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Flavour-nutrient learning in humans:

An elusive phenomenon?

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ABSTRACT

One widely cited model of how humans acquire liking for different foods is flavour-nutrient learning, where associations between the orosensory properties of the ingested food or drink (the flavour CS) and positive consequences of nutrient ingestion (the UCS) leads to acquired liking for the flavour (flavour-nutrient hedonic learning: FNL-H). Likewise, an association between the CS and the post-ingestive effects of ingested nutrients has been suggested to lead to learning about how satiating a particular food is (flavour-nutrient satiety learning: FNSH). However, whereas there is evidence for both FNL-H and FNL-S in experimental studies with non-human animals, evidence in humans is less convincing, with many failures to find the predicted changes in liking, preference or intake following repeated flavour-nutrient pairings. The present short review considers how subtle differences in experimental design might underlie this inconsistency, and identifies key design features which appear to increase the likelihood of success in human flavour-nutrient learning studies. Key factors include CS novelty, the level of nutrients ingested during training, the appetitive state of the consumer and individual consumer characteristics. A further complication is competition between FNL-H and FNL-S, and with other associations such as flavour-flavour learning. From this it is possible to make important inferences about the nature of human flavour-nutrient learning which firstly suggest that it has important similarities to that seen in other species, but secondly that the laboratory investigations of both FNL-H and FNL-S in humans can be compromised by subtle but important variations in experimental design.

Running Head:

Human flavour nutrient learning

Key terms:

Flavour, conditioning, learning, nutrients

Humans and other animals, in particular omnivores, have a remarkable ability to identify which of the almost endless supply of potential foods in their environment are nutritious and worth consuming and which are poisonous and so should be avoided. However, as described in the Omnivore's Paradox [1], humans are not born with a pre-set menu of preferred foods, but instead have to learn what is safe and nutritious by balancing neophobia and inquisitiveness. Arguably the only component of our food preferences that appears to reflect an innate like is the case of a general preference for sweet tastes in humans and other species [2]. But evidence that not all humans do like sweet tastes [3] implies that even our initial liking for sweet tastes can be modified by experience, in line with reversal of an aversion to bitter tastes when the bitterness predicts a benefit, as is the case with caffeinated products [4].

So how then do humans acquire liking for nutrient-rich foods? Historically, many different models for how humans acquire liking for novel flavours have been proposed [5, 6] but in this brief review only one model, flavour-nutrient hedonic learning (FNL-H), will be considered as many have implied that this is likely to be the most important driver of acquired flavour liking [5]. In many reviews this form of learning is usually referred to purely as Flavour-Nutrient Learning (FNL), however here the acronym FNL-H is used to separate studies examining changes in liking or preference from those looking at how satiating different foods are. FNL-H was conceived as a form of classical conditioning where the orosensory characteristics of a novel food or drink acted as a conditioned stimulus (CS) predicting what the organism experienced post-ingestion (the detected effects of nutrient ingestion, acting as unconditioned stimuli UCS). In this way, FNL-H was a logical extension of the classic discovery of conditioned taste aversions (CTA), where a specific taste acted as a predictor of subsequent gastro-intestinal illness [7]. It followed that if a flavour could predict illness and

lead to an acquired aversion then ingestion of a novel flavoured food which was a useful source of nutrients should lead to an acquired preference.

Although liking for food is a major influence on ingestion [8], and therefore acquired liking may be an important driver of short-term intake, flavour-nutrient associations also provide the opportunity for acquisition of other associations that may also impact on ingestion. One such learned association has been described as learned satiety [hereafter referred to as Flavour Nutrient Satiety Learning, FNL-S: 9, 10]. Learning that a flavour predicts how filling a food might be has been suggested to both lead to adjustments in the size of meals taken with that food (sometimes referred to as learned satiation) or enhancements in the degree to which the food suppresses subsequent appetite (learned satiety). An important, and in humans unresolved, question is whether FNL-H and FNL-S reflect separate, competing forms of associations or reflect two expressions of the same learning process. Since the focus of the present review is on what factors appear to lead to successful experimental demonstrations of both forms of learning in humans, the first part of this review treats FNL-H and FNL-S as separate competing associations since in non-human animals there is evidence that these processes operate to some degree independently [11, 12]. The actual nature of these associations, and how they may be confounded in many studies, is then discussed.

In non-human animals there is abundant evidence for FNL-H [13, 14]. The most convincing studies, run over many years by Sclafani and colleagues, has rats drinking from two tubes of flavoured water. In this instance, consumption of one flavoured water leads to intra-gastric infusion of a nutrient solution, while consumption of the second flavoured solution leads to intra-gastric water infusion. The outcome of these studies is both clear and highly reproducible: intra-gastric infusions of fat [15, 16], various sugars [17-19], protein [20] and

alcohol [21, 22] all support a profound and enduring increase in preference for the nutrient-paired flavour. In animal studies, it has been argued that the important distinction between FNL-H and FNL-S is in the way these processes moderate behaviour, with the suggestion that FNL-S results in small meals that cannot be explained by reduced preference [23]. With this criterion, fewer studies have demonstrated FNL-S than FNL-H in non-human animals, but there have been several reports that fit with this conceptualisation of FNL-S [24-26] and other studies that also provide evidence of learned satiety in the rat [11, 27].

So if both FNL-H and FNL-S have been demonstrated in rats and other animals under controlled laboratory conditions, surely the same phenomena will be found in studies with humans? The main motivation for the present review is increasing awareness that studies of flavour-nutrient associations in humans appear to have had mixed success. While some studies do report changes in behaviour following repeated exposure to flavours paired with nutrient consumption that could be considered as consistent with FNL-H and/or FNL-S [28-35], increasing numbers of studies which follow very similar designs fail to find evidence of either FNL-H [36], FNL-S [10, 36] or both [37-39]. Overall, 9/14 (64%) of studies that report changes in liking or preference provided evidence for FNL-H, but only 3/12 (25%) reported changes consistent with FNL-S. Moreover, failures to find effects tend to be under-reported as they can often be dismissed by the researchers as inadequately controlled designs, and certainly there are several studies in our laboratory and elsewhere [40] that add to the body of evidence that both types of flavour associations are elusive in humans. Some possible explanations for this variability in study outcome have been the focus of recent reviews which have explored the importance of conscious awareness of the underlying association for learning to proceed [41], and whether there may be a critical developmental period during which this learning typically occurs [40], and these issues are not considered

further here. Instead the focus here is on potential methodological explanations for different outcomes of apparently very similar studies.

1.0 The design features of human flavour-nutrient studies

Table 1 summarises the key design characteristics and outcomes of studies that have explored both FNL-H and FNL-S in humans that we are aware of. The essential design of all of these studies is similar: the key contrast is how repeated consumption of a novel flavoured food or drink that has been supplemented with additional nutrients (interpreted as the positive conditioned stimulus, CS+) alters liking or preference for (FNL-H), or measures of intake of the flavoured food or a subsequent meal (FNL-S), for that flavoured food relative to changes in behaviour to a different flavour which predicts ingestion of less nutrients (the low-nutrient control, usually interpreted as the negative conditioned stimulus or CS- since it predicts a relative absence of nutrients). Some studies base these contrasts on exposure of the same participant to two distinct products, one paired with nutrient and one control (within-participants designs), while others rely on between-participants contrasts. In the present context, although some of the studies reviewed here are presented as examinations of liking acquisition while others are primarily focussed on learned satiety or satiation, the essential design of both types of studies are the same, with only the measures used to assess changes in behaviour differing. Thus if a study examines how much of a nutrient rich food is voluntarily consumed over repeated trials, the prediction from FNL-H might be that liking for that flavour will increase, although this measure is not always reported in studies of learned satiety. Conversely, studies that examine effects of repeated consumption on changes in hedonic evaluation of the flavour without allowing any opportunity to assess voluntary intake or subsequent appetite may find evidence of FNL-H but cannot assess FNL-S even though there was clear opportunity for satiety-related learning. Thus we review all studies, both

those focussed on FNL-H and FNL-S, as current theory suggests that both outcomes should be seen following repeated consumption of foods differing in nutrient content. There are a large number of experimental factors that might contribute to the likely success of such studies, and those considered here are:

1. How many training trials were used
2. Was a between or within-participants design used?
3. How novel was the test flavour(s)
4. How much additional energy was used to supplement the CS+
5. The motivational state of the consumer
6. The specific group of consumers being tested

The likely importance of each of these design issues is discussed to try and determine whether apparent failures to detect FNL-H or FNL-S could be explained by specific design features of successful and unsuccessful studies.

1.1. How many trials are needed to change behaviour towards a novel flavour through FNL-H or FNL-S?

Table 1 suggests wide variation in the numbers of trials used in studies of flavour-nutrient associations in humans. The basic principle of Pavlovian conditioning is that learning should proceed until a maximum association has been achieved, at which point behaviour should stabilise. Thus with FNL-H and FNL-S as with other models of Pavlovian learning the prediction might be that studies with more training trials (defined as occasions when experience of the CS is contingently paired with nutrient ingestion) will be more successful than those with fewer trials. Inspection of Table 1 does not support this contention. For studies focussing on FNL-H, some successful trials used only one or two pairings of flavour

and nutrient ingestion [34, 35, 42], whereas several of the studies which found no changes in liking used multiple trials [10, 39], and one notable study that was well-powered but found no changes in liking after exposure used 20 pairings each with CS+ and CS- [36]. One possible explanation for the relative lack of success in studies with more trials might be that monotony with the test food masks any possible increase in liking through FNL-H, since monotony has been shown to decrease liking in general following repeated exposure [43-47]. However, since some studies which used extended training have reported significant increased CS preference [28, 30], any effects of monotony cannot prevent FNL-H but may make them harder to detect. A similar pattern of results tends to emerge with FNL-S: some of the early successful studies used relatively few trials [e.g. 48], but more recent studies which found no effects used many more trials [10, 39]. It is harder to explain this finding through monotony. Some studies suggest that the sensory cue is important here: textural cues appear more effective than flavour per se [10]. Advances in sensory science have allowed for greater control over orosensory stimuli, and early studies lacked the sensory sophistication to test for such effects and so may have had stronger textural cues, for example. Regardless of the cause, the finding that both FNL-H and FNL-S are less easily observed with extended rather than brief training clearly confirm that it is not possible to dismiss studies that failed to find evidence for altered behaviour to flavour CS, either in terms of liking or intake, as due to insufficient training trials. Some have argued that, as with classic findings from the study of CTA [49], the importance of learning about nutrient effects may also result in a single trial being adequate for FNL-S [9]. Since both the flavour CS and nutrient consequence are relatively long-lasting stimuli, unlike the brief stimuli used in more traditional forms of learning, this idea is not inconsistent with current theories of the nature of association learning. Indeed, studies looking at changes in flavour liking through flavour-caffeine

associations have reported effects even after single CS-UCS pairings [50], clearly demonstrating that effects can be learned very quickly.

1.2. Was a between or within-participants design used?

Another design feature that varies between studies is whether the same participant is trained on different days with the nutrient-paired flavour (CS+) and control (CS-) in a within-participant design, or whether the same flavour is trained paired with nutrients in one group (CS+) and as a control in other groups (CS-) in a between participant design. Some have argued that within-participant designs offer a stronger test of the underlying associative nature, and are less prone to “pseudo-conditioning” effects where liking change reflects changes in the evaluation of the test foods which are due to factors other than flavour-nutrient associations [40]. Most of the studies reviewed here have used within-participant designs, but there is no clear difference in success rate of studies depending on this design feature: success rates for studies of FNL-H were 7/11 (64%) using within- and 100% (3/3) for between participants, and for FNL-S just 1/8 (15%) using within- and 50% (2/4) for between participants designs. Thus failures to find evidence of flavour nutrient learning is not easily attributable to the design style. The overall higher success rate for between-participant designs could be interpreted either as consistent with the principles of conditioning or as evidence in support of the claim that such designs are prone to pseudo-conditioning effects, with no way to discriminate such explanations from the literature as it stands. In terms of a learning explanation, within-participant designs would be predicted to require greater training as in the initial stages there is a tendency for generalisation between the CS+ and CS- stimuli, with discrimination (i.e. learning which cue predicts the UCS) emerging after multiple trials. With no competition between cues, differences between CS+ and CS- should be evident more

quickly in between-participant designs. Overall there is no evidence in the current data for any superiority of within-participant designs.

1.3. How novel was the test flavour?

Latent inhibition is a principle feature of Pavlovian learning, and refers to the consistent observation that prior knowledge that a cue (the CS) predicts nothing slows down (retards) the rate at which new associations are acquired. In relation to both FNL-H and FNL-S, no study in humans has specifically tested whether prior exposure to a flavour slows down subsequent acquisition of flavour-liking through FNL-H or learned changes in eating through FNSH probably because existing models are not consistent enough to allow such tests to be made with any confidence. However, in broader studies of affective learning (i.e. where pairings of a novel stimulus with a second stimulus which is already liked), stimulus pre-exposure attenuated subsequent changes in liking for the novel stimulus when paired with affectively-valenced stimuli [51-53]. However, all of these studies looked at liking change in the context of the picture-picture model of evaluative learning. One study which provided data consistent with latent inhibition in the context of human flavour-based learning came through the study of acquisition of liking for novel flavours by association with ingestion of caffeine. A large body of evidence suggests that people rapidly acquire liking for flavours paired with caffeine ingestion provided that they are in need of caffeine at the time of ingestion [4, 54]. One study noted in particular that the degree to which participants rated the tested flavour as novel predicted overall liking change in those studies [50], with those who were less familiar with the flavour at the start of testing showing the largest changes in liking, consistent with latent inhibition. However, this was based on a correlation between perceived novelty and subsequent liking change: a more robust test would have been to pre-expose one group of participants to a flavour without consequence.

One way of testing whether latent inhibition might occur in this context is to see whether learning is slower for more familiar flavours. To test this we re-analysed data from three of our recent studies of FNL-H in humans where we had collected ratings of flavour novelty at baseline and where there was an overall increase in flavour liking [33, 35, 55]. In these analyses, we calculated overall change in liking for the flavour used as CS+ only and looked at the correlation between novelty rating and change in the rated pleasantness for this CS+. If latent inhibition impacted liking change, then these correlations should be positive since greater novelty should have reduced the impact of latent inhibition and so resulted in faster learning. Table 2 shows the relevant correlations. It is notable that all three studies yielded positive correlations, but that lack of study power meant that these were only marginally significant. When the three studies were combined, however, the overall correlation (controlling for study) was positive and significant, consistent with the idea that those who were least familiar with the flavour CS showed the largest behavioural change in line with latent inhibition. This analysis makes many assumptions since the three studies differed in nutrient UCS, number of training trials and flavour CS. However, the positive value does give a clear indication that studies which failed to provide novel flavour CS, and crucially which did not test explicitly that the test participants evaluated the test CS as novel, are less likely to be successful.

Might a failure to control adequately for possible effects of CS familiarity explain some of the failures to find evidence of FNL-H or FNL-S in humans? As can be seen (Table 1), many studies describe the CS they use as novel but fail to provide evidence that their participants actually rated the test flavour as novel. Unless the experience of the novel CS in the overall flavour context is treated as novel, flavour-learning is likely to be retarded, and this level of

novelty therefore needs to be confirmed for each participant. For FNL-S, evidence that textural cues may be more important than flavour [10, 39] also makes studies more prone to latent inhibition if the flavour is novel but texture familiar, and so familiarity with the more salient cue in relation to FNL-S (texture) could prevent new learning about the actual satiating effects of the food. As consumers acquire a wider experience of flavours and textures through exposure in their diet, so it becomes more difficult to produce test stimuli that are truly novel to support these types of learning. Thus one possible explanation for the greater success seen in studies with children relative to adults is that prior experience leads to increased latent inhibition and so greatly reduces the likely success of studies in older consumers. Future studies thus need to do more than assume that stimuli are novel: they need to include specific tests of CS novelty in the study design.

1.4 How much additional energy was used to supplement the CS+

In humans, studies of flavour-nutrient associations rely on the incorporation of the additional nutrients in the CS+ condition into the ingested food or drink, in contrast to the elegance of infusing nutrients directly into the gut in the classic studies with rats [14, 56]. Adding additional nutrients in this way does raise serious issues. Firstly, in order to ensure that the presence of the additional nutrients is not obvious to participants, as this would confound any conclusions that could be drawn about changes in flavour evaluation, studies attempt to disguise any changes in sensory quality the nutrients generate. This places clear limitations firstly on what nutrients can be added in a way that can be effectively disguised, and secondly on the difference in the level of nutrient in the low-nutrient CS-condition and nutrient-rich CS+ condition. Table 1 shows that studies vary on both these measures. Moreover, many studies fail to report data demonstrating that nutrients were adequately disguised. Since it is becoming clear that subtle sensory differences impact on the degree to which nutrients

generate satiation [57-59] and satiety [60], orosensory cues generated by differences in viscosity between CS+ and CS- training conditions may therefore confound interpretation of consequent changes in liking and intake. Thus the failure to characterise sensory differences between CS+ and CS- conditions, and to consider these purely as differences in post-ingestive energy sensed after consumption could lead to misinterpretation of study outcomes.

An additional possible explanation for the variable outcome of the studies in Table 1 might also be that the relative difference in nutrient signal generated between the CS+ to CS- conditions was not easy to discern by participants in some studies. Support for this contention came from a recent study where we contrasted the effectiveness of two versions of a breakfast food (porridge) varying in energy density on subsequent liking and intake [35]. In that study, participants evaluated and consumed the foods ad libitum at two baseline sessions, and were then given two training sessions with each food to allow an opportunity to associate flavour and nutrient ingestion. Changes in liking and intake were re-assessed at two post-training sessions, thus allowing a test of both hedonic and satiety-based associations. During the training phase, the amount of food that was consumed differed between groups, with either a small (150g) or large (300g) training portion. The outcome was evidence for increased flavour liking in two conditions: for the higher energy cereal (the CS+) when trained as a smaller portion and the lower energy version (the CS-) trained as the larger portion. At face value these results do not fit with a general model of FNL-H because liking for the flavour of a food with less nutrients (the CS- in the larger serving condition) increased, whereas liking for the higher nutrient intake (the CS+ in the large portion condition) did not. That conclusion however is based on an implicit idea that it is the absolute level of nutrient ingestion that is the underlying reinforcer of changes in flavour liking through FNL-H. Our data suggest that the ability of nutrients to reinforce changes in flavour liking are biphasic, as

illustrated in Figure 1. Essentially the suggestion here is that there is an optimal level of nutrients that produce effects that are perceived as gratifying. Low levels of nutrients are unlikely to cause sufficient changes in the signalling systems that monitor post-ingestive effects of nutrient ingestion to allow contingent associations between flavours and these effects to be reinforced. Large quantities of nutrients in contrast might produce such strong post-ingestive signals that these are seen as aversive. Here a contingent association may form, but it is between a flavour and a mildly aversive post-ingestive state, which may lead to an acquired flavour dislike (in effect a mild CTA). Only where the nutrient load produces a post-ingestive effect that is large enough to be detected but not so large as to be aversive will FNL-H studies result in acquired flavour liking. Further support for this contention came from two studies in our laboratory where ingestion of a large amount of nutrients (a large bowl of energy-dense porridge cereal) resulted in both a decrease in rated flavour pleasantness across trials and evidence that the participants experienced some degree of nausea post-ingestion [34, 35]. Again some caution is needed in interpretation of the post-ingestive effect, with the suggestion that the key signal may arise through effects of maltodextrin on gastric osmolarity [61]. Since no study has specifically measured such changes, these ideas are clearly speculative. However, regardless of mechanism, the finding that there was an optimal level of nutrient difference between CS+ and CS- training conditions does provide a further complication in interpretation of human studies of FNL-H. In contrast, FNL-S might be expected to increase as nutrient load increases since satiety would be predicted to be proportional to the amount of ingested nutrients. The relative lack of success of studies examining FNL-S however means that this suggestion cannot be reliably tested from the literature as it stands.

Could these considerations of absolute levels of nutrients explain some of the variability in outcome of studies of FNL-H in humans? For this explanation to hold, it would be predicted that either the CS- had sufficiently high nutrient content to promote FNL-H by itself, or the CS+ had such a high level of nutrients that participants had to consume to produce aversive post-ingestive effects. Most of the studies that found no changes in behaviour post-training used relatively low nutrient content in the CS- condition, and a moderate level of energy in the CS+ condition so it seems unlikely that the lack of positive findings could be attributed to inadequate nutrient differences. In some studies whose focus was FNL-S but which included hedonic measures, over-satiation was prevented by allowing consumers to determine the amount of food consumed during training, but again found no evidence of learned changes in either liking or intake. One key study did use a large nutrient load which resulted in data that was interpreted as conditioned satiation but which may have also generated an aversive level of over-satiation [62].

Overall nutrient load does appear to be an important element of the outcome of laboratory-based studies of FNL-H in humans, and an assumption of “more is better” is inappropriate. However, this design feature alone does not readily discriminate successful and unsuccessful studies of FNL-H in humans.

1.5 The importance of current motivational state

In the context of changes in evaluation or intake of a flavoured product consumed in the context of investigation of flavour-based learning, one component of the design that may be critical is the extent to which the participant was in an appropriate motivational state at the point of consumption, and that the ingested nutrients reduced that state in a way that was either perceived as beneficial in the case of FNL-H or which was interpreted as satiety in the

case of FNL-S. Some research into flavour-nutrient associations in other animals has found evidence of state-sensitivity [63, 64], however other studies found comparable changes in flavour preference for flavours paired with intragastric nutrient infusions in both food-deprived and undeprived rats, suggesting that deprivation state is not necessary for learning in that situation in rats [65, 66]. However, some data in human studies provide evidence that current deprivation state is critical for changes in liking or preference [28, 33, 42]. For example, repeated consumption of a novel yoghurt with added fat lead to a clear increase in preference for the high fat flavour over a second flavour paired with low fat, but expression of this preference was dependent on the hunger state at test [28]. Likewise, liking for the flavour of a novel drink with added sucrose increased more when the drink was consumed in a hungry than sated state [33]. The general lack of positive findings with FNL-S does not allow any clear test of whether learning about satiety is also dependent on motivation state, although it was claimed in one study that the pattern of acceptance did depend on hunger state [62]. Also, although brief experience (1 training trial only) of a novel flavour with higher protein content did not alter behaviour (propensity to eat or intake), for those few participants who did increase desire to eat a high-protein food after training, this effect disappeared if they had consumed a high protein meal beforehand [42]. Could failure to test participants in an appropriate motivational state have confounded some studies of FNL-H or FNL-S? This seems unlikely since the majority of studies in Table 1 took care to control the level of intake before training trials to ensure that participants are receptive to the effects of ingestion. Overall there is some evidence that motivational state impacts on expression of FNL-H in humans, but little evidence that a failure to control for appetitive state can explain the mixed outcome of past studies of FNL in humans.

1.6 Individual differences in sensitivity to flavour-based learning

Could different sub-groups of participants show differential sensitivity to the specific CS-UCS contingencies that underlie both FNL-H and FNL-S? Certainly many of the studies include analyses that suggest that only some participants showed changes that were consistent with learning [42, 62], but have not been able to identify the cause of this variation.

Individual differences in flavour novelty are clearly one factor, as discussed earlier. But these can be explained by differences in diet history alone, and do not reflect a consistent group difference in sensitivity to these types of association. One individual difference which has been shown to modify sensitivity to FNL-H is dietary restraint, defined as the tendency to restrict food intake as a consequence of concerns about body weight. The original concept of dietary restraint suggested that the eating behaviour of weight-concerned individuals was regulated more by cognitive than physiological controls, with artificial cognitive limits imposed on how much they were allowed to consume [67]. The finding that women who scored highly on dietary restraint also showed insensitivity to manipulated energy in a study of FNL-H [31] suggests that habitual use of diet-related rules to control eating may lead to a relative insensitivity to internal cues needed to learn about consequences of ingestion. It is notable that although some of the studies summarised in Table 1 control for the influence of restraint, many do not. If the study participants comprised a mixture of restrained and unrestrained eaters, then the outcome would be much greater variance in the behavioural changes produced by CS-UCS pairings and this in turn would greatly reduce the power to detect effects. Although it is hard to dismiss the lack of evidence for changes in behaviour in some studies on this basis since there was no evidence of a non-significant change in behaviour [36], future studies should control for this possibility by either pre-screening participants for restraint and only testing unrestrained participants, or by stratifying the design to allow contrasts of behaviour by restraint status.

2.0 Multiple associations

2.1 Flavour preferences, learned satiation and satiety.

So far the review has treated FNL-H and FNL-S as two competing associations and it is now important to consider whether this assumption is supported by our broader understanding of how humans make dietary decisions. FNL-H suggests that every time a food is consumed that provides an acceptable level of nutrition then liking should increase. This in turn implies that liking should be positively related with energy density, and analyses of food preference data suggests this is so [68]. Greater liking usually results in increased food intake [8]. Thus a clear prediction of FNL-H would be that people will consume more of foods or drinks which have flavours that have been associated with nutrients, at least when tested in a hungry state..

But the concepts of learned satiation, satiety and acquired control of meal-size all suggest something different. In this review we defined learned satiation as enhanced reduction in appetite during an eating episode as a consequence of learned associations between the sensory quality of the food and its nutrient content. Learned satiety is then the enhanced suppression of appetite after ingestion of a food brought about by associations between the sensory quality of the food and its post-ingestive content. In practice learned satiation and satiety probably represent the same underlying associations but with different behavioural outcomes: either reduced appetite and intake of the food itself or reduced intake and decreased appetite at a subsequent meal. For that reason, both types of study were defined as examples of FNL-S. Classically, based on the detailed analysis of meal patterns in rats experiencing a change from a low to high protein diet, it was found that rats developed a preference for the high-protein diet but ate smaller meals with this diet than the low-protein diet [24], which can be interpreted as co-expression of an acquired flavour preference and

learned satiation. But the effect of reduced intake is the converse of the predicted effects of enhanced flavour liking on intake.

How do we reconcile these two apparent different effects on intake, and which is then seen in human studies? Firstly, is there evidence that acquired liking leads to enhanced intake for liking acquired by associations between flavour and nutrient? Several recent studies suggest this is so [34, 35, 55]. For example, liking for the flavour of a drink that had been acquired by repeated experience of that drink with either added non-sweet (maltodextrin) or sweet (sucrose) carbohydrate resulted in increased intake of a novel food (sorbet) with the same flavour [55]. Is there also evidence that flavour-nutrient associations result in increased flavour liking but reduced intake? One study could be interpreted as evidence supporting that idea [62], and a further study provided evidence of an acquired ability to regulate intake after repeated consumption of a higher nutrient food [69], although changes in liking were not reported in that study. Other studies however have failed to find evidence of conditioned satiety [10, 36, 37, 39]. One study in particular has proved contentious [35]. In that study, repeated consumption of a large energy-dense breakfast resulted in reduced intake at post-test, replicating an earlier related study [34]. It was subsequently suggested that this was clear evidence to support the conditioned satiety concept [61]. However, in both studies the factor that best predicted intake was the change in pleasantness for the test flavour at the outset of eating, with no evidence that people grew to like an energy-rich food but then consume less of it. In contrast, where the effects of ingestion were a reduction in appetite in the absence of over-satiation (i.e. no experience of uncomfortable levels of fullness and consequent nausea), liking when the food was first tasted and intake both increased. Thus there was no evidence of increased liking and more rapid satiation when flavours predicted higher nutrient content in those studies.

It has been claimed that a critical issue here is the timing of flavour-nutrient associations relative to the stage of a meal [9]. The argument has been that FNL-S reflects relative expression of dislike for flavours that are associated with greater satiety if those flavours are experienced later in a meal. For this reason, studies which purport to demonstrate FNL-S in humans explicitly examined acceptance of nutrient-paired flavours when experienced in the latter phases of a meal [48, 62]. Although the outcome of these studies implies, at least in those participants who showed some evidence of having acquired the flavour-satiety association, reduced acceptance of flavours under these conditions, it does not preclude acquired liking for the same flavour if tested when hungry. But this does raise a puzzling issue: if expression of liking and intake for foods which people have learned to be satiating is reduced in a replete state, surely this implies that diet selection at the end of a meal would be for reduced energy foods? Culinary practice, in contrast, is to end meals with foods with very high energy density (sweetened desserts and cheese) where learned satiety would predict low acceptance. If our liking for these energy-dense foods is through prior associations of flavours and nutrients through FNL-H, and this acquired liking drives intake, then it could be argued that conditioned hedonic response seems more important than learned satiety in controlling our normal eating habits.

The discussion of FNL-S so far does not preclude the possibility that separate hedonic and satiety associations develop, but instead argues that under the specific conditions of laboratory based testing, hedonic associations appear somewhat easier to measure than do effects on satiation and satiety. There is now evidence that people do have clear and testable expectations about satiety that are clearly learned [70, 71], and indeed there is specific evidence that repeated consumption of foods varying in nutrient content both alter these

expectations and the anticipated portion size that would be needed to make people full [72], perhaps the most elegant demonstration of FNL-S in humans to date. However, critically the same study did not measure whether the same learning resulted in actual increased liking for the flavour of the food, and actual intake was unaltered by these changes in expected satiety. Further studies of this type are needed to test more fully whether hedonic and satiety-based learning operate as part of a single underlying learning process or as separate dissociable processes.

2.2 Flavour-flavour learning (FFL)

Alongside the potential for separate FNL-H and FNL-S associations complicating interpretations of effects of repeated consumption, there are other possible associations that are based more on the effects of added nutrients on the sensory quality of the ingested food. Repeated experience of novel flavour elements alongside known liked or disliked tastes can modify liking for the novel element when tested alone (flavour-flavour learning, FFL: [73-76]. In these studies, pairing of flavours with aversive flavour elements leads to an acquired flavour dislike whereas pairing with a liked sweet taste can lead to an acquired flavour like, although the latter is less consistent across studies [see 77 for recent review]. The issue here is whether inadequate disguise of added nutrients could lead to subtle flavour cues that themselves lead to changes in response to the flavour CS which is then interpreted as evidence of either FNL-H or FNL-S, but is actually a consequence of FFL. The use of sugars or other sweet-carbohydrates as UCS in some of the studies in Table 1 may be a particular issue since sweet-likers who experience enhanced sweetness during training may increase liking through FFL which could be interpreted as nutrient effects supporting FNL-H. It has been possible to dissociate these two effects experimentally: liking and intake of a novel food (sorbet) was increased more in a condition where both FFL and FNL-H were likely (when the CS+ flavour

had been associated with ingestion of sucrose) than either for FNL-H (non-sweet carbohydrate maltodextrin) or FFL (aspartame) alone [55]. Another recent study also looked at the issue of these types of interactions. Participants consumed crackers with cream cheese with an added bitter taste either in high or low fat versions, and subsequently evaluated liking for low fat cream cheese in the absence of the bitter taste [32]. The authors interpreted the cream cheese/cracker combination as CS and either bitter taste or nutrient content as competing UCS. At test participants trained with the high fat crackers rated the low-fat crackers as less aversive than did those trained with low fat. This was interpreted as evidence that a nutrient-based association was more effective than FFL, although the absence of non-aversive training conditions did not allow an assessment of whether the bitterness had impacted on the hedonic evaluations. The paper also did not report the amount consumed in training or nutrient differences between high and low fat conditions. Overall, the limited literature looking at interactions between FFL and flavour-nutrient associations to date suggest that these processes seem to operate in tandem.

Could inadequate control for the effects of adding nutrients result in training stimuli that have overall aversive or pleasant hedonic qualities that confound interpretation of nutrient-based learning? Many studies fail to report the extent to which attempts to disguise the added nutrient was successful, and/or fail to report hedonic ratings for the training CS/UCS pairings. Such studies need to be interpreted with particular caution since apparent support for changes in behaviour arising from nutrient-associations may be confounded by inadvertent effects of FFL.

3.0 The optimal design of studies of flavour-nutrient learning in humans

Overall, the discussion how the minutiae of design of studies of human FNL-H and FNL-S may impact on outcome allows some suggestions of how future studies should be designed. Inspection of Table 1 might suggest, as discussed previously [40], that the most important feature determining the outcome of these studies in humans is participant age: only one of the published studies that failed to find changes in outcome was conducted in children. As already discussed, latent inhibition remains a plausible basis for this difference between age groups, although cannot readily explain the one study with children that found no evidence of FNL-H or FNL-S [e.g. 38]. Testing children in this context does raise issues however. Firstly, young children are less able to make subtle discriminations between cues to allow more sensitive measures of liking and intake to be made, and secondly ethical considerations limit the types of manipulations that can be conducted with younger participants. Moreover, if latent inhibition is the key difference between different age groups, then the solution is to ensure that flavour CS are truly novel for the actual participant both by the use of truly novel foods and drinks, and with specific tests built into the study design to confirm that participants did experience these as novel. There are increasing numbers of studies clearly providing evidence of FNL-H in adults, but it is also clear that particular care is needed in such studies to guard against latent inhibition. In contrast, FNL-S remains evasive in humans, with many more failures to find such effects even in well designed and powered studies.

The level of nutrients in both the baseline (CS-) and test (CS+) also appear critical, and ensuring that there is adequate difference between CS+ and CS- is important, while ensuring the absolute level of nutrients in the CS+ is not so great as to be perceived as generating aversive post-ingestive effects, such as nausea. Likewise, ensuring that participants are sufficiently hungry to perceive effects of nutrient ingestion as a positive experience is

important. There also has to be clear evidence that the addition of nutrients was adequately disguised so that the CS alone and combined CS/UCS combination is equally pleasant, and ideally as neither liked or disliked, else the study confounds FFL with flavour-nutrient effects. Finally, characterising individual participants in terms of restraint, and possibly other individual differences such as body-size, and the extent to which food is significant for that consumer (for example using the sub-scales of the Power of Food Scale [78]) will be important. Ensuring all of these features are in place in future studies of human FNL-H and FNL-S may not guarantee successful study outcomes, but at least guard against some of the pitfalls in experimental design identified in this review.

4.0 Summary

Both FNL-H and FNL-S in humans remain elusive, with several large, well-designed studies failing to find effects that smaller, older studies reported. This review suggests that FNL-S is the more difficult phenomena to find, with a much higher proportion of studies examining FNL-S reporting negative outcomes than is the case for FNL-H. Some caution is needed in interpretation of this observation, however, since several of the more recent studies of FNL-S did not report measures of hedonic change alongside measures of satiety, and discussion with the authors of some of those studies suggest that there was no hedonic change either, implying that the failure rate for FNL-H is much higher than the published data suggest. However, rather than treating these findings as evidence that these learning processes are not relevant to the everyday behaviour of adult consumers as some have implied [36], what this review highlights is that careful consideration of the design of studies in the human flavour learning literature has allowed identification of some of the key design features of successful studies. Some of these features, such as the critical importance of CS novelty and importance of current appetitive state, are precisely what would be predicted from theoretical consideration

of the learning process underlying flavour-nutrient associations. Others, such as the resistance to FNL-H seen in restrained eaters, are less easy to explain.

For the sake of brevity, the present review has not discussed how different outcome measures such as the use of rated liking, ranked preference or intake might also be critical for FNL-H, and there may be many other key features of design that will emerge as new findings are published. One such issue which might be examined in a future review would be the degree to which the variability in the human literature reflects differences in response to macronutrients. It is now widely accepted that different macronutrient sources have different impacts on satiety [79, 80]. For example, protein is generally found to be more satiating than carbohydrate [e.g. 81, 82], and fat has often been reported as least satiating [e.g. 83, 84], although as with the learning studies reviewed here not all studies find evidence of differences in effects of macronutrients on satiety [85-87]. Other studies have reported differences within macronutrient classes but depending on specific nutrient types: for example whey protein has been reported as more satiating than casein [88]. The studies in Table 1 use a wide variety of nutrient sources, and again this variation may have impacted on study outcome although notably when maltodextrin was used, there are more positive [30, 34, 35, 62, 89] than negative [38] outcomes, but several [10, 39], but not all [28], of those studies those using fat as the energy source failed to find evidence of learning.

Overall, the use of failures to find evidence for flavour nutrient learning to dismiss these types of association as an important component of how we acquire liking for, and regulate intake of, different foods and drinks is clearly not justified given the increasing numbers of studies which show changes in behaviour through these types of associations. Future studies might

use the suggested design features outlined here to increase the likelihood that we will better understand the nature of flavour-nutrient associations in humans.

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Table 1. Key design features and outcomes of studies examining changes in flavour liking or preference, or for evidence of enhanced satiation or satiety, through flavour-nutrient associations in humans.

Study	FNL-H, FNL-S or both? ^a	Design	Flavour CS	Nutrient UCS	Novelty?	Trials	Participants	Measures	Outcome
Booth et al. 1976 [48]	FNL-S	Changes in intake of a test meal after consumption of a disguised high- or low- nutrient load within participants.	Distinctly flavoured yoghurt desserts consumed as part of a test lunch	Energy content of preload consumed prior to lunch: actual energy values not specified	Implied but not tested	2 with each energy preload	Sample size not specified	Change in lunch intake	Reduction in lunch intake in response to disguised energy manipulation increased with training.
Booth et al. (1982) [62]	FNL-S	Experiment 1 (E1): changes in intake either after preload or with one lunch item augmented with starch within subjects Experiment 2 (E2): changes in liking for and intake after soup preload within subjects	Distinctly flavoured soup preload and dessert test food	Energy enhanced by addition of starch but quantity not specified	Not tested but unlikely	E1: 4 pairings E2: 1 trial with each energy load	E1: 8 men and 8 women E2: 14 men and 18 women	E1: change in intake and liking for lunch E2:	E1: Reduced intake after enhanced soup preload increased over trials but no effect for enhanced dessert. E2: no significant change in soup pleasantness
Birch et al. (1990 [30])	FNL-H	Ranked preference of 6 drinks before and after exposure to one drink (CS+) with added energy and one (CS-) without.	2 distinct flavoured drinks	High 155 kcal: Low 5 kcal: Energy added as maltodextrin	Implied but not tested	8 with each stimulus	5 boys and 6 girls aged 3-5 years	Rank preference for CS+ and CS-	Preference for CS+ increased but remained unchanged for CS-
Johnson et al. (1991) [90]	FNL-H	Two experiments both measuring ranked preference of 5 novel yoghurts before and after exposure to one yoghurt (CS+) with added energy and one (CS-) without.	2 distinct flavoured yoghurts	Experiment 1: High 265 kcal: Low 136 kcal. Experiment 2: High 230 kcal: Low 110 kcal	Not tested	8 with each stimulus	Experiment: 1: 5 boys and 7 girls aged 3-4 2: 6 boys and 3 girls aged 2-3	Rank preference for CS+ and CS-	In both studies, larger increase in preference for CS+ than for CS-
Kern et al. (1993) [28]	FNL-H	Liking for high-fat and fat-free yoghurts evaluated before and after six exposures to each version within participant.	5 distinct colour/flavour yoghurts ranked at pre-test: two mid-ranked flavours used as	High 228 kcal: Low 66 kcal: Energy added as canola oil	Not reported	6 with each stimulus	15 boys and 12 girls aged 4-5 years	Rank preference for CS+ and CS-	Ranked preference for CS+ increased more than for CS-

			CS						
Gibson et al. (1995) [42]	FNL-S	Rated desire to eat and intake of novel soup and blancmange differing in protein content	Novel foods varying in flavour and colour	Minimal energy difference: difference in protein content between High and Low conditions	Not tested	1 with each stimulus	7 men and 10 women	Change in rated propensity to eat and intake	No significant overall change in propensity to eat or intake between high and low protein conditions.
Specter et al., (1998) [37]	FNL-H and FNL-S	Preference for and intake of two different flavoured ice-creams, high fat CS+ and low-fat CS-, before and after repeated exposure within participants.	Ice-cream varying in fat content	High: c. 408kcal Low: 2.93 kcal	Not tested	6 with each stimulus	16 men	Rank preference for and intake of CS+ and CS-	No significant difference in flavour preference
Zandstra et al. (2002) [36]	FNL-H and FNL-S	Effects of exposure on preference for and intake of yoghurts with high or low energy within participants.	Distinctly flavoured yoghurt	High: 273 kcal Low: 67 kcal Energy added as fat + sugar	Not tested	20 with each stimulus	31 men and 38 women	Compensatory eating at subsequent test meal plus liking	No significant differences between CS+ and CS- on any measure
Yeomans et al., (2005) [34]	FNL-H and FNL-S	Effects of exposure on liking for low or high energy breakfast cereal contrasted within-participant (Experiment 2).	Distinctly flavoured cereal (porridge)	High 614 kcal: Low 261 kcal Energy added as maltodextrin + sugar	Implied but not reported	2 with each stimulus	16 men	Intake and rated liking for CS+ and CS-	CS- significantly more pleasant than CS+ post training.
Appleton et al. (2006) [29]	FNL-H	Two levels of energy combined with two levels of energy requirement tested within-participant.	Eight novel yoghurt flavours	High 162 kcal. Low 105 kcal. Energy added as skimmed milk powder:	Tested	5 with each pairing	12 students (no gender details)		Increased liking for CS+ both in laboratory and real world setting
Mobini et al. (2007) [33]	FNL-H	Evaluation of novel fruit tea before and after home consumption of tea in one of 3 training conditions: 1. Sweetened with sucrose (energy CS+) 2. Sweetened with aspartame (sweetness only CS+) 3. Unsweetened (CS-) Drinks consumed at home when	Novel fruit tea	High 132kcal: Low 40kcal. Energy added as sucrose	Tested	4	18 men and 42 women, all sweet likers	Rated liking for CS+ and CS-	Largest increase in liking for CS+ when drink had been consumed with added sugar when hungry.

		hungry or sated using between-participant design.							
Brunstrom & Mitchell (2007) [31]	FNL-H	Evaluation of liking for novel high- and low-energy desserts before and after training using within-participant design.	Two distinctive novel desserts	High 450 kcal: Low 54 kcal.	Tested	3 with each stimulus	44 normal weight women classified as high or low in dietary restraint		Increased liking for the CS+ in unrestrained women only.
Yeomans et al. (2008) [55]	FNL-H and FNL-S	Evaluation of novel food (sorbet) before and after training with the same flavour in one of four conditions in between participants design: 1. Energy added as sucrose (CS+ energy and sweetness, 159kcal) 2. Energy added as maltodextrin (CS+ energy, 159kcal) 3. Aspartame (CS+ sweetness, 7kcal) 4. CS- (7kcal)	Novel cranberry and mandarin sorbet and drink	High 159kcal: Low 7kcal. Energy added as sucrose or maltodextrin.	Tested	4	60 women, all sweet likers, unrestrained and normal weight	Intake and liking for CS+ and CS-	Increased liking for CS+ in sucrose condition, marginal increase for CS+ paired with maltodextrin
Yeomans et al. (2009) [35]	FNL-H and FNL-S	Effects of exposure on liking for low or high energy breakfast cereal contrasted within-participant, with trained portion size between participants.	Distinctly flavoured cereal (porridge)	High either 255 or 510 kcal. Low 95 or 189 kcal. Energy added as maltodextrin + sucrose	Tested	2 with each stimulus	48 unrestrained normal weight men	Intake and liking for CS+ and CS-	Increased liking for CS+ in small portion condition, but for CS- in large portion condition
Zeinstra et al. (2009) [38]	FNL-H and FNL-S	Preference and intake of two novel fruit juices before and after training tested within participants.	Novel vegetable juice	High: 170-187 kcal Low: 20-37 kcal Energy added as maltodextrin	Implied but not tested	7 with each stimulus	7 boys and 12 girls	Intake and ranked preference	No change in intake or ranked preference.
Mars et al. (2009) [39]	FNL-H and FNL-S	Preference ranking and compensatory eating following repeated exposure	Yoghurt beverages	High: 150 kcal/100g Low: 50	Implied but not tested	10	8 men and 38 women, Normal	Intake and preference for CS+/CS-	No change in relative preference between high and low energy

		to high and low energy yoghurts with low or high viscosity tested within participants.		kcal/100g. Energy added as cream			weight.		flavours
O'Sullivan et al. (2010) [91]	FNL-H	Consumption of reduced energy (374 kcal) or standard energy (567 kcal) serving of familiar food, between participants.	Spaghetti Bolognese flavour plus novel food label	Meal consumed as lunch differing in energy content	Familiar flavour, novel label	5	9 men and 27 women, Normal weight		Liking for CS- (reduced energy lunch) decreased.
Hogenkamp et al. (2010) [10]	FNL-S	Mixed design with intake and liking for high-energy (CS+) and low energy (CS-) contrasted within participant but mode of consumption tested between group.	Novel flavoured yoghurts	High: 150kcal/100g Low: 41kcal/100g Energy added as cream	Tested in pilot but not reported for test.	10 with each stimulus	105 healthy young adults divided into 3 groups	Intake of CS+/CS-	No changes in intake with exposure.
Zandstra et al. (2011) [89]	FNL-H	Within participants high CS+ versus low CS- breakfast yoghurt drink	Identical drinks but visual cue to discriminate	High 255kcal: Low 57 kcal. Energy added as maltodextrin	No	5 CS+, 5CS-	22 men and 22 women. Normal weight		Greater choice of high-energy labelled (CS+) drink after training: CS+ rated as more pleasant.

Footnotes: (a) This column denotes the primary focus of the study: liking change (FNL-H) or altered satiation/satiety (FNL-S)

Table 2. Correlations between flavour novelty and change in liking in three studies of flavour-nutrient learning in humans.

Study reference	CS+ condition	Sample	Correlation	Significance (p)
[33]	Novel fruit tea with added sucrose (additional 92 kcal)	10	0.37	0.29
[55]	Flavoured drink with added sucrose (additional 152 kcal)	12	0.48	0.056+
[35]	Novel flavoured porridge with energy added as maltodextrin (additional 160 kcal)	12	0.40	0.091
Combined data	Partial correlation controlling for study.	32	0.41	0.008

Figure 1. Schematic representation of how the relative size of a nutrient load may impact on conditioned flavour liking and consequent intake based on the design of [35]. Conditions varied in energy density (low: LED, high: HED) and portion-size (small or high).

