantness ratings between conditions in Study Five.

10.4.2.1.1 Pleasantness ratings across days

A number of predictions were made regarding the influence of the different label conditions. An acquired liking would be expected in the HE control group in comparison to the LE control group, in line with previous research and the finding from Study Four. When calorie information was provided, very little change in liking was predicted based upon the findings from Study Four. Finally, based upon findings from the pilot study, the hedonic label was expected to enhance the acquired liking effect predicted in the HE control group.

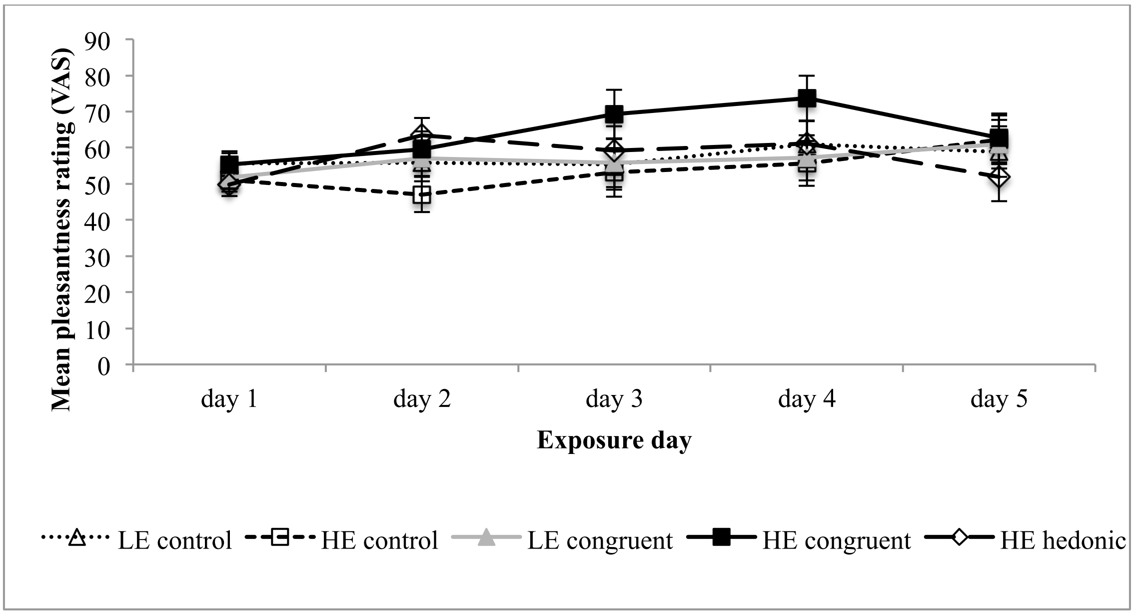


Figure 10.8: Mean (±SEM) pleasantness ratings across the five experimental sessions in Study Five.

A two way mixed ANOVA (day x condition) revealed no significant differences in pleasantness between conditions $F(4,54) = 0.72$, $p = .587$. There was a significant main effect of day $F(3.92,211.40) = 3.63$, $p = .007$, with ratings on day four ($M = 61.7 \pm 2.8$) significantly higher than those on day one ($M = 52.7 \pm 1.5$, $p = .015$) with no other differences between days. There was no significant day*condition interaction $F(15.66,211.40) = 1.31$, $p = .193$.

As in Study Four, the critical tests were the within-subjects linear contrasts, as liking was predicted to change as a function of time. Contrasts revealed there was a significant linear effect of day $F(1, 54) = 7.17$, $p = .010$, but no day*condition interaction $F(4, 54) = 0.62$, $p = .650$.

The pattern of the data contradicted the hypothesis, as those in the HE control condition did not appear to demonstrate the predicted acquired liking in comparison to the other conditions, and contrary to the findings in Study Four those in the HE congruent condition showed a trend for the pattern of acquired liking that would be predicted by

FNL. The hedonic label did not appear to enhance liking, with ratings over time remaining fairly stable (Figure 10.8).

10.4.2.1.2 Change from baseline pleasantness data

As predictions were based on changes over time, change from baseline ratings were also analysed, by deducting pleasantness rating on day one (baseline) from pleasantness ratings on subsequent test days. As discussed in Section 10.4.2.1, those in the HE control condition were expected to show a large increase from baseline pleasantness, as were those in the hedonic label condition. Very small changes in pleasantness were predicted in the LE conditions, and in the HE congruent condition.

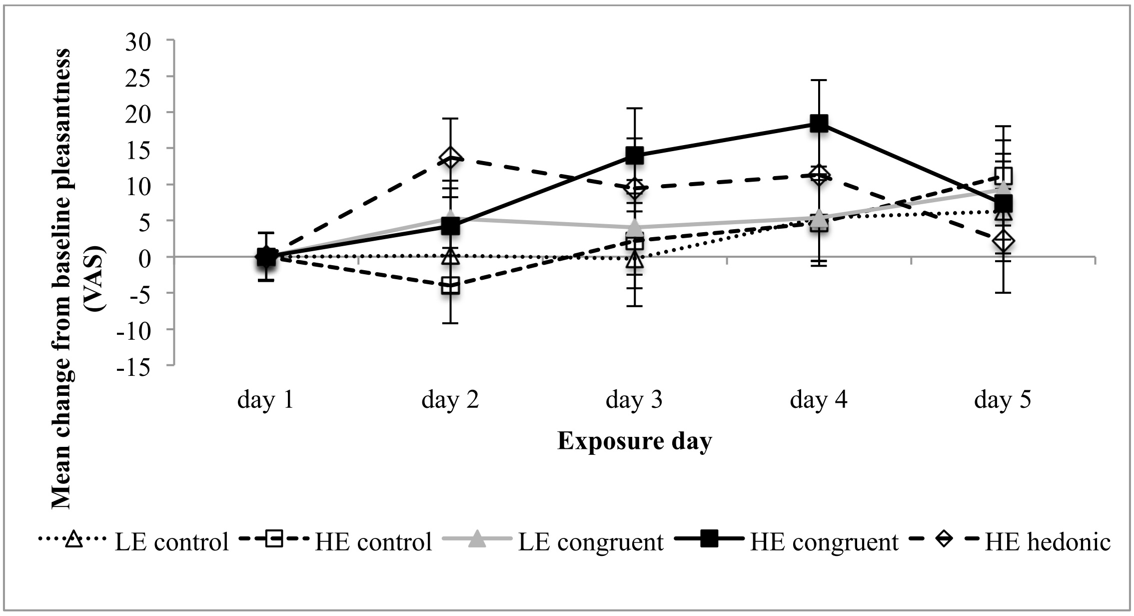


Figure 10.9: Mean (±SEM) change from baseline pleasantness between conditions in Study Five.

There were no significant differences in change from baseline pleasantness across days $F(3,162) = 1.69$, $p = .170$ or conditions $F(4,54) = 0.47$, $p = .754$. There was a significant day*condition interaction $F(12, 162) = 1.80$, $p = .052$. A Bonferroni corrected repeated measures ANOVA revealed these differences were not significant for any condition across time, and the same was demonstrated when each time point was analysed using Bonferroni corrected one-way independent ANOVAs.

When linear contrasts were examined, day was non-significant $F(1,54) = 2.31$, $p = .134$ but day*condition was significant $F(4,54) = 2.99$, $p = .026$.

It can be seen in Figure 10.9 that those who consumed the LE breakfast showed very small changes from baseline pleasantness. Both HE control and congruent conditions tended to show some increases from baseline pleasantness, with those in the congruent condition showing a decrease on the final session. The hedonic label showed some increase in pleasantness for the initial sessions but remained relatively stable over time.

10.4.2.1.3 Expected pleasantness

Based upon the pilot study findings, it was predicted that conditions would differ significantly in the rated expected pleasantness on day one. Expected pleasantness was predicted to be higher in the HE hedonic condition compared to all other conditions.

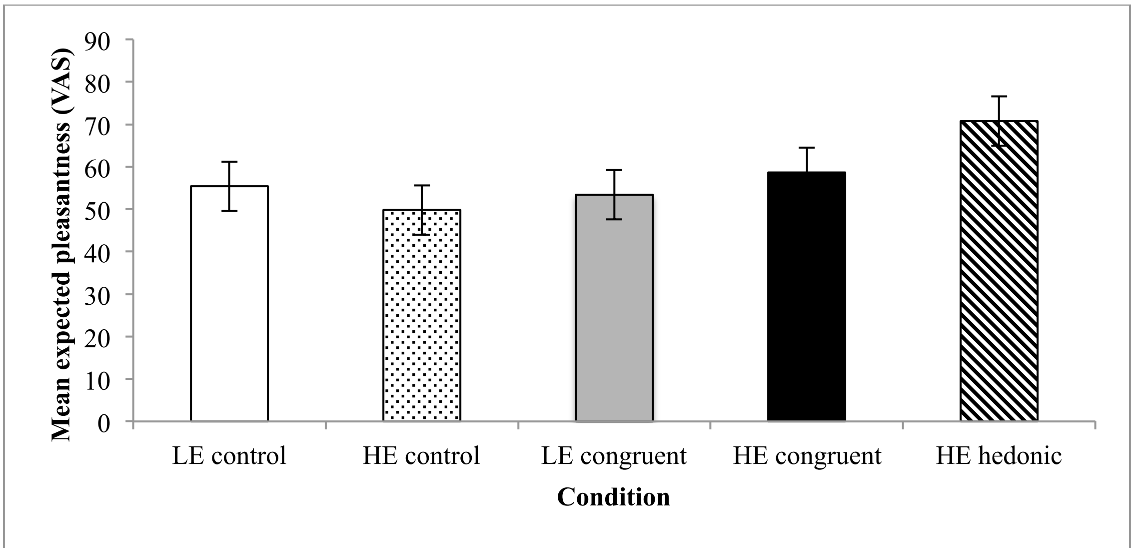


Figure 10.10: Mean (±SEM) expected pleasantness of the yoghurt on day one of Study Five.

A one way ANOVA found no significant differences between conditions, $F(4, 55) = 1.89$, $p = .126$. To test the directional hypothesis mentioned above (and hinted at in Figure 51), contrasts were interpreted, with all conditions tested against the hedonic label condition. Those who were provided with the hedonic label ($M = 70.8 \pm 5.8$) expected the yoghurt to be significantly more pleasant than those with the HE control ($M = 49.8 \pm 5.8$, $p = .014$) and LE congruent ($M = 53.4 \pm 5.8$, $p = .040$) labels. The difference between the hedonic and the LE control ($M = 55.4 \pm 5.8$, $p = .068$) was approaching significance, and there was no significant difference to the HE congruent ($M = 58.7 \pm 5.8$, $p = .149$).

Expected pleasantness ratings over time were then analysed to investigate how experience with the label and energy content influenced these expectations. Based upon the principles of flavour nutrient learning, it was predicted that expected pleasantness would increase in the HE control condition, and remain high in the HE congruent and HE hedonic conditions, with perhaps a decrease in the LE conditions.

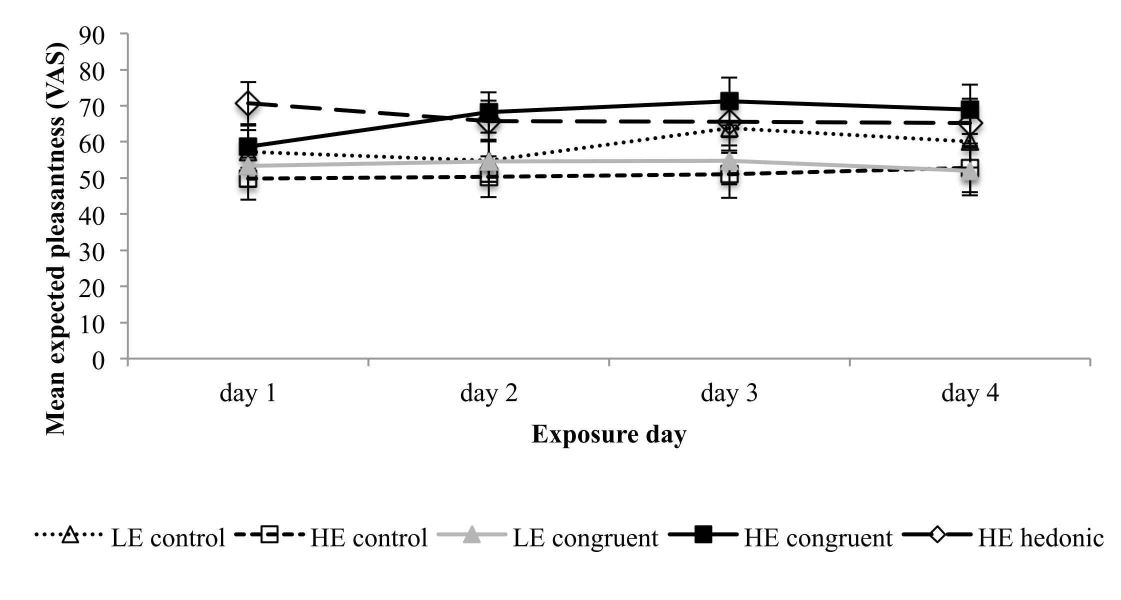


Figure 10.11: Mean (±SEM) expected pleasantness ratings across sessions in Study Five.

There were no significant differences in expectations across the days $F(2.06,111.09) = 0.80$, $p = .455$, between conditions $F(4,54) = 1.82$, $p = .139$ or day*condition interaction $F(8.23,111.09) = 0.88$, $p = .537$. As can be seen in Figure 10.11, the HE congruent condition increased in expected pleasantness, and the HE hedonic remained fairly high as predicted. Unexpectedly, the HE control demonstrated little change across the sessions whereas the LE control showed a slight increase in the middle of the exposure days.

10.4.2.1.4 Differences between expected and actual pleasantness

It could be that there was a discrepancy between expected and actual pleasantness, therefore a difference score was calculated and compared between conditions.

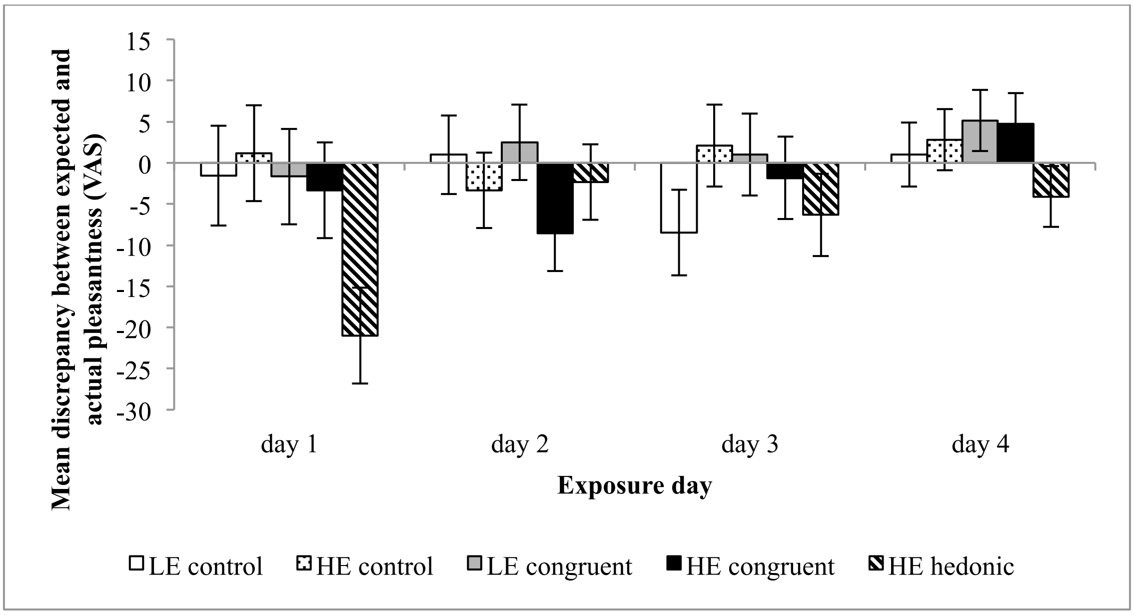


Figure 10.12: Mean (±SEM) difference between expected and actual pleasantness ratings in Study Five.

The difference between expected and actual pleasantness was significantly different between the days $F(3,162) = 2.78$, $p = .043$, and within subjects contrast showed this was a linear difference $F(1,54) = 6.48$, $p = .014$. Bonferroni post hoc tests showed this significant difference was between days one and four; the negative difference between expected and actual pleasantness on day one ($M = -5.3 \pm 2.6$), was significantly different to the positive difference on day four ($M = 1.9 \pm 1.7$, $p = .030$). There were no significant differences between conditions $F(4,54) = 1.35$, $p = .262$ and no significant day*condition interaction $F(12,162) = 1.47$, $p = .140$. However, as Figure 10.12 shows, there was a substantially larger discrepancy between expected and actual pleasantness for those with the hedonic label, the yoghurt was much less pleasant ($M = -21.0 \pm 5.8$) on day one than on any of the other days, and compared to all of the other conditions. By day four, all except the hedonic condition rated the yoghurt as slightly more pleasant than expected (with those in the congruent conditions showing the largest values), with the hedonic condition rating the yoghurt as less pleasant than expected but this difference was smaller than on day one.

10.4.2.2 Appetite ratings: Hunger

Before change in hunger over session was assessed, baseline hunger ratings for each day were analysed between conditions to check for differences. There were no significant differences on days one $F(4,55) = 0.78$, $p = .542$, two $F(4,55) = 1.66$, $p = .173$, three $F(4,55) = 0.71$, $p = .590$ or four $F(4,54) = 0.21$, $p = .929$ (see Table 10.7 for the descriptives).

Condition	**Day one**	**Day two**	**Day three**	**Day four**
LE Control	66.8 ± 4.7	67.0 ± 4.3	73.3 ± 3.8	68.8 ± 3.6
HE Control	59.6 ± 4.0	55.6 ± 7.7	60.3 ± 6.1	65.4 ± 6.9
LE Congruent	56.8 ± 8.1	57.8 ± 5.9	64.7 ± 7.3	64.9 ± 7.0
HE Congruent	64.1 ± 6.0	73.1 ± 5.5	69.8 ± 4.1	69.0 ± 4.2
HE Hedonic	52.1 ± 8.7	53.4 ± 8.2	62.4 ± 9.0	62.7 ± 6.3

Table 10.7: Mean (±SEM) hunger ratings at the start of each session in Study Five.

10.4.2.2.1 Hunger change over session

Hunger rating at the end of the session was subtracted from the rating at the start to calculate hunger change over the session. It was predicted that those consuming the HE yoghurt would show a greater reduction in hunger particularly in the later sessions, as the flavour became associated with the energy content.

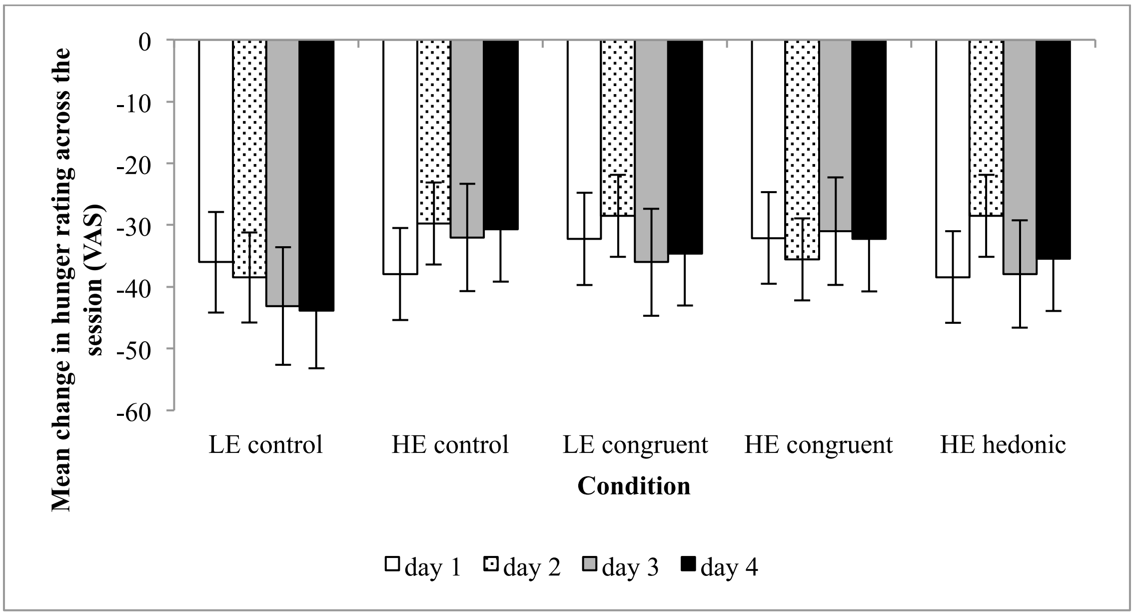


Figure 10.13: Mean (±SEM) hunger change across each session between conditions in Study Five.

There was no significant effect of day $F(2.84,150.25) = 0.43$, $p = .721$, condition $F(4,53) = 0.26$, $p = .901$ or day*condition interaction $F(11.34,150.25) = 0.35$, $p = .975$. As can be seen in Figure 10.13, all conditions reported reduced hunger across each session to relatively stable level across days. Contrary to predictions, those who consumed the LE breakfast with no label showed slightly larger reductions in hunger as the sessions progressed rather than those consuming the HE versions.

10.4.2.2.2 One hour post test

Change data from baseline hunger to one-hour post test were also calculated. Perhaps those who consumed the HE breakfasts would have shown a larger reduction in hunger one-hour post test to those who consumed the LE breakfasts.

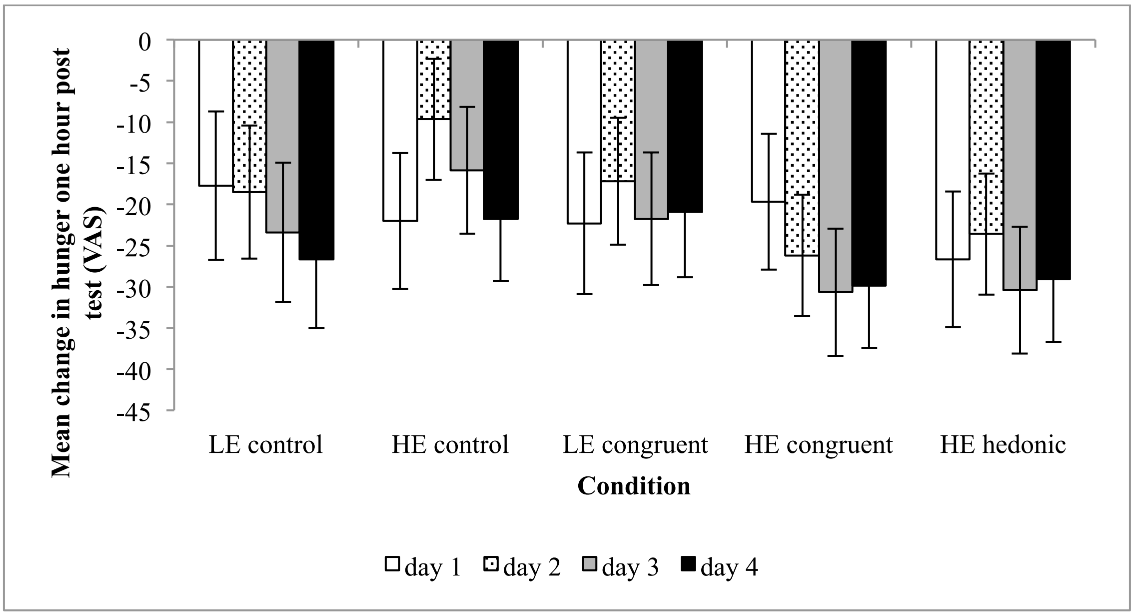


Figure 10.14: Mean (±SEM) change in hunger one hour post test across days and between conditions in Study Five.

There was no significant effect of day $F(3,156) = 1.43$, $p = .237$ or difference between conditions $F(4,52) = 0.47$, $p = .756$ for hunger change one hour post test. There was also no significant day*condition interaction $F(12,156) = 0.40$, $p = .961$. From Figure 10.14, it can be seen that hunger reduction appeared to be larger in the HE labelled conditions on days three and four, in comparison to the other conditions, suggesting that perhaps the information provided could have had an impact on subjective hunger.

10.4.2.3 Appetite: Fullness ratings

As with the hunger ratings, fullness ratings were initially analysed to check for baseline differences at the beginning of each session. Homogeneity of variance was violated for days one and three, therefore Welch and Brown-Forsythe statistics were reported. There were no baseline fullness differences between conditions on any days: day one; $F(4, 7.15) = 0.70$, $p = .597$, $F(4,47.15) = 0.88$, $p = .482$, day two; $F(4, 55) = 0.87$, $p = .487$, day three; $F(4,27.03) = 1.75$, $p = .168$, $F(4,42.0) = 1.35$, $p = .269$, or day four; $F(4, 54) = 0.73$, $p = .578$. Table 10.8 shows the descriptives.

Condition	Day 1	Day 2	Day 3	Day 4
LE Control	22.0 ± 5.1	17.8 ± 3.8	15.3 ± 3.8	24.3 ± 4.3
HE Control	19.9 ± 3.5	30.5 ± 7.0	30.0 ± 7.7	19.2 ± 4.5
LE Congruent	30.4 ± 6.2	28.9 ± 6.8	23.7 ± 5.1	18.6 ± 4.2
HE Congruent	18.2 ± 3.9	21.8 ± 4.4	26.2 ± 4.0	27.3 ± 3.3
HE Hedonic	22.8 ± 5.7	26.5 ± 5.3	32.6 ± 7.1	24.2 ± 5.3

Table 10.8: Mean (±SEM) fullness ratings at the start of each session in Study Five.

10.4.2.3.1 Fullness change over session

Change data were then analysed, with the prediction that those consuming the HE versions would report a higher increased fullness than those consuming the LE version, with information enhancing this effect.

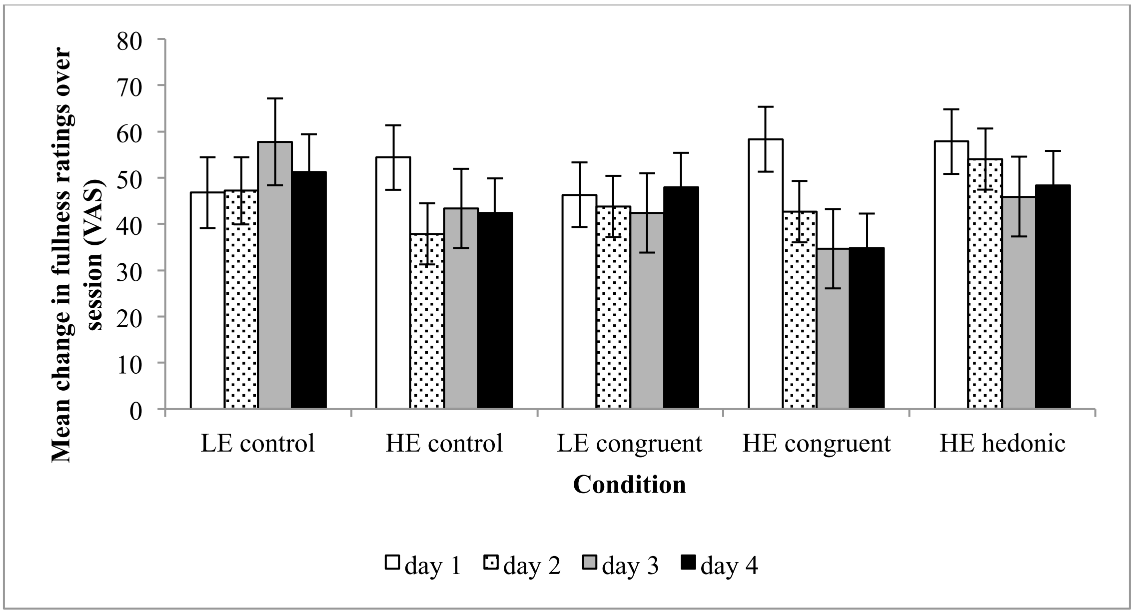


Figure 10.15: Mean (±SEM) change in fullness ratings across the session between conditions in Study Five.

There was no significant difference between days $F(2.97,159.52) = 2.36$, $p = .074$, but the linear within subjects contrast was borderline significant $F(1,53) = 3.97$, $p = .052$. There was no significant difference in fullness change between conditions $F(4,53) = 0.47$, $p = .759$, and no interaction effect $F(11.89,157.52) = 1.21$, $p = .283$, although again, there was a trend in the linear within subjects contrast $F(4,53) = 2.16$, $p = .087$. Fullness change across sessions in general appeared to be higher on day one than the other days, perhaps reflecting a change in expectation after the initial exposure. From Figure 10.15, it can be seen that fullness change seemed to be smaller in the HE congruent condition than the other conditions after day one and fullness appeared to increase the most in the HE hedonic and LE control conditions over time.

10.4.2.3.2 One-hour post test

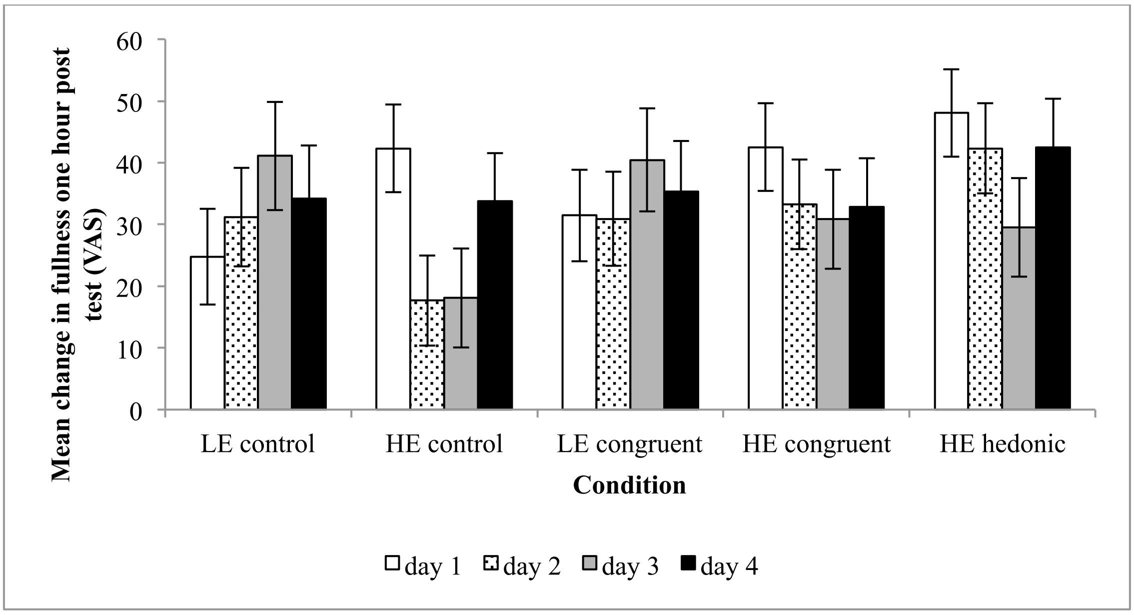


Figure 10.16: Mean (±SEM) change in fullness one-hour post test across days and conditions in Study Five.

As with the hunger ratings, change in fullness between the start of the session and one-hour post test was calculated. There was no significant difference between days $F(3,56) = 1.62$, $p = .187$ or conditions $F(4,52) = 0.58$, $p = .678$ but there was a significant day*condition interaction $F(12,156) = 1.99$, $p = .028$. Linear within subjects contrasts were not significant for day $F(1,52) = 0.20$, $p = .655$ or the day*condition interaction $F(4,52) = 1.43$, $p = .239$. A series of Bonferroni corrected one way ANOVA's for each day showed that the there was no significant differences between conditions on any of the exposure days. When split by condition, the HE control condition was the only group to significantly differ across days $F(3,33) = 4.92$, $p = .006$, with the fullness change one hour post test on day one ($M = 42.3 \pm 7.0$) significantly larger than day three ($M = 18.1 \pm 9.3$, $p = .034$) and approaching significantly larger than day two ($M = 17.7 \pm 4.4$, $p = .056$) with no other significant differences.

10.4.2.4 Appetite ratings: Desire to eat

As desire to eat ratings were also taken in this study, changes in ratings across days and conditions were examined. As in all other analyses, before change data were analysed, baseline data were checked for significant differences. There were no significant differences in desire to eat ratings between conditions on day one $F(4,55) = 1.86$, $p = .131$, day two $F(4,55) = 0.59$, $p = .674$, day three $F(4,55) = 0.92$, $p = .460$, day four $F(4,54) = 0.19$, $p = .944$ or day five $F(4,55) = 0.38$, $p = .823$. Descriptive statistics for baseline desire to eat ratings are displayed in Table 10.9.

Condition	Day 1	Day 2	Day 3	Day 4	Day 5
LE Control	65.3 ± 5.5	66.1 ± 5.0	71.8 ± 3.3	63.7 ± 4.3	67.5 ± 3.0
HE Control	66.0 ± 4.7	57.7 ± 7.4	61.8 ± 4.5	58.0 ± 7.9	66.3 ± 4.0
LE Congruent	59.1 ± 6.3	58.9 ± 7.7	59.3 ± 7.5	64.6 ± 7.3	59.3 ± 8.4
HE Congruent	70.5 ± 4.4	68.6 ± 6.6	67.5 ± 6.7	64.8 ± 6.7	60.5± 6.7
HE Hedonic	50.3 ± 7.2	57.2 ± 7.5	56.3 ± 9.1	61.3 ± 6.2	60.2 ± 7.4

Table 10.9: Mean (±SEM) desire to eat ratings between conditions at the start of each day in Study Five

10.4.2.4.1 Change in desire to eat across session

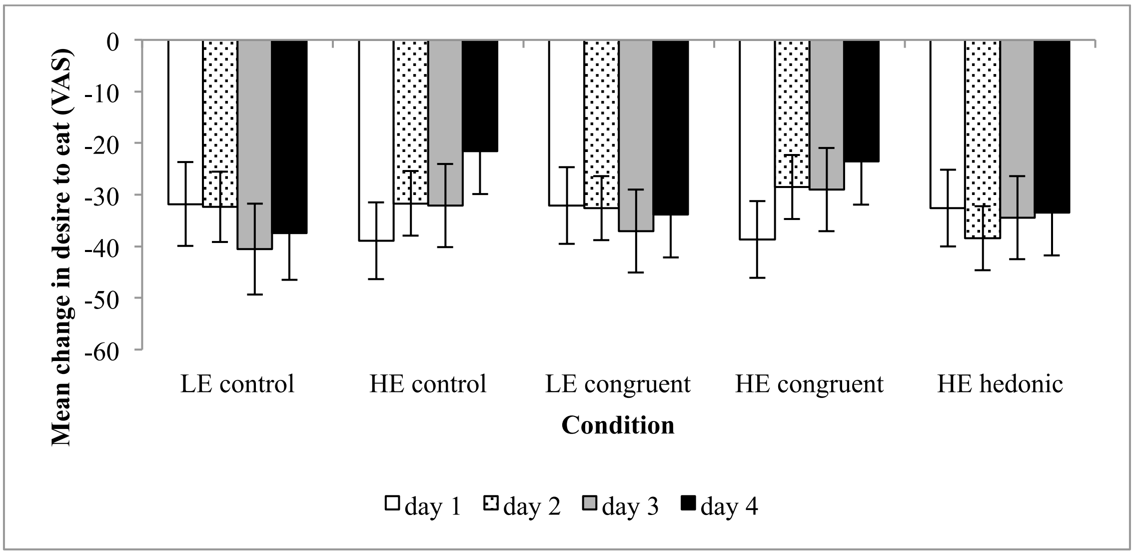


Figure 10.17: Mean (±SEM) change in desire to eat across the session between conditions in Study Five.

There were no significant differences in the change in desire to eat across days $F(3,159) = 0.88$, $p = .451$ or between conditions $F(4,53) = 0.15$, $p = .962$. There was also no significant day*condition interaction $F(12,159) = 0.82$, $p = .628$. As can be seen in Figure 10.17, changes in desire to eat were relatively similar across conditions although those in both the HE control and congruent labelled condition reported a smaller reduction in desire to eat as the sessions progressed, whereas those in the LE control showed an increased reduction over time. Those in the hedonic labelled condition remained stable in their reduction in desire to eat.

10.4.2.4.2 One hour post test

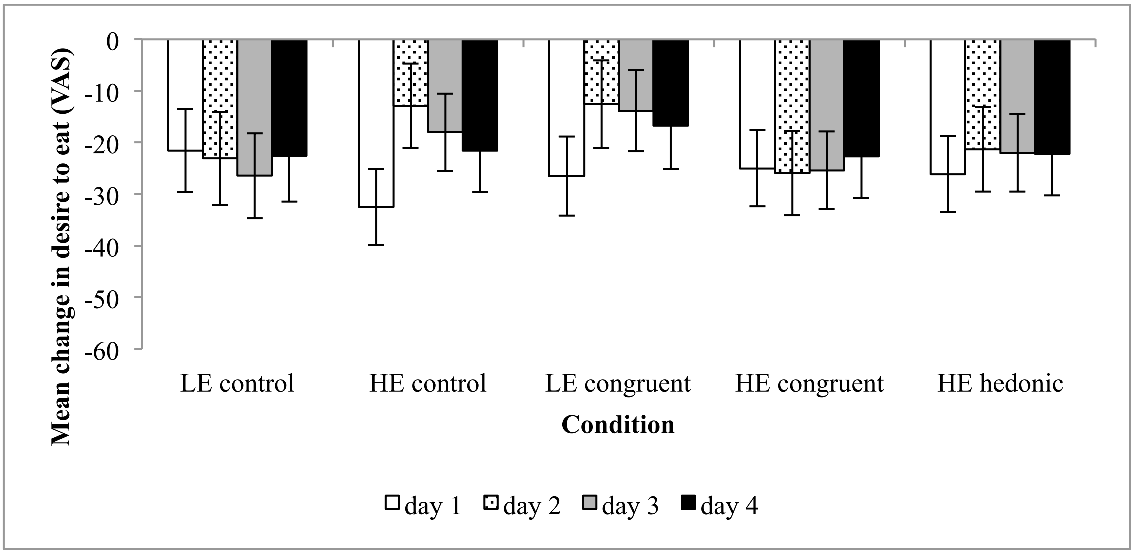


Figure 10.18: Mean (±SEM) change in desire to eat one hour post test between conditions in Study Five.

As with the other appetite ratings, change data were calculated between the start of each session and the rating given one hour post test. There was no significant difference in change in desire to eat one hour after consumption across the days $F(3,156) = 1.40$, $p = .244$ or between conditions $F(4,52) = 0.20$, $p = .935$, and no significant day*condition interaction $F(12,156) = 0.54$, $p = .884$. Figure 10.18 indicates that changes in desire to eat were relatively similar between conditions and days, although smaller reductions in desire to eat were demonstrated over time in the HE control and LE congruent conditions.

10.4.2.5 Prospective consumption

Participants were also asked to rate how much they could eat right now (prospective consumption), so change data were calculated and analysed. There were no significant baseline differences between conditions on any of the test days; day one $F(4,55) = 1.32$, $p = .273$, day two $F(4,55) = 0.62$, $p = .650$, day three $F(4,55) = 0.56$, $p = .695$, day four $F(4,54) = 0.53$, $p = .714$ or day five $F(4,55) = 0.39$, $p = .815$. Descriptive data are presented in Table 10.10.

Condition	Day 1	Day 2	Day 3	Day 4	Day 5
LE Control	63.3 ± 5.3	64.5 ± 4.7	65.3 ± 3.7	65.5 ± 3.0	68.3 ± 3.3
HE Control	55.8 ± 3.9	57.2 ± 7.5	55.8 ± 5.9	66.2 ± 6.2	65.7 ± 5.4
LE Congruent	61.7 ± 6.7	58.7 ± 7.2	62.3 ± 8.3	66.3± 6.9	60.9 ± 7.9
HE Congruent	63.2 ± 3.5	68.2 ± 5.0	66.8 ± 4.1	70.2 ± 4.7	69.4 ± 4.8
HE Hedonic	49.9 ± 5.4	57.0 ± 6.9	57.9 ± 8.0	58.4 ± 7.3	62.8 ± 6.4

Table 10.10: Mean (±SEM) prospective consumption ratings between conditions at the start of each day in Study Five

10.4.2.5.1 Change in prospective consumption across session

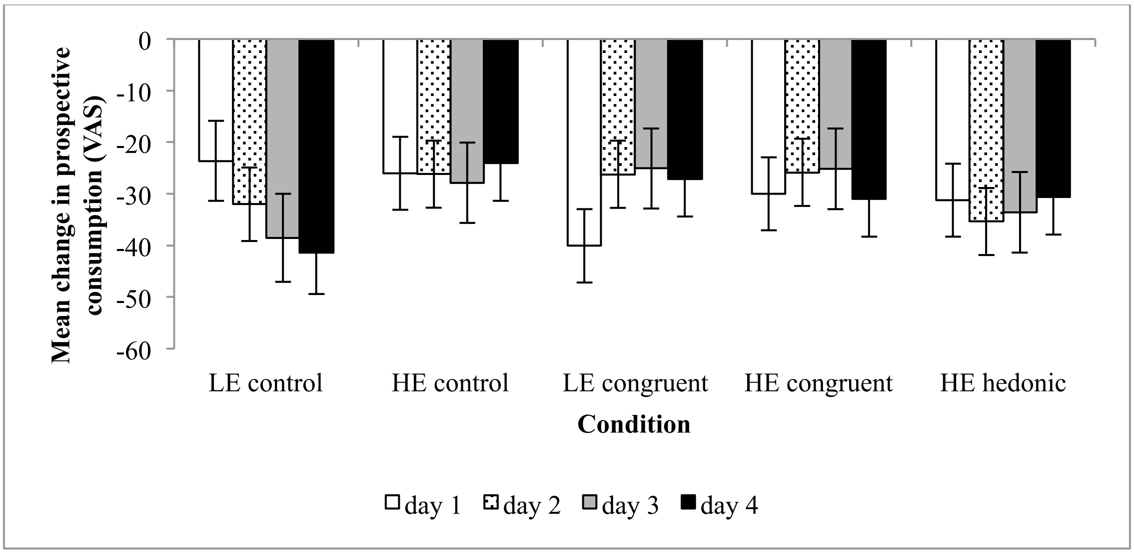


Figure 10.19: Mean (±SEM) change in prospective consumption between conditions and across days in Study Five.

There were no significant differences in change in prospective consumption across days *F*(2.95,156.25) = 0.09, *p* = .965, between conditions *F*(1,44) = 0.32, *p* = .866, and no significant day*condition interaction *F*(11.79,156.25) = 1.06, *p* = .398.

Figure 10.19 shows that those in the LE control condition showed an increased reduction in prospective consumption over time, whereas those in the LE congruent condition showed a decrease. All other conditions remained relatively stable across time and were similar in change.

10.4.2.5.2 One hour post test

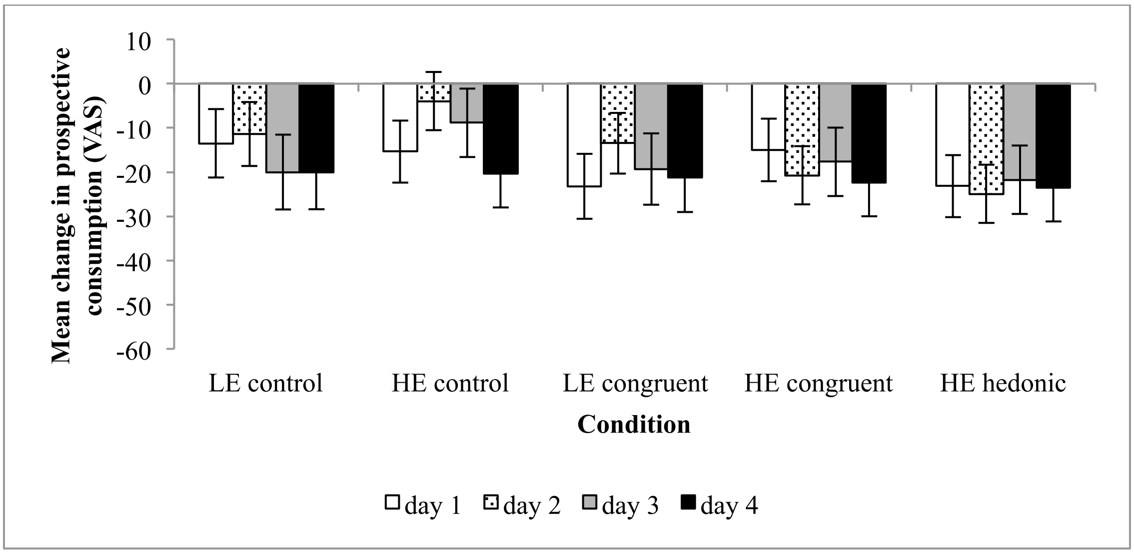


Figure 10.20: Mean (±SEM) change in prospective consumption between conditions and across days at one hour post test in Study Five.

At one hour post test there remained no significant differences in prospective consumption across days *F*(3,156) = 1.40, *p* = .246, between conditions *F*(4,52) = 0.50, *p* = .734 or day*condition interaction *F*(12,156) = 0.62, *p* = .824. As seen in Figure 10.20, all changes in prospective consumption were similar between conditions and across days.

10.4.2.6 Relationship between appetite ratings

As in Study Four, the relationship between appetite ratings was explored through Pearson *r* correlations. Mean change in each appetite rating were calculated for immediately, and one hour, after consumption. Table 10.11 shows the coefficients and their associated significances.

a)

	1	2	3
1. Hunger			
2. Full	-.76*		
3. Desire to eat	.93*	-.73*	
4. PC	.90*	-.78*	.90*

b)

	1	2	3
1. Hunger			
2. Full	-.75*		
3. Desire to eat	.91*	-.69*	
4. PC	.89*	-.73*	.80*

Table 10.11: Pearson *r* correlation coefficients for appetite ratings in Study Five a) immediately after consumption and b) one hour post consumption. * denotes $p < .001$.

As can be seen in Table 10.11, all changes in appetite ratings significantly correlated with each other, with changes in hunger, desire to eat and prospective consumption positively correlating with each other, and negatively correlating with changes in fullness.

10.4.2.7 Other expectations

Measures of expected calories and how filling the portion would be were also taken. From the pilot study (Part a) it was predicted that on day one, the hedonic label (which provided no information regarding energy content) would fall in the middle of the other two label conditions in terms of expected calorie content. Over the sessions, the congruent label conditions should remain stable in these ratings, whereas the control groups, and perhaps the hedonic condition, may have adjusted their expectations in line with the actual energy content. Although the pilot showed significant differences in terms of how filling the breakfast was expected to be, this may not emerge on day one when the portion itself was present but over the sessions, the LE may be expected to be less filling than the HE conditions, which would be enhanced in the labelled conditions.

10.4.2.7.1 Number of calories the portion was expected to contain

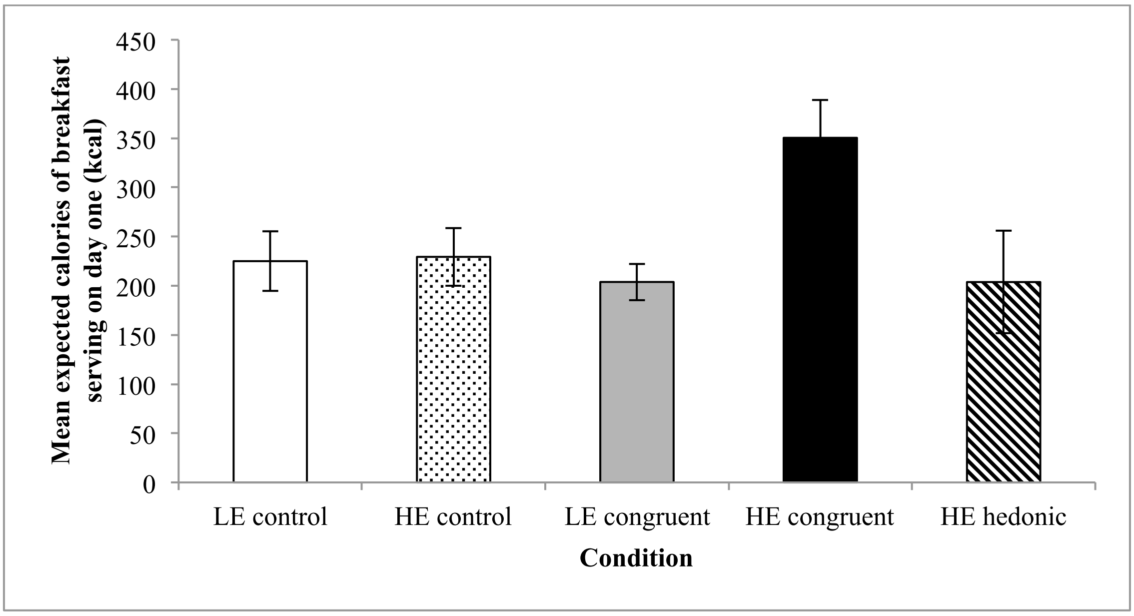


Figure 10.21: Mean (±SEM) calorie content expected in the breakfast across conditions on day one of Study Five.

There was a significant difference between conditions in the expected calories on day one $F(4,54) = 2.98$, $p = .027$. The HE congruent label ($M = 350.4 \pm 38.7$) generated the expectation of significantly more calories per serving than both the LE congruent ($M = 203.8 \pm 18.5$, $p = .052$) and the HE hedonic ($M = 203.8 \pm 52.2$, $p = .052$) labels. Therefore, contrary to predictions, before consuming the portion, the HE hedonic label did not generate expectations of additional calories in comparison to the LE congruent condition.

When investigating expected calorie content over the sessions, there was no significant effect of day $F(2.17,14.63) = 1.34$, $p = .266$ or day* condition interaction $F(8.65,114.63) = 1.09$, $p = .375$ but there was a significant difference between conditions $F(4,53) = 2.69$ $p = .041$. Games Howell post hoc tests revealed that the only groups that differed significantly in expected calories were HE congruent ($M = 334.7 \pm 33.9$) and LE congruent ($M = 187.6 \pm 33.9$, $p = .022$), with the latter expected to contain significantly less calories. This would be expected as the information explicitly stated how many calories were in the serving. Exposure to the yoghurt over time did not appear to moderate these expectations.

10.4.2.7.2 Filling

Condition	Expected filling rating
LE control	75.4 ± 4.4
HE control	69.9 ± 16.4
LE congruent	71.7 ± 4.4
HE congruent	67.2 ± 5.5
HE hedonic	71.9 ± 2.9

Table 10.12: Mean (±SEM) expected rating of filling across the conditions on day one of Study Five.

There was no significant difference between conditions in ratings of how filling the breakfast was expected to be on day one $F(4,55) = 0.45$, $p = .77$.

When tested over the exposure sessions, these differences remained non significant between conditions $F(4,54) = 0.20$, $p = .938$, and also across days $F(2.73,147.17) = 0.20$, $p = .878$, with no significant day*condition interaction effect $F(10.90,147.17) = 1.04$, $p = .415$.

10.4.2.8 Exposure versus non-exposure flavour

The taste test that was completed on day one was repeated on day five, to see if the difference between exposure and non exposure flavour had changed, and whether this was influenced by the condition the participant was in. It was predicted that a greater increase in pleasantness would be seen for the exposed rather than the non-exposed flavour, and that this would be enhanced for those who consumed the HE version.

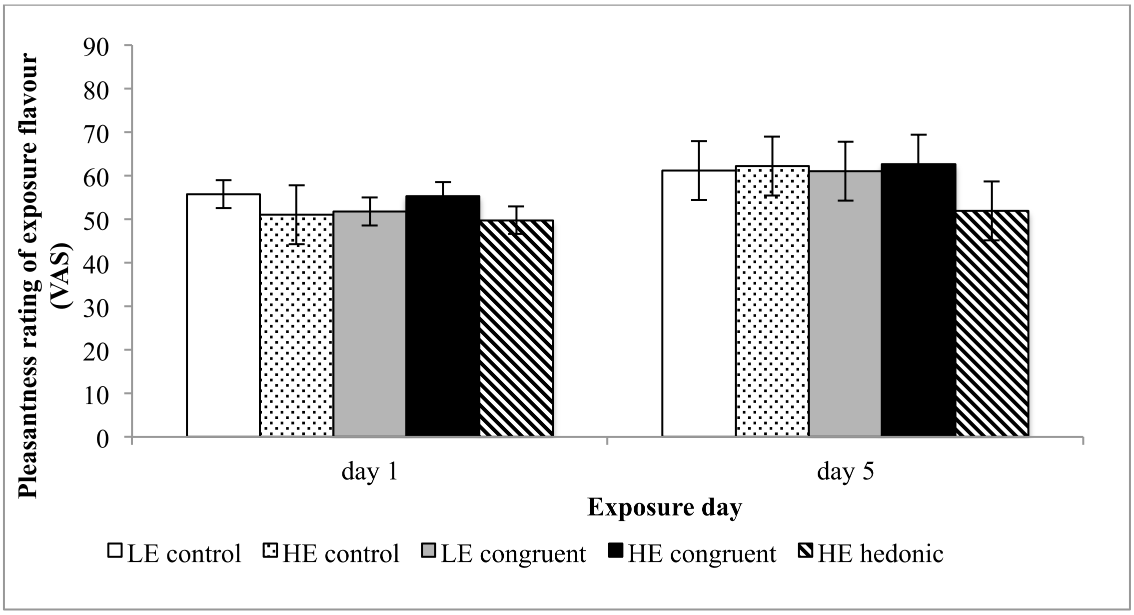
Condition	Exposure flavour	Non-exposure flavour
LE control	55.8 ± 2.0	65.7 ± 4.5
HE control	51.0 ± 3.7	46.1 ± 10.9
LE congruent	51.8 ± 3.4	63.0 ± 6.4
HE congruent	55.3 ± 2.5	69.0 ± 5.3
HE hedonic	49.8 ± 39	68.8 ± 6.3

Table 10.13: Mean (±SEM) pleasantness of flavours on day one of Study Five.

Baseline differences between conditions for pleasantness of both flavours were analysed, and as homogeneity of variance was violated, Welch and Brown-Forsythe statistics were reported. There were no differences between conditions in pleasantness of the flavour that would be the exposure flavour; $F(4,27.02) = 0.77$, $p = .555$ and $F(4,46.91) = 0.71$, $p = .592$, or the non-exposure flavour; $F(4,27.11) = 0.94$, $p = .457$ and $F(4,36.19) = 1.83$, $p = .145$. As can be seen in Table 24, the mean pleasantness of the non-exposure was slightly lower in the HE control condition than the others, although there was large variability in these ratings. Generally, pleasantness was higher for the non-exposure flavour than the exposure on day one.

A three way mixed ANOVA (day*condition*sample) was conducted to explore difference in pleasantness between the exposure and non-exposure flavours over time and between conditions. There was a significant difference in the pleasantness ratings of the two samples, $F(1,55) = 5.63$, $p = .021$ and a difference approaching significance between days $F(1,55) = 3.74$, $p = .058$. When ignoring all other factors, the non-exposure sample was rated as significantly more pleasant ($M = 63.2 \pm 2.8$) than the exposure sample ($M = 56.3 \pm 1.8$), and ratings were higher on day five ($M = 61.8 \pm 2.3$) than day one ($M = 57.6 \pm 1.9$). There were no significant differences in pleasantness ratings between conditions $F(4,55) = 0.73$, $p = .573$, and no significant interactions; sample*condition $F(4,55) = 1.56$, $p = .198$, day*condition $F(4, 55) = 0.83$, $p = .516$, sample*day $F(1, 55) = 1.85$, $p = .180$ or sample*day*condition $F(4, 55) = 0.47$, $p = .756$.

a)



b)

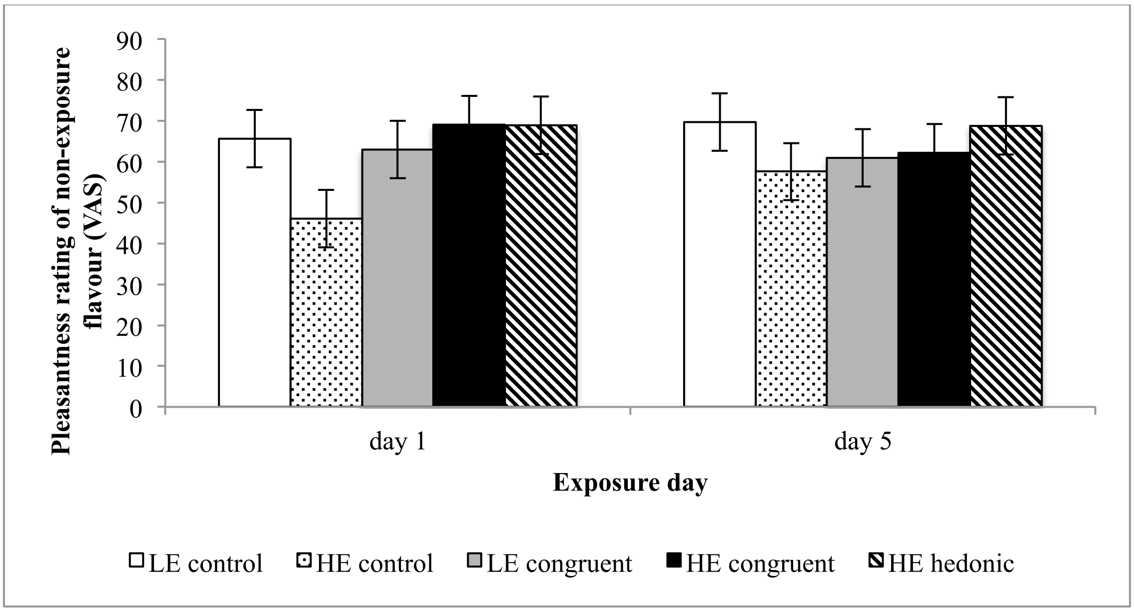


Figure 10.22: Mean (±SEM) pleasantness ratings of the a) exposure and b) non-exposure samples in Study Five.

As can be seen in Figure 10.22, all conditions showed an increased pleasantness for their exposure flavour between days one and five. The non-exposure remained relatively stable in pleasantness for most conditions, with an increased pleasantness demonstrated in both control conditions. There was little evidence that the exposure flavour became more pleasant than the non-exposure flavour over time.

10.4.3 Discussion

The present study aimed to further investigate the influence of explicit information on FNL, and to expand upon the findings from Study Four. Although differences in pleasantness between conditions were not significant, the observed liking changes in the HE congruent condition did not support the blocking effect demonstrated in the previous study. Instead, pleasantness changes were more in line with the behaviour that would originally have been predicted by the FNL literature (as reviewed by Brunstrom, 2007; Yeomans, 2006b; Yeomans, 2008).

There was some evidence for an acquired liking in the HE versions, with small, although not significant, increases in pleasantness in the HE control and congruent label conditions. There were no consistent differences in appetite ratings between the conditions, suggesting that neither labelling nor energy content had an impact. This mainly supports the findings in Study Four, as most of the appetitive changes in that study were seen in the *ad libitum* sessions not those with fixed consumption. Contrary to Study Three, the additional appetite change ratings of prospective consumption and desire to eat were correlated with hunger and fullness changes, suggesting that perhaps these concepts are measuring different aspects of appetite dependent upon the context in which the energy is consumed. This will be discussed in more detail in Chapter 11.

Expectations regarding the pleasantness of the yoghurt followed those predicted in Part a) except for how filling the breakfast was expected to be, and these remained stable across time regardless of whether the breakfast was high or low in energy content. It is perhaps not surprising that the expected filling ratings did not initially follow the predicted pattern from Part a), as once the breakfast portion was also presented alongside the label, this may have been perceived to be a larger portion than the norm in terms of yoghurt. However, some dissociation between the low and high energy versions may have been predicted as time progressed, with the association between flavour and nutrient perhaps leading to increased ratings for the HE and/or decreased ratings for the LE.

Contrary to prediction, hedonic labelling did not appear to enhance the effect or lead to increased pleasantness contradicting research showing that descriptive labels can bias evaluations of food to be rated as more pleasant (e.g., Wansink, Painter, & Van Ittersum, 2001). However, the discrepancy between expected and actual pleasantness was larger than in the other conditions, suggesting that the yoghurt did not meet the initial high expectations and indicating that the flavour generated a contrast rather than assimilation effect. This supports an assimilation-contrast model (as reviewed by Anderson, 1973) where a large discrepancy that is deemed unacceptable results in a contrast effect. This could explain the failure to find the predicted acquired liking for the breakfast for those who received the hedonic label and supports the finding by Yeomans et al., (2008a) where a contrast effect occurred when participants were told a product was ice cream, evoking expectations of a sweet fruity flavour, but actually given a smoked salmon ice cream. The present study indicated that even when an expectation is merely generated by the use of the word 'luxury', if this is not met this can impact greatly upon the liking and acceptance of a product.

Finally, there were also no differences in pleasantness of the exposed flavour over that of the non-exposed, suggesting that a preference for the exposed did not emerge. However, as questions regarding preference were not explicitly asked this couldn't be measured. In a future study it would be beneficial to include a more direct measure of preference, especially as there is evidence that a more pronounced change may be demonstrated in terms of preference rather than liking (Zandstra & El-Deredy, 2011) which warrants further investigation.

A potential confound of this study was the taste test and the use of different flavours for the test stimuli. Although the flavours were matched in Part Two, perhaps a contrast effect may have influenced the final ratings on day five, with a flavour that had not been exposed actually causing the exposed flavour to be rated as less pleasant as it had been consumed in large quantities over the previous exposure days.

In summary, the influence of labelling over FNL in this study was not clear, with no suggestion of the blocking effect demonstrated in Study Four, and no firm evidence that a flavour paired with HE acquired liking more than if paired with LE.

Chapter 11: General Discussion for Part Two

Study Three found that manipulating oro-sensory cues to be predictive of energy did not appear to enhance FNL, with no evidence of an acquired liking for a HE drink in either sensory condition. Appetite ratings were influenced more by actual energy content rather than sensory cues, although the high sensory drinks were expected to be more filling. Studies Four and Five went on to manipulate expectations in a more explicit manner by using labels. Although findings were inconsistent between the two studies, it was evident that labelling did affect FNL, although not always in the directions that were predicted.

In Part One, the role of context was discussed, as little evidence for FNL was demonstrated within a beverage context. The findings from Study Three indicated that even when oro-sensory cues were manipulated to be more viscous, increasing oral exposure time and signalling the presence of energy (thought to be important factors in why learning is often not demonstrated within a liquid, De Graaf, 2011), this did not lead to an acquired liking for a HEHS drink. Although evidence is mixed (e.g., Almiron-Roig, et al., 2003) calories consumed in a liquid form have frequently been shown to be less satiating and to lead to weaker compensation in subsequent intake (Mattes, 2005). Perhaps any post ingestive effects of nutrients consumed in this study were not attributed to consumption of the test drinks and this lack of association meant that the predicted increases in pleasantness were not observed, even when expectations were manipulated to signal energy. Previous research has found that enhancing the satiating quality of a beverage leads to greater satiation and subsequent compensation of intake at test meal (Yeomans & Chambers, 2011), but Study Three found little influence of sensory manipulations on appetite, with energy being the more salient factor (which partly supports the study by Yeomans and Chambers, (2011) as satiety relevant cues alone were not sufficient in producing greater satiation, the energy content was also important).

Labels were used in Studies Four and Five to generate expectations more explicitly, and these labels did alter the process of FNL, although not in a consistent way between studies and not always in the predicted direction. Congruent information about a HE yoghurt led to enhanced initial pleasantness ratings in comparison to the other HE yoghurts in Study Four, and this pleasantness did not change across test days, whereas in Study Five, this condition showed some evidence of FNL where other conditions did not. Previous research has shown that providing information about the calorie content or satiating quality of a food can lead to changes in rated pleasantness of that food (Wansink & Park, 2002; Yeomans, et al., 2001) although this is not always the case, particularly as knowledge of the nutritional content of a food does not always mean that a food will be more or less pleasant (Eiser, et al., 1984). The different effects of the labels on acquired liking in the two breakfast studies is interesting and warrants further investigation, as evidently providing explicit information did influence FNL, but methodological differences between the studies make any conclusions difficult to make.

Studies Three, Four and Five demonstrated that expectations about a food or drink can be manipulated using both sensory cues and explicit information provided through labelling. High sensory drinks were expected to be more filling, and yoghurt breakfasts labelled as ‘luxury’ were expected to be more pleasant than unlabelled or calorie labelled breakfasts. However, these expectations did not necessarily map onto actual ratings, with the high sensory drinks not influencing appetite to a larger extent than the low sensory, and the hedonic labelled breakfasts not delivering the increased pleasantness that was expected prior to consumption. These studies highlight the importance of expectations and what happens when these expectations are not met. This was particularly salient in Study Five where the hedonic label generated much higher expectations of pleasantness than the actual product delivered, and this resulted in lower ratings of pleasantness indicating a contrast effect which supports findings from Yeomans et al., (2008a) and suggests that the discrepancy was large enough to result in a contrast rather than assimilation effect (Zellner, et al., 2004).

In Studies Three and Five measures of desire to eat and prospective consumption were also taken. The relationship between these measures and the other appetite measures of hunger and fullness were different between the studies. When the energy was consumed in liquid form (Study Three), changes in desire to eat and prospective consumption were significantly correlated with each other, but not with changes in hunger and fullness, whereas when the energy was consumed in a food form (Study Five) all change ratings were significantly correlated with each other. This suggests that desire to eat and prospective consumption are tapping into a different (more motivational) aspect of appetite than hunger and fullness (perhaps more physiologically driven). In a study by Hill et al*.*, (1984) it was found that desire to eat and prospective consumption were influenced by the sight of food whereas hunger was only influenced upon consumption, which could be a factor in the differences in the correlational data within this thesis. Perhaps the lack of relationship in the drink context, and the significant relationship within the food context, could be explained by the presence of 'food' in Study Five compared to Study Three.

Exploring the interaction between oro-sensory cues and more explicit cues such as labelling, would be the logical extension of the research reported in Part Two, as we experience these in combination throughout our lives. The studies suggested that there could be the potential to manipulate expectations through these cues in order to influence liking and subjective appetite, although further investigation is needed in order to clarify these effects. Chambers, Ells and Yeomans (2012) used labels with explicit messages about how satiating a yoghurt beverage preload would be, in a within subjects study where a HE version was consumed on one day and a LE on another. The beverages were identical to the ones detailed in Study Three and for those in the label condition the HE was labelled as "Stayfull" and the LE as "Lighter" to highlight the satiating power of the preload. The study found that the sensory properties did influence the satiating power of the preload, with lower consumption of a test meal after the high energy high sensory preload than the low sensory version, and that both energy and sensory factors influenced subjective appetite. However, labelling had no influence over appetite, which could suggest that the sensory and energy effects were sufficiently powerful which rendered the labels unnecessary. In terms of pleasantness, the only variable that influenced ratings was energy content, with the LE version actually rated as more pleasant than the HE. This partially supports the findings in Study Three, where, contrary to prediction, those who consumed the LELS drink rated it as more pleasant.

In addition, perhaps the pattern of liking changes demonstrated in response to the different labels within Studies Four and Five would be clearer if participants were restrained eaters rather than unrestrained. There is some evidence to suggest that restrained eaters respond more to external cues (such as labels) and as these individuals are also cognitively restricting their intake they are likely to be more sensitive to calorie or nutritional information than unrestrained eaters (e.g., Ogden & Wardle, 1990). Restrained eaters have been reported to be less sensitive to energy manipulations with regards to FNL (Brunstrom & Mitchell, 2007) but perhaps the presence of oro-sensory cues, such as texture, may provide a salient cue for energy content, facilitating the achievement of cognitive restriction. Further investigation comparing restrained and unrestrained eaters in their response to information and oro-sensory cues may be beneficial in examining the influences that these factors have on liking and appetite changes.

In summary, Part Two has highlighted that expectations do influence FNL but it is unclear as to the mechanisms underlying this process and clarification of what influence they have is needed. The final section of this thesis aims to address some wider theoretical implications for the experimental work that has been discussed and some of the methodological issues that have arisen throughout.

Chapter 12: General discussion

12.1 Introduction

Exploring the mechanisms involved in the development and persistence of liking for flavours could help to change food choice behaviour and reduce overconsumption of palatable foods. This thesis set out to explore two key aspects of this process: extinction of acquired liking after the removal of energy, and how expectations could facilitate the learning process, with particular focus on FNL. It has been suggested that flavour learning is resistant to extinction (De Houwer, et al., 2001), with liking often persisting despite the removal of the functional ingredient in both animal (e.g., Harris, et al., 2004) and human (e.g., Stevenson, et al., 2003) research, although the majority of human studies have focused on liking acquired through FFL. Therefore, it was warranted to investigate this process further within a FNL context, which would have particular relevance in the current obesogenic environment where removal of energy in products is a common procedure. Additionally, manipulating expectations about a product, for example through labelling or the enhancement of sensory cues to signal the presence of energy, have been shown to alter perceptions, hedonic ratings and appetite responses (e.g., Cassady, et al., 2012; Crum, et al., 2011), and these expectations could potentially facilitate flavour learning.

This discussion will provide an overview of the main findings, which have been discussed in Chapters 6 and 11, and address some theoretical implications, limitations and further research.

12.2 Main findings: a summary

12.2.1 Study One: Extinction within a drink context

Unfortunately, and surprisingly, in Study One there was little evidence of an acquired liking for a flavour paired with energy, which meant that conclusions about extinction were difficult to draw. However, those in the extinction condition showed little change in pleasantness ratings after the energy was removed and behaved in line with those who continued to consume the HE version. Appetite changes also remained stable after the removal of energy, giving support to the tentative suggestion that extinction did not occur. There were also numerous gender effects influencing the clarity of changes within the study, with tentative evidence for FNL in females.

12.2.2 Study Two: Extinction within a sorbet context

Study Two introduced a paradigm where previous evidence of learning had been demonstrated (Yeomans, et al., 2008b) in an attempt to overcome methodological issues recognised in Study One, and the decision was taken to recruit only female participants. Unfortunately, low participant numbers prevented firm conclusions from being made from Study Two, but unlike in Study One, there was some evidence for an acquired liking for a sorbet after the flavour had been paired with energy between days one and six, but this liking was not maintained between days six and eleven when this association continued. Conversely, those who underwent extinction continued to demonstrate an acquired liking for the sorbet over this second period, as did those who consumed the training drink in a LE form throughout the training days. Intake data showed that those in the extinction condition demonstrated similar changes to those in the HE condition, with an increase over days one to six but a decrease between days six and eleven. No consistent effects of energy were found in terms of appetite change. Mixed conclusions could be made regarding extinction, as the pleasantness of sorbet was maintained over time but this followed a different pattern to that displayed in the HE condition.

12.2.3 Study Three: Manipulating oro-sensory cues to be predictive of energy

Study Three attempted to investigate the apparent lack of FNL in a liquid context that occurred in Studies One and Two, but there remained very little evidence of learning despite the manipulation of oro-sensory cues of a drink to be predictive of energy (i.e. thick and creamy). Low sensory drinks appeared to be rated as more pleasant over time than high, and this was enhanced within the LE version, contrary to hypothesis. Generally, appetite changes appeared to be influenced more by the energy content than the sensory characteristics of the drink, and desire to eat and prospective consumption changes were not related to hunger and fullness changes. Although appetite changes were not as influenced by sensory as predicted, ratings of how filling the drink would be were significantly higher in the high sensory than low sensory conditions.

12.2.4 Study Four: The impact of labelling calorie content in a breakfast context

Perhaps the subtle manipulation of expectations in Study Three were not sufficient in translating to pleasantness differences and the influence of expectations could be enhanced using more explicit methods. Study Four was the first of two studies exploring the impact of labelling on FNL in a breakfast context. An acquired liking was demonstrated for a HE compared to a LE yoghurt based breakfast, and this was moderated by information, although not in line with prediction. Higher baseline pleasantness was shown for those receiving congruent information about a HE breakfast, but there were no subsequent changes in liking over time. Incongruent labelling resulted in initial confusion in ratings, but towards the end of the test sessions ratings were becoming in line with those demonstrated in the no label control conditions. No differences were observed in intake or hunger ratings, with higher ratings of fullness after the HE breakfast than after the LE. Some change in matched expected satiety was observed, but this was an overall decrease in the amount of calories (in the form of porridge or Crunchy Nut Cornflakes) that would be expected to match the breakfast in terms of satiety.

12.2.5 Study Five: the impact of labelling calorie content and hedonic factors within a breakfast context

Study Five looked to expand upon the findings from Study Four, by investigating the expectations that were generated by the labels used, and to extend the information to a hedonically labelled condition alongside the energy labels used in Study Four. Part a) of Study Five showed that the expectations generated by the labels were different, with the hedonic (luxury) label generating expectation that the yoghurt would be significantly more pleasant, creamy, fruity and thick than the other labels, and also more filling than the LE label. The HE label generated the expectation that the yoghurt would be significantly higher in calorie content than both other labels, and also creamier, thicker and more filling than the LE label. Therefore the labels appeared to generate the desired expectations. Part b) showed that the two flavours that were created for the yoghurt breakfasts in the main study were matched in a number of attributes including pleasantness, novelty and sweetness. Part c) of Study Five was the main part of the study. There was no evidence that the congruent label led to a blocking of liking in the

HE breakfast, so the results from Study Four were not supported. There were some small changes in pleasantness for the HE over the LE breakfast, although these were not significant, with inconsistent effects on appetite changes, and there was no evidence that the exposed flavour was any more liked than the non-exposed. The hedonic labelled condition actually resulted in a contrast effect, suggesting that the actual yoghurt was not meeting the high expectations that were generated by the label.

Study	Evidence of liking change?	Evidence of appetite change?
Study One	No - although hint in the predicted direction for females	Yes – significant differences
Study Two	No - hint in predicted direction when non-responders excluded	No – means in the predicted direction
Study Three	Yes - but just general increase over time, no energy/sensory effects	No – some indication of energy differences being more influential
Study Four	Yes – significantly different between HE and LE when no information	Some – significant effects of incongruent label, and in energy, for some measures
Study Five	No – a hint of energy differences	Yes – but only differences in fullness changes between energy conditions

Table 12.1: Summary of key outcomes in relation to liking and appetite changes within this thesis.

12.3 Theoretical implications

The general discussions of Part One and Part Two (Chapters 6 and 11) have explored some theoretical implications for each section. There are wider theoretical implications that relate to the thesis as a whole, in particular, contingency awareness and the robustness of FNL.

12.3.1 Contingency awareness

It has long been debated as to whether contingency awareness is necessary for learning to occur and this has important implications for dietary learning, as if associations are made without awareness this implies that it is out of an individual's voluntary control (Brunstrom, 2004). If this was the case it could suggest that the prevalence of obesity and overeating could be attributed to exposure to CS-US pairings from food companies without consumers recognising that these associations have been made. Contingency awareness can be defined as knowing that the CS predicts a particular US (Lovibond & Shanks, 2002) so for example, a flavour predicts a post-ingestive outcome. Brunstrom (2004) reviews the literature and suggests that the issue of contingency awareness within dietary learning (which incorporates FFL, FNL and learned satiety) has not been investigated in the same robust manner as in other forms of learning (as reviewed by De Houwer, 2001; Field, 2000), and needs clarification before firm conclusions can be made. There are indications that FFL and FNL may be unique forms of learning that occur automatically rather than with awareness, and if this were the case it could help to explain the unreliable findings that have emerged within the literature, and indeed this thesis. An evaluative conditioning study by Baeyens et al., (1990b) demonstrated a double dissociation between awareness and evaluative conditioning where conditioning was demonstrated in CS-Flavour conditions but with no explicit knowledge of stimulus pairings with reinforcers and CS-Colour conditions displayed explicit knowledge of pairings but showed no evidence of conditioning. This finding has been supported by other studies (Baeyens, Eelen, & Vandenbergh, 1990a; Dickinson & Brown, 2007) but has been questioned by other researchers (Field & Davey, 1997, 1998) and replication has not always been successful (as reported in Lovibond & Shanks, 2002). However, Stevenson et al., (Stevenson, et al., 1998; Stevenson, et al., 1995) have shown that an odour can acquire the taste of a flavour through FFL without awareness and the combination of findings make some suggestion that FFL can occur without the presence of contingency awareness.

If awareness is necessary for learning to occur then beliefs, expectations and attitudes could mediate the learning process (Brunstrom, 2004; Gibson & Brunstrom, 2007). There was some evidence in Studies Four and Five that this was happening as it did appear that labels were influencing FNL, although the effects of the individual labels were not consistent across the studies. In Study Three, when expectations were manipulated in a more implicit manner there was very little evidence of a change in liking so it is interesting that the largest evidence for FNL was demonstrated in the studies where explicit reference to energy content was made. However, no measure of contingency awareness was taken throughout this thesis so perhaps inclusion of a measure of this nature would be recommended in future work, although this poses methodological problems in human research as it is difficult to limit the explicit information about characteristics such as volume, colour, density, to which participants are exposed. Additionally oral consumption, rather than intragastric infusion, makes it difficult to separate the sensory impact of the nutrient from its post-ingestive consequence, making a measurement of contingency awareness difficult to detect (Brunstrom, 2004).

12.3.2 How robust is FNL?

Another issue that has been raised from the research in this thesis is the robustness of FNL, as generally there were only hints of this type of learning within the studies reported (See Table 12.1). As discussed in Chapter One, animal research demonstrates consistent evidence for FNL but findings with human participants are mixed, much of which could be attributed to the number of other factors that are present during every eating episode. As discussed previously, a recent review (Yeomans, 2012) highlights some important considerations that should be taken into account when running studies of this nature, and where inconsistencies within these considerations could explain why such mixed findings occur within human research. Many of these considerations will be discussed below in Section 12.4.

12.3.3 Context

The influence of context on FNL has been discussed in detail within Chapters 6 and 11, but the general conclusions from this thesis suggest that learning within a liquid context was much more difficult to condition than within a more solid context. This was reflected in pleasantness changes and also in differential effects on appetite changes, both across studies and between measures (i.e. desire to eat/prospective consumption were significantly correlated with hunger/fullness within the solid context study but not within the liquid context study).

12.4 Methodological considerations

Throughout the research presented in this thesis, the measurement of liking, or indeed pleasantness, has been used as the main indicator of whether or not FNL had occurred. However, perhaps this is not the most effective or sensitive method in determining whether or not an association has been made. Recently, the measurement of preference alongside pleasantness has been incorporated into some flavour learning studies, with interesting outcomes. Zandstra and El-Deredy (2011), who published a study after the outset of the main studies reported in this thesis, were one of the first researchers to attempt to measure both liking and preference in humans, with an explicit question asking participants which drink they preferred, alongside measurements of liking for each stimuli. Small differences were observed between high and low energy versions in terms of liking, with the behavioural measurement of preference being more sensitive to these energy differences. This highlights an interesting suggestion for future research, although it is important to note that colour, not flavour, was used as the cue for energy in this study. Whilst there was no evidence in Study One or Study Five for increased pleasantness of an exposed compared to a non-exposed flavour, no explicit measure of preference was taken, and perhaps if this was included into the design differences may have been observed, as it is possible to prefer something without finding it more pleasant.

It has previously been suggested that monotony effects due to multiple exposure sessions may make the detection of FNL more difficult (e.g., Hetherington, et al., 2002; Meiselman, et al., 2000). Certainly in Studies One, Two and Three monotony could be a potential explanation for an overall lack of acquired liking, although studies with long exposure times have found evidence of FNL in the past (e.g., Birch, et al., 1990; Kern, et al., 1993) and in the majority of the studies within this thesis pleasantness ratings remained stable as opposed to decline, at least suggesting there was no negative effect of number of exposure sessions on pleasantness.

A mixed design was used throughout this thesis with participants only experiencing one form of energy in each study. As discussed in Section 2.3.1 there has been much debate as to whether a between or within subjects design is more appropriate as each has strengths and weaknesses. Perhaps individuals would have been more sensitive to energy differences if exposed to flavours paired with both HE and LE versions although this would have been difficult to implement for the extinction studies in particular and would mean additional exposure sessions in studies that already required a large number of repeated exposures. Additional sessions in the studies where labelling was used may have inhibited learning as information from one label may have generalised across breakfasts, and would have made it challenging to test all label and energy conditions a sufficient number of times for associations to be made for all combinations.

There were potential issues with statistical power within this thesis due to small participant numbers, and perhaps increasing participant numbers may have clarified some of the patterns of change that were observed, and allowed for detection of the subtle effects of FNL studies. Reduced participant numbers in Study Two may have limited the statistical power of the study to find any differences in pleasantness change across the three conditions but these were out of experimenter control due to discontinuation of the test drink and these data had to be analysed using smaller participant numbers than anticipated. Methodologically studies of this nature pose a number of challenges in terms of power due to the large numbers of sessions required (especially in the extinction studies) and the time/expense constraints that are involved. Participants are free to withdraw at any point in the schedule which can mean that data sets are incomplete without anticipation, where replacing the individual is not feasible within the timeframe, and the repeated measures nature of the session aimed to reduce the need for larger participant numbers, increasing the number of exposures. Previous studies within the laboratory have detected differences in pleasantness changes between energy conditions using similar designs, energy differences and sample sizes to the ones in this thesis (e.g., Yeomans, Gould, Leitch, & Mobini, 2009a; Yeomans, et al., 2008b) and these were used as a basis for the studies reported.

12.5 Future design of FNL studies

From the review by Yeomans (2012) there are a number of suggestions that have been made for how FNL designs could be maximised to detect a genuine effect, some of which have been discussed, and many of which an attempt has been made to address, throughout this thesis. Evidence of learning appeared to be maximised in studies where maltodextrin was used as the macronutrient added to HE versions, and this was the primary source of energy used within three out of the five studies in this thesis. Sufficient energy differences between the CS+ and CS- whilst matching for pleasantness is important, with the majority of studies in this thesis based upon previous designs where energy differences have elicited the predicted differences in pleasantness changes. Individual differences between participants in terms of restraint score on the TFEQ (Stunkard & Messick, 1985), BMI and hunger upon arrival at the laboratory were controlled for as strictly as possible, and the CS was designed to be as novel as possible without being liked or disliked on the initial test session. In some studies other factors also impacted upon such initial ratings, particularly those where expectations were manipulated preventing a truly novel evaluation of the test food.

12.6 Future work

All of the studies reported in this thesis focused on how consumption of a food or drink altered appetite within a small time frame (within the laboratory and one hour post consumption) but in reality this consumption may have influenced subsequent consumption throughout the day. This has important implications for learning research, as perhaps what is learned during the exposure sessions does influence behaviour but this is not demonstrated until a later time point. A recent study (Yeomans & Chambers, 2011) using the same test drinks as in Study Three of this thesis found that subsequent consumption of a test meal was lower after the HE than the LE drink, and this was dependent upon the sensory context. So it is interesting that despite no differences in pleasantness or appetite changes within the consumption period in this study, there is suggestion that subsequent differences may have been observed.

Along a similar line, it is important to extend research outside of the laboratory setting, as behaviour that is observed or not observed within this setting may be different from a real world setting. Multiple factors and experiences influence an eating episode and any learning that occurs about a new food or drink. Some studies have been conducted outside of the laboratory and have found evidence of flavour-learning (Appleton, et al., 2006; Mobini, et al., 2007; Mobini, et al., 2005), which also provide support for the validity of laboratory research, although others have failed to find evidence of flavour learning outside of the laboratory (Zandstra, Stubenitsky, De Graaf, & Mela, 2002).

Context has emerged as an unexpected discussion point within this thesis, which could have important implications for how learning transfers between products. If learning about a product occurs during one context (whether it be, for example, the context of branding, or a liquid vs. solid form) does this learning generalise onto other similar products, or is the information we learn about a particular product limited only to that product?

12.7 Conclusions

In conclusion, this thesis aimed to explore the impact of extinction on the hedonic response to a previously experienced flavour-nutrient pairing and investigate the potential role for cognitive expectations in the development of FNL in humans. There was tentative evidence to suggest that removing the nutrient from a drink that was previously experienced with a nutrient did not result in a decrease in rated pleasantness of that drink, suggesting resistance to extinction. There seemed little evidence of acquired liking when energy was delivered in liquid form, and this was not improved by manipulating oro-sensory cues to be predictive of energy. FNL did appear to be influenced by expectations, with labelling altering the pattern of liking change although effects were not consistent between studies. Crucially, the findings within this research are based upon patterns and trends in behaviour and therefore conclusions are tentative. However, it is clear that the robustness of FNL needs future investigation, and without a robust learning model, what happens when a nutrient is removed from a product (but the sensory properties remain the same) cannot be fully understood. The interaction between expectations and the processes involved in FNL offer an interesting avenue of future research and this thesis highlights theoretical underpinnings that need additional clarification from researchers working in the field of FNL.

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Name ____
Gender Male ○ Female ○
Date of Birth (dd/mm/yyyy) ____
Contact Details:
Email at Sussex ____ Other email ____
Mobile Number ____ Landline ____
Status UG ○ PG ○ Staff ○ Other ____
Degree Programme ____
Year expect to leave Sussex (if known) ____
Today's date ____
Please can you give an estimate of your **current weight and height**
Weight ____ Height ____
Do you smoke? Yes ○ No ○ If yes, on average, how many do you smoke a day? ____
Do you speak English as a first or 'native' language? Yes ○ No ○
Have you ever participated in any previous studies in the food lab? Yes ○ No ○
If so please give brief details:
____

Proceed

DRINKING HABITS AND PREFERENCES

53. On average, how many cups, mugs or glasses of the following drinks do you drink in a typical day?

	0	1	2	3	4	5	6	7	8	9	10	11+
Instant Coffee	○	○	○	○	○	○	○	○	○	○	○	○
Decaff Instant Coffee	○	○	○	○	○	○	○	○	○	○	○	○
Filter Coffee	○	○	○	○	○	○	○	○	○	○	○	○
Decaff Filter Coffee	○	○	○	○	○	○	○	○	○	○	○	○
Espresso	○	○	○	○	○	○	○	○	○	○	○	○
Other Coffee (please specify) ____	○	○	○	○	○	○	○	○	○	○	○	○
Regular Tea	○	○	○	○	○	○	○	○	○	○	○	○
Decaff tea	○	○	○	○	○	○	○	○	○	○	○	○
Herbal Tea	○	○	○	○	○	○	○	○	○	○	○	○
Fruit Tea	○	○	○	○	○	○	○	○	○	○	○	○
Green Tea	○	○	○	○	○	○	○	○	○	○	○	○
Cola	○	○	○	○	○	○	○	○	○	○	○	○
Decaff Cola	○	○	○	○	○	○	○	○	○	○	○	○
Fruit Drinks	○	○	○	○	○	○	○	○	○	○	○	○
Red Bull	○	○	○	○	○	○	○	○	○	○	○	○

54. How many of the following do you drink in a typical week?
Pint of lager ____ Glass of wine ____ Pint of cider ____ Pint of ale ____
Single measure of spirit ____ Alcopop ____

55.What sort of drink do you normally have when you first get up in the morning? (Please describe accurately e.g. mug of instant coffee with milk and one sugar).
____

Back Proceed

FOOD HABITS

56. How regularly do you eat breakfast in the mornings?
Never ○ Rarely ○ Sometimes ○ Usually ○ Always ○

57. How pleasant would you rate the following foods for breakfast?
Crunchy Nut cornflakes with milk: Very pleasant ○ Moderately pleasant ○ Very unpleasant ○
Special K with milk: Very pleasant ○ Moderately pleasant ○ Very unpleasant ○

58. Which of the following best describes your eating habits?
○ Eat all meat
○ Eat only white meat and fish
○ Eat fish only
○ Strict vegetarian
○ No animal products (vegan)
○ Halal
○ Kosher
○ Other (please give details)

59. Are you currently on a diet?
Yes ○ No ○

60. Do you have any food allergies? Yes ○ No ○
If yes, please give brief details;

61. Do you have any food aversions? Yes ○ No ○
If yes, please give brief details;

Thank you very much for taking the time to complete this questionnaire.
Please click the Submit button below to send us your responses.
Your details will be stored on our secure database and you will be contacted when suitable studies arise.

Back Submit

PLEASE COMPLETE THIS ONE HOUR AFTER LEAVING THE LAB, AND RETURN AT YOUR NEXT SESSION

Name: ____________________ Date:__/__/20__ Session & time:__________

Please complete the following rating scales by marking an 'X' at the point on the line which most accurately reflects how you feel at present. Please be as accurate as possible.

Calm

Not at all ____ Extremely

Full

Not at all ____ Extremely

Lively

Not at all ____ Extremely

Hungry

Not at all ____ Extremely

Thirsty

Not at all ____ Extremely

Tired

Not at all ____ Extremely

Clear headed

Not at all ____ Extremely

Nauseous

Not at all ____ Extremely

Information for subjects **Name**_________________

The purpose of the experiment
Examining relationships between food and mood

What you will be required to do
This study runs over 8 sessions, when you will be asked to report to the laboratory for breakfast between 8 and 10 and return again 3 hours later. During this second part you will be instructed to taste 5 solutions, and to consume a fixed amount of a drink. This session will last a maximum of 40 minutes. Prior to each session, you will be required not to eat or drink anything except for water from 11pm the night before, for the 3 hours between sessions, and for one hour post test.

Throughout the study you will complete a series of digital, or paper, mood scales.

Upon completion of all sessions, you will receive £30, or you can opt for 24 course credits, or a combination.

IT IS IMPORTANT THAT YOU DO NOT SIGN UP TO ANY OTHER FOOD STUDY WHILST PARTICIPATING IN THIS ONE

Precautions
You should not take part if you:

1. are diabetic
2. are currently taking prescription medication, excluding the contraceptive pill
3. have been diagnosed as having an eating disorder
4. are pregnant
5. smoke more than 5 cigarettes a day
6. have any known allergies or aversions to the following common foods and food additives:
   sugar (sucrose, glucose, fructose), artificial sweeteners (aspartame, acesulfame, saccharin), natural food flavourings, natural colourings, nuts, wheat products, dairy products.

If you have any queries or concerns please contact:

Miss Natalie Gould, DPhil student, n.gould@sussex.ac.uk, 01273 877031

Professor Martin Yeomans, Principal Investigator, martin@sussex.ac.uk, 01273 678617

Please remember, you may withdraw from the study at any time, without giving a reason.

Volunteer Consent Form

I have read and had explained to me the attached information sheet, which I have signed and of which I retain a copy. The nature and purpose of the psychological testing has been explained to me. I am aware that I have the right to withdraw from the experiment at any time, without giving a reason.

I fully understand the nature and purpose of the study and give my consent to participate.

Name: ____________________________

Signed: ____________________________

Date: ___ / ___ / __

Phone Number: ____________________________

NAME: ………………………………………………………
AGE: ………………………………………………………
HEIGHT: ………………………………………………………
WEIGHT: ………………………………………………………

What do you think was the purpose of the study?

Do you have any observations to make about the study, for example how you felt after consuming the drink, whether you noticed any differences…?

NAME: ……………………………………………………………
AGE: ……………………………………………………………
HEIGHT: ……………………………………………………………
WEIGHT: ……………………………………………………………

What do you think was the purpose of the study?

Are there any comments you would like to make about the study?

Information for subjects **Name_________________**

The purpose of the experiment
Examining relationships between food and mood

What you will be required to do
This study runs over 11 days, when you will be asked to report to the laboratory at a time between 11.30 and 1.30 for a sandwich lunch, returning 3 hours later for the test session. On some of these sessions you will be asked to taste and rate a sorbet, and on other sessions you will be asked to consume a drink. Prior to each session, you will be required to consume your normal breakfast, and then refrain from eating and drinking (except water) until you come to the lab. You will also be asked to consume only water for the 3 hours between lunch and afternoon session, and for one hour after the session.

Upon completion of all sessions, you will receive £50, or you can opt for 8 hours course credits, or a combination.

IT IS IMPORTANT THAT YOU DO NOT SIGN UP TO ANY OTHER FOOD STUDY WHILST PARTICIPATING IN THIS ONE

Precautions
You should not take part if you:

1. are diabetic
2. are currently taking prescription medication, excluding the contraceptive pill
3. have been diagnosed as having an eating disorder
4. are pregnant
5. smoke more than 5 cigarettes a day
6. have any known allergies or aversions to the following common foods and food additives:
   sugar (sucrose, glucose, fructose), artificial sweeteners (aspartame, acesulfame, saccharin), natural food flavourings, natural colourings, nuts, wheat products, dairy products.

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Please remember, you may withdraw from the study at any time

Volunteer Consent Form

I have read and had explained to me the attached information sheet, which I have signed and of which I retain a copy. The nature and purpose of the psychological testing has been explained to me. I am aware that I have the right to withdraw from the experiment at any time, although no payments will be made unless the experiment is completed.

I confirm that I will not be taking part in any other eating behaviour experiements at any time during my participation in this study.

I fully understand the nature and purpose of the study and give my consent to participate.

Name: ______________________________

Signed: ______________________________

Date: ___ / ___ / ____

Phone Num: ____________________________

NAME: ……………………………………………………
AGE: ……………………………………………………
HEIGHT: ……………………………………………………
WEIGHT: ……………………………………………………

What do you think was the purpose of the study?

Do you have any observations to make about the study, for example how you felt after consuming the drink, whether you noticed any differences…?

Information for subjects **Name_________________**

The purpose of the experiment
Examining relationships between food and mood

What you will be required to do
This study runs over 6 days (minimum 2 a week), when you will be asked to report to the laboratory for breakfast between 8.15 and 10am and return again 3 hours later. During this second part you will be instructed to consume and rate a drink, and then complete some additional ratings in the lab after 30 and 60 minutes. Prior to each session, you will be required not to eat or drink anything except for water from 11pm the night before, for the 3 hours between sessions, and for one hour post test.

Throughout the study you will complete a series of digital mood scales.

Upon completion of all sessions, you will receive £50

IT IS IMPORTANT THAT YOU DO NOT SIGN UP TO ANY OTHER FOOD STUDY WHILST PARTICIPATING IN THIS ONE

Precautions
You should not take part if you:

1. are diabetic
2. are currently taking prescription medication, excluding the contraceptive pill
3. have been diagnosed as having an eating disorder
4. are pregnant
5. smoke more than 5 cigarettes a day
6. have any known allergies or aversions to the following common foods and food additives:
   sugar (sucrose, glucose, fructose), artificial sweeteners (aspartame, acesulfame, saccharin), natural food flavourings, natural colourings, nuts, wheat products, dairy products.

If you have any queries or concerns please contact:

Miss Natalie Gould, DPhil student, n.gould@sussex.ac.uk, 01273 877031

Professor Martin Yeomans, Principal Investigator, martin@sussex.ac.uk, 01273 678617

Please remember, you may withdraw from the study at any time, without giving a reason.

Volunteer Consent

I have read and had explained to me the attached information sheet, which I have signed and of which I retain a copy. The nature and purpose of the psychological testing has been explained to me. I am aware that I have the right to withdraw from the experiment at any time, although no payments will be made unless the experiment is completed.

I confirm that I will not be taking part in any other experiments at the Psychopharmocology Unit at any time during my participation in this study.

I fully understand the nature and purpose of the study and give my consent to participate.

Name: ______________________________

Signed: ______________________________

Date: ___ / ___ / ___

Phone Number: ______________________________

Name

Age

Height

Weight

What did you think was the purpose of the study?

Do you have any comments you would like to make about the drinks?

How did you feel after consuming the drink?

Information for Subjects **Name:_______________**

The Purpose of the Experiment

To investigate the interaction between mood and appetite

What you will be required to do

On four occasions you will be asked come to the lab for testing at a time between 8.15 and 10.00 in the morning. Each session should take no more than 30 minutes, usually less.

From 11 pm the night before each session you will be required to drink only water until you arrive at the lab for testing. On arrival at the lab, you will be required to rate your mood and then consume breakfast. You will be asked to consume only water for 1 hour after leaving the laboratory, when you will be asked to complete a paper mood scale.

The Breakfast

The breakfast will consist of yoghurt and fruit, made using standard ingredients. During some sessions you will be free to eat as much as you like, in other sessions you will be required to eat a set amount.

You will receive £15 upon completion of all sessions. If you are a psychology undergraduate you can opt to receive 12 course credits, or a combination of credits and money instead.

Exclusion criteria
You should not take part if you:

1. Have participated in a previous study by Natalie Gould or Lauren Upjohn
2. are diabetic
3. are pregnant
4. are currently taking prescription medication (excluding the contraceptive pill)
5. have any known allergies or aversions to these common foods and food additives: sugar, nutrasweet, saccharin, food flavourings, food colourings, maltodextrin, dairy products (milk, cheese etc), fruit
6. have been diagnosed as having an eating disorder
7. smoke more than 5 cigarettes per day

IT IS IMPORTANT THAT YOU DO NOT SIGN UP TO ANY OTHER FOOD STUDIES WHILST PARTICIPATING IN THIS ONE.

If you have any queries or concerns, please contact:

Professor Martin R Yeomans, Department of Psychology, University of Sussex, BN1 9QH. Tel: 01273 678617, email martin@sussex.ac.uk

Natalie Gould, Department of Psychology, University of Sussex, BN1 9QH. Tel: 01273 877031, email: n.gould@sussex.ac.uk

Remember, you may withdraw from the study at any time, without giving a reason

Volunteer Consent

I have read and had explained to me the attached information sheet, which I have signed and of which I retain a copy. The nature and purpose of the psychological testing has been explained to me. I am aware that I have the right to withdraw from the experiment at any time, although no payments will be made unless the experiment is completed.

I confirm that I will not be taking part in any other experiments at the Psychopharmocology Unit at any time during my participation in this study.

I fully understand the nature and purpose of the study and give my consent to participate.

Name: ______________________________

Signed: ______________________________

Date: ___ / ___ / ___

Phone Number: ______________________________

PLEASE COMPLETE THIS ONE HOUR AFTER LEAVING THE LAB, AND RETURN AT YOUR NEXT SESSION

Name: ___________________ **Date:__/__/20__ Session & time:__________**

Please complete the following rating scales by marking an 'X' at the point on the line which most accurately reflects how you feel at present. Please be as accurate as possible.

How Calm do you feel right now?

Not at all calm ____ Extremely calm

How Full do you feel right now?

Not at all full ____ Extremely full

How Lively do you feel right now?

Not at all lively ____ Extremely lively

How Hungry do you feel right now?

Not at all hungry ____ Extremely hungry

How Thirsty do you feel right now?

Not at all thirsty ____ Extremely thirsty

How Tired do you feel right now?

Not at all tired ____ Extremely tired

How Clear headed do you feel right now?

Not at all clear headed ____ Extremely clear headed

How Nauseous do you feel right now?

Not at all nauseous ____ Extremely nauseous

Name

Age

Height

Weight

What did you think was the purpose of the study?

Do you think the label accurately described the yoghurt in terms of energy content?

Did your opinion of the yoghurt change over the sessions?

Do you have any other observations?

Information for Subjects **Name:____________**

What you will be required to do

On five occasions you will be asked come to the lab for testing at a time between 8.15 and 10.00 in the morning. Each session should take between 15-30 minutes.

From 11 pm the night before each session you will be required to drink only water until you arrive at the lab for testing, and for one hour after leaving the lab.

Day 1 will involve a 10 minute screening session where you will be asked to taste and rate some yoghurt samples. This will determine whether you continue with the rest of the study (you will receive £2 or a course credit if not).

If you do continue with the study, you will be asked to complete some mood ratings, and then consume and rate a fixed portion of yoghurt (this will then be the same for days 2, 3 and 4). You will be required to rate your mood after consuming 5 will involve a taste test, and a debrief session.

The Breakfast

The breakfast will consist of yoghurt and fruit, made using standard ingredients.

You will receive £15 upon completion of all sessions. If you are a psychology undergraduate you can opt to receive 12 course credits, or a combination of credits and money instead.

Exclusion criteria
You should not take part if you:

1. Have participated in a previous study by Natalie Gould or Lauren Upjohn
2. are diabetic
3. are pregnant
4. are currently taking prescription medication (excluding the contraceptive pill)
5. have any known allergies or aversions to these common foods and food additives: sugar, aspartame, saccharin, food flavourings, food colourings, maltodextrin, dairy products (milk, cheese etc), fruit
6. have been diagnosed as having an eating disorder
7. smoke more than 5 cigarettes per day

IT IS IMPORTANT THAT YOU DO NOT SIGN UP TO ANY OTHER FOOD STUDIES WHILST PARTICIPATING IN THIS ONE.

If you have any queries or concerns, please contact:

Professor Martin R Yeomans, Department of Psychology, University of Sussex, BN1 9QH. Tel: 01273 678617, email martin@sussex.ac.uk

Natalie Gould, Department of Psychology, University of Sussex, BN1 9QH. Tel: 01273 877031, email: n.gould@sussex.ac.uk

Remember, you may withdraw from the study at any time, without giving a reason

Volunteer Consent

I have read and had explained to me the attached information sheet, which I have signed and of which I retain a copy. The nature and purpose of the psychological testing has been explained to me. I am aware that I have the right to withdraw from the experiment at any time, although no payments will be made unless the experiment is completed.

I confirm that I will not be taking part in any other experiments at the Psychopharmocology Unit at any time during my participation in this study.

I fully understand the nature and purpose of the study and give my consent to participate.

Name: ______________________________

Signed: ______________________________

Date: ___ / ___ / ___

Phone Number: ______________________________

PLEASE COMPLETE THIS ONE HOUR AFTER LEAVING THE LAB, AND RETURN AT YOUR NEXT SESSION

Name: ___________________ Date:__/__/20__ Session & time:__________

Please complete the following rating scales by marking an 'X' at the point on the line which most accurately reflects how you feel at present. Please be as accurate as possible.

How Full do you feel right now?

Not at all full ______________________________ Extremely full

How Lively do you feel right now?

Not at all lively ______________________________ Extremely lively

How much do you think you could eat right now?

Nothing at all ______________________________ A large amount

How Calm do you feel right now?

Not at all calm ______________________________ Extremely calm

How Thirsty do you feel right now?

Not at all thirsty ______________________________ Extremely thirsty

How strong is your desire to eat right now?

Very weak ______________________________ Very strong

How nauseous do you feel right now?

Not at all nauseous ______________________________ Extremely nauseous

How Hungry do you feel right now?

Not at all hungry ______________________________ Extremely hungry

Name Height
Age Weight

1) What did you think was the purpose of the study?

2) On days 1 and 5, you tasted small samples of yoghurt. Do you think either of these samples were the same yoghurt as the breakfast you later consumed?

3) Do you think the label accurately described the breakfast? (please circle)

Yes

No

Why/why not?

4) When you read the label, what were your expectations of how it would:

a) taste?

b) make you feel?

5) If a food is described as "high energy" or "low energy", what do you understand by the term 'energy'?

6) Do you think you were given the same yoghurt for breakfast every day? (please circle)

Yes

No

Why/why not?

7) How many calories do you think were in the breakfast you consumed each day? (Circle the answer you think is correct)

82kcal 164kcal 247kcal 330kcal 412kcal

8) Did your perception of the breakfast change across the sessions?

9) Was there anything you liked or disliked about the breakfast?

10) I would expect a " high energy" breakfast to: (please circle an answer for each)

a) contain sugar –	none	some	lots
b) give me energy –	none	some	lots
c) contain calories -	none	some	lots
d) increase alertness	-never	sometimes	alot

Please indicate which option from a)-d) you think is the most relevant

10) Any other observations/comments?

Name
Age

Height
Weight

1) What did you think was the purpose of the study?

2) On days 1 and 5, you tasted small samples of yoghurt. Do you think either of these samples were the same yoghurt as the breakfast you later consumed?

3) When you were given your breakfast, what were your expectations of how it would:

a) taste?

b) make you feel?

4) If a food is described as "high energy" or "low energy", what do you understand by the term 'energy'?

5) Do you think you were given the same yoghurt for breakfast every day? (please circle)

Yes
No

Why/why not?

6) How many calories do you think were in the breakfast you consumed each day? (Circle the answer you think is correct)

82kcal	164kcal	247kcal	330kcal	412kcal

7) Did your perception of the breakfast change across the sessions?

8) Was there anything you liked or disliked about the breakfast?

9) I would expect a " high energy" breakfast to: (please circle an answer for each)

a) contain sugar –	none	some	lots
b) give me energy –	none	some	lots
c) contain calories -	none	some	lots
d) increase alertness	-never	sometimes	alot

Please indicate which option from a)-d) you think is the most relevant

10) Any other observations/comments?