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Body weight loss, effective satiation and absence of homeostatic neuropeptide compensation in male Sprague Dawley rats schedule fed a protein crosslinked diet

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ABSTRACT

Food structure contributes to the induction of satiation and the maintenance of satiety following intake of a meal. There is evidence from human studies that protein-crosslinking of a milk-protein based meal may enhance satiety, but the mechanism underpinning this effect is unknown. We investigated whether a rat model would respond in a similar manner and might provide mechanistic insight into enhanced satiety by structural modification of a food source. Rats were schedule fed a modified AIN-93M based diet in a liquid form or protein-crosslinked to produce a soft-solid form. This was compared to a modified AIN-93M solid diet. Average daily caloric intake was in the order solid > liquid > crosslinked. Body composition was unaltered in the solid group, but there was a loss of fat in the liquid group and a loss of lean and fat tissue in the crosslinked group. Compared to rats fed a solid diet, acute responses in circulating GLP-1, leptin and insulin were eliminated or attenuated in rats fed a liquid or crosslinked diet. Quantification of homeostatic neuropeptide expression in the hypothalamus showed elevated levels of *Npy* and *Agrp* in rats fed the liquid diet. Measurement of food intake after a scheduled meal indicated that reduced energy intake of liquid and crosslinked diets is not due to enhancement of satiety. When continuously available ad-libitum, rats fed a liquid diet showed reduced weight gain despite greater 24 h caloric intake. During the dark phase, caloric intake was reduced, but compensated for during the light phase. We conclude that structural modification from a liquid to a solidified state is beneficial for satiation, with less of a detrimental effect on metabolic parameters and homeostatic neuropeptides.

1. Introduction

Both human and rat studies show that satiation and satiety are a complex integration of the characteristics of food, including form, volume, caloric and macronutrient content (Gerstein, Woodward-Lopez, Evans, Kelsey, & Drewnowski, 2004; Marmonier, Chapelot, & Louis-Sylvestre, 2000; Phillips & Powley, 1996; Rolls et al., 1998; Westerterp-Plantenga, Lejeune, Nijs, Van Ooijen, & Kovacs, 2004). Evidence from human studies shows that caloric intake and subsequent satiety properties of a meal are directly influenced by the structural and textural characteristics of the food presented which can have effects on hormonal profiles, metabolic responses and appetite ratings (Martens, Lemmens, Born, & Westerterp-Plantenga, 2012, 2011; Mattes & Rothacker, 2001; Mattes, 2008; Moukarzel & Sabri, 1996; Wilkinson, Dijksterhuis, & Minekus, 2001).

There are various ways that food structure and texture can be altered for different requirements. Although the percentage content and amino acid composition of protein in a diet can be varied, dietary protein also provides an opportunity to manipulate the physical characteristics of food, for example following enzymatic crosslinking (Buchert et al., 2010). Several enzymes have been employed for protein crosslinking, including the naturally occurring enzyme transglutaminase (TG) (Kuraishi, Yamazaki, & Susa, 2001; Simpson, Rui, & XiuJie, 2012, pp. 327–361). TG can alter the structure and texture of food high in protein by increasing viscosity and causing gelation (Buchert et al., 2010; Kuraishi et al., 2001). Although the functional characteristics of most foods that are high in protein can be altered by TG, casein is optimal for

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protein crosslinking due to its open structure (Bönisch, Lauber, & Kulozik, 2004; Lorenzen, Schlimme, & Roos, 1998). TG is used in various forms in the food industry to alter the characteristics of food, for example in the processing of meat and fish products (Kuraishi et al., 2001; Motoki & Seguro, 1998; Yokoyama, Nio, & Kikuchi, 2004), in baking (Autio et al., 2005), and in dairy produce (Jaros, Partschefeld, Henle, & Rohm, 2006).

Recently emerging evidence has shown that crosslinking dietary protein to modify texture and structure without detriment to nutritional quality may have beneficial effects on human metabolic profile and appetite ratings (Juvonen et al., 2011, 2012). However, the mechanism underpinning this response is unknown, although there is some evidence to suggest that crosslinking proteins with TG increases the resistance of proteins to enzymatic breakdown (Flanagan, Gunning, & FitzGerald, 2003; Lorenzen et al., 1998; Monogioudi et al., 2011) which could delay digestion and increases transit time. Other evidence suggests that TG crosslinked diet has an effect on the upper gastrointestinal (GI) tract, modulating the digestive process through physical means such as gelation and aggregation in the stomach (Juvonen et al., 2009; Kong & Singh, 2008; Kristensen & Jensen, 2011), which can then trigger a cascade of hormonal responses.

Currently there is no information on the effect that crosslinking has on the appetite regulatory regions of the brain. One key area is the arcuate nucleus (ARC) in the hypothalamus of the forebrain which harbours two important populations of neurons pertinent to the homeostatic regulation of food intake. These are the orexigenic neurons that express neuropeptide Y (NPY) and agouti-related peptide (AGRP) and the anorexigenic neurons that express cocaine and amphetamine regulated transcript (CART) and the product of pro-opiomelanocortin (POMC) cleavage, α-melanocyte-stimulating hormone (α-MSH) (Coll, Farooqi, & O'Rahilly, 2007; Morton, Cummings, Baskin, Barsh, & Schwartz, 2006).

Protein crosslinking to enhance satiation or satiety without increasing energy density and macronutrient content could have numerous applications in assisting an overall objective of decreasing energy intake. The aim of this study was to utilise TG to crosslink protein in a casein supplemented rodent diet (AIN-93M) without the exclusion of water, providing a direct comparison of a solid and liquid form without changing volume or energy density. In using the Sprague Dawley rat model we allow for a mechanistic exploration of how the crosslinking of dietary protein affects food intake, body weight, hormonal profile and neuropeptide levels in the ARC. To achieve this we have modified a schedule feeding protocol (Johnstone, Fong, & Leng, 2006) which facilitates immediate food consumption on presentation of food, thus enabling gene expression and metabolic parameters to be determined relative to food intake.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats of 7–8 weeks of age were purchased from Charles River, UK. On arrival, rats were housed on a 12 h light: 12 h dark cycle, with lights on at 7 a.m. (Zeitgeber (ZT) 0) and lights off at 7pm (ZT12). Rats were acclimatized to an *ad libitum* standard chow diet (Special Diet Services, UK, #871505 CRM (P); 69% carbohydrate, 22% protein, 9% fat by energy, 3.58 kcal/g) for 7 days. The rats were then housed individually with *ad libitum* access to pelleted AIN-93M (Special Diet Services, UK, 75.9% carbohydrate, 14.7% protein, 9.4% fat; 3.77 kcal/g) with water being freely available throughout the study. Average daily caloric intake on AIN-93M was calculated for each individual rat. This value was used to provide the daily (100%) caloric intake for each individual rat during scheduled feeding. On the day prior to the start of the scheduled feeding, the rats were scanned by EchoMRI™, (Echo Medical Systems, Houston, TX, USA) to determine body composition. Animal husbandry and experiments were carried out under a Project Licence approved by the Home Office in accordance with the Animals (Scientific Procedures) Act of 1986. The experiments also received ethical approval from the Rowett Institute Ethics Committee.

2.2. Dietary manipulations

AIN-93M diet was also purchased in powdered form for use in the preparation of modified diets. The 'solid' diet consisted of 78% powdered AIN-93M, 18% H_2O, 2% additional casein, 0.8% emulsifier, and 1% inactivated transglutaminase (TG, Activa MP, Ajinomoto foods) and was moulded into a pellet with a caloric value of 2.98 kcal/g. 'Liquid' and 'crosslinked' diets were composed of (per 100 g before solubilisation) 55.1% powdered AIN-93M, 43.1% casein, 0.8% emulsifier (Grinsted® SSL P 55 VEG, Danisco Nutrition & Health,UK) and 1% inactivated or active TG. The mixture (minus TG) was dissolved in water, then for gelation, the TG enzyme was added to the solubilized constituents and the mixture was allowed to crosslink at 4 °C for 16 h. For the liquid diet, heat inactivated TG was added to maintain proportional content of constituents and protein. The caloric value of the diet was 1 kcal/ml. After an overnight incubation with TG at 4 °C, the diet solidifies with little or no change in volume resulting in a semi-rigid gel. Evidence of crosslinking by active TG and the absence of crosslinking with inactivated TG in the liquid diet were assessed by testing the viscosity at 20 °C with a stress controlled rotational rheometer (AR-G2, TA instruments, UK). The steady state viscosity was measured in duplicate with an increasing stress range, which resulted in Shear rates from 0.01 to 30 s^{-1} (Fig. 1A). The test product crosslinked with the active TG showed a higher viscosity than the liquid with the inactivated TG and could therefore be considered to be solidified to a semi-rigid gel.

Study 1. response to schedule fed experimental diets

The protocol is outlined in Fig. 1B (study 1). The scheduled feeding program involved limiting access to food to two periods of food availability, both during the dark phase, such that the consumption of food is well co-ordinated between all rats once access is restored. In Study 1, groups received one of the 3 individual diets (solid, liquid or crosslinked). Each rat received 100% of ad-libitum intake. This was calculated for each individual rat from their daily intake over 12 days of feeding stock AIN-93M pellet, which showed little variation over the 12 day period for each group (between day 1 and 12–100 ± 1.2%). All food for one day was presented from the start of scheduled feeding at two intervals - ZT13-15 for the first meal with food left over from this meal being made available at ZT20-22 for a second scheduled meal. This paradigm of scheduled feeding facilitated immediate intake on presentation of food whilst maintaining sufficient food intake to prevent weight loss after adaptation to scheduled feeding. Echo MRI scanning for body composition was performed on days 12 and 18. On the final day of the study, the rats were killed either at time 0 min (n = 7), or had free access to the daily ration of 100% of *ad libitum* energy intake of the specified diet for 90 min and before being killed (n = 7). Rats were anaesthetised with isoflurane, followed by decapitation. Trunk blood (approximately 10 ml) was collected into 15 ml polypropylene tubes containing heparin and 8 mM DPP-IV inhibitor (KR-62436 Hydrate, Sigma) and stored on ice until processing for plasma. Brains were removed and frozen on dry ice. Blood was centrifuged at 1000 g for 15 min at 4 °C and the plasma was removed to microfuge tubes and stored as aliquots at −80 °C.