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Restriction of dietary protein leads to conditioned protein preference and elevated palatability of protein-containing food in rats

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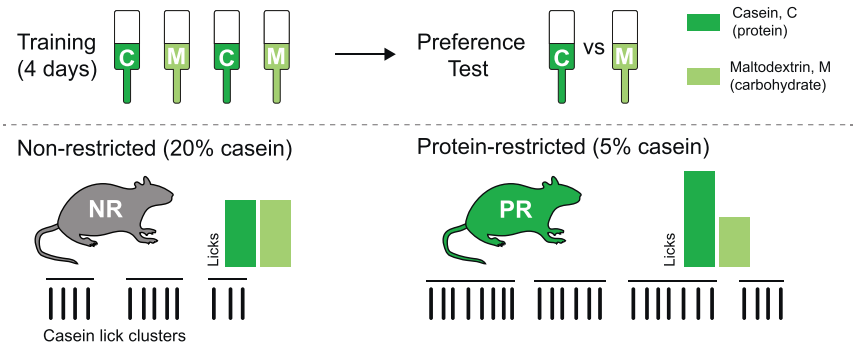
GRAPHICAL ABSTRACT



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ABSTRACT

The mechanisms by which intake of dietary protein is regulated are poorly understood despite their potential involvement in determining food choice and appetite. In particular, it is unclear whether protein deficiency results in a specific appetite for protein and whether influences on diet are immediate or develop over time. To determine the effects of protein restriction on consumption, preference, and palatability for protein we assessed patterns of intake for casein (protein) and maltodextrin (carbohydrate) solutions in adult rats. To induce a state of protein restriction, rats were maintained on a low protein diet (5% casein) and compared to control rats on non-restricted diet (20% casein). Under these dietary conditions, relative to control rats, protein-restricted rats exhibited hyperphagia without weight gain. After two weeks, on alternate conditioning days, rats were given access to either isocaloric casein or maltodextrin solutions that were saccharin-sweetened and distinctly flavored whilst consumption and licking patterns were recorded. This allowed rats to learn about the post-ingestive nutritional consequences of the two different solutions. Subsequently, during a preference test when rats had access to both solutions, we found that protein-restricted rats exhibited a preference for casein over carbohydrate whereas non-restricted rats did not. Analysis of lick microstructure revealed that this preference was associated with an increase in cluster size and number, reflective of an increase in palatability. In conclusion, protein-restriction induced a conditioned preference for protein, relative to carbohydrate, and this was associated with increased palatability.

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1. Introduction

There is considerable evidence that of the three macronutrients dietary protein is most tightly regulated [1–3]. As such, when presented with diets that differ in macronutrient content, rats will adjust their consumption to ensure that protein intake meets a baseline level [4]. The mechanisms by which these adjustments occur are still not fully understood.

An important outstanding question is whether the drive for protein is immediate and innate or whether there is a role for learning using post-ingestive consequences [5,6]. Some evidence suggests that when protein-restricted a specific appetite for protein arises, similar to the appetite for sodium that arises under conditions of sodium depletion. Rats have been shown to rapidly increase their intake of a number of protein sources when protein-restricted in a manner that precludes using post-ingestive effects to guide their intake [7]. Further research suggested these rapid effects on protein appetite were driven by olfactory cues [8]. However, a large body of evidence indicates that adjustments to protein intake are slow, require experience with each food/diet, and likely involve post-ingestive feedback. For example, when allowed to select between diets that differ in protein content, it takes rats several days to adjust their intake appropriately [9]. This adaptation is more rapid in young rats, although still not immediate, presumably because protein requirements are elevated early in development and positive post-ingestive feedback is enhanced.

The majority of the above studies have assessed food intake and diet selection in home cage tests in which diets are given ad libitum. This arrangement does not allow precise monitoring of lick patterns over time. Sophisticated analysis of lick patterns, or lick microstructure, is a key method for assessing palatability of solutions in rodents [10]. As such, when individual licks are grouped into runs based on interlick intervals (termed bursts, clusters and bouts), increases in palatability are associated with longer runs of licking. Importantly, with respect to protein appetite, lick microstructure has not yet been investigated.

Learned shifts in the palatability of protein or protein-containing foods could contribute significantly to increased protein intake under protein-restriction. As a striking example, when rats are sodium-depleted normally aversive concentrations of sodium chloride become highly palatable [11]. Moreover, learning an association between conditioned flavors and intragastric infusions of glucose leads to an increase in palatability of the flavors paired with positive post-ingestive consequences [12,13]. However, increased intake is not always associated with shifts in palatability. For example, rats made deficient in a single essential amino acid increase their intake of the missing amino acid but this is not associated with an increase in palatability [14].

Here, we have used analysis of lick patterns to assess the effect of protein restriction on intake and palatability of isocaloric protein- and carbohydrate-containing solutions in adult rats. We find that protein-restricted rats, relative to controls, develop a learned preference for protein-containing solutions over carbohydrate and this is associated with an increase in relative palatability.

2. Materials and methods

2.1. Animals

Forty adult male Sprague-Dawley rats were used for experiments (Charles River; > 275 g at start of experiment). Twenty-four of these rats were used for the main behavioral experiment and a further sixteen contributed to the food intake data. Rats were group-housed (2–3 per cage) in IVCs with bedding materials as recommended by NC3R guidelines. Temperature was 21 ± 2 °C and humidity was 40–50% with 12 h:12 h light/dark cycle (lights on at 07:00). Water was available ad libitum; chow containing different protein:carbohydrate ratio was available ad libitum (details below). All experiments were covered by the Animals [Scientific Procedures] Act (1986) and carried out under the appropriate license authority (Project License: 70/8069).

Table 1
Experimental diets used in study. List of ingredients (upper) and macronutrient breakdown (lower) in control diet (#D11051801; 20% casein) and protein-restricted diet (#D11092301; 5% casein).

	D11051801 (control, 20% casein)	D11092301 (protein-restricted, 5%)
Ingredient	g/kg	g/kg
Casein	200	50
L-Cystine	3	0.75
Corn starch	375.7	485
Maltodextrin 10	125	150
Sucrose	107.1	107.1
Cellulose	50	50
Soybean oil	25	25
Lard	75	75
Mineral mix S10022C	3.5	3.5
Calcium carbonate	12.5	8.7
Calcium phosphate, dibasic	0	5.3
Potassium citrate	2.48	2.48
Potassium phosphate, monobasic	6.86	6.86
Sodium chloride	2.59	2.59
Vitamin mix V10037	10	10
Choline Bitartrate	2.5	2.5
FD&C Yellow dye #5	0.05	0
FD&C Red dye #40	0	0.05

	g (%)	kcal (%)	g (%)	kcal (%)
Protein	18	18	5	4
Carbohydrate	62	60	76	74
Fat	10	22	10	22

2.2. Diet manipulations

All rats were initially maintained on standard laboratory chow containing 20% dietary casein. To induce a state of protein restriction in half of the rats, standard chow was switched for one of two experimental diets based on modified AIN-93G that differed in protein:carbohydrate ratio (Table 1) but were isocaloric (4.1 kcal/g). Non-restricted diet (#D11051801, Research Diets, New Brunswick, NJ) contained 20% casein whereas protein-restricted diet (#11092301, Research Diets) contained 5% casein. Body weight data were collected daily throughout the experiments. As rats were group-housed, food intake data were collected by cage and divided by the number of rats in the cage to give an average intake per animal. Conditioning experiments started 2 weeks following diet switch.

2.3. Behavioral testing

All testing took place within standard operant chambers (in cm: 30.5L, 24.1D, 21.0H; Med Associates, St. Albans City, VT) equipped with a house light and two bottles. Each bottle was connected to a contact lickometer calibrated to detect individual licks. Licks were recorded on a computer for all sessions as a measure of intake. All sessions lasted for one hour. For one to three days at the start of each experiment, rats were placed in the chambers with 0.2% sodium saccharin in both bottles to familiarize them with the apparatus. Following this, rats underwent a series of conditioning sessions and a preference test. In conditioning sessions, which occurred in a block of 4 days, only one bottle each day was available and was filled with either protein-containing solution (4% casein + 0.21% methionine + 0.2% sodium saccharin + 0.05% flavored Kool-Aid) or an isocaloric carbohydrate-containing solution (4% maltodextrin + 0.2% sodium saccharin