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Short-term Effects of Macronutrient Preloads on Appetite and Energy Intake in Lean Women

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POPPITT, S. D., D. MCCORMACK AND R. BUFFENSTEIN. *Short-term effects of macronutrient preloads on appetite and energy intake in lean women.* PHYSIOL BEHAV **64** (3) 279–285, 1998.—This study investigated the relative satiating hierarchy of the four energy-providing macronutrients (fat, carbohydrate (CHO), protein, and alcohol) in lean women. On four separate occasions, the composition of an iso-energetic lunch preload was manipulated in 12 lean (BMI < 25 kg/m^2) women. The four treatments comprised a 1-MJ baseline meal and drink (40% fat, 48% CHO, 12% protein) to which was covertly added: 1) +1MJ protein; 2) +1MJ alcohol; 3) +1MJ CHO; and 4) +1MJ fat. Prior to and at 30-min intervals, subjects completed 100-mm visual analogue scales rating subjective hunger and satiety. Ninety min following the preload, an ad lib. lunch meal was given (40% fat, 48% CHO, and 12% protein) and energy intake (EI) measured. Energy intake at the lunch meal was 2195 (880, SD) kJ, 2772 (1191, SD) kJ, 2502 (681, SD), kJ and 2558 (1050, SD) kJ for the protein, alcohol, CHO, and fat preloads, respectively. There was no significant difference between the pleasantness of the preloads ($p > 0.05$). Macronutrient composition had a significant effect on short-term hunger ($F = 3.19$; $p < 0.05$), subjects being less hungry after the protein preload. Subjects also had a lower energy intake after the protein preload ($F = 3.11$; $p < 0.05$). We conclude that only protein has a differential short-term satiating effect when incorporated iso-energetically and at a similar energy density into the diet. © 1998 Elsevier Science Inc.

Appetite Hunger Satiety Energy intake CHO Fat Protein Alcohol

THE REGULATION of appetite and energy intake (EI) is fundamental to the control of energy balance and maintenance of body weight. An inability to match hunger, satiety, and consequently food intake to energy expenditure may lead to the gradual increase in body fatness and the development of obesity. The individual metabolic fuels of fat, carbohydrate (CHO), protein, and alcohol appear critical in controlling both hunger and satiety and thus energy balance.

While little work has been done on the role of alcohol, numerous studies suggest that there may be a hierarchy in satiation between protein, CHO, and fat in the diet, with protein being the most and fat the least satiating. These range from epidemiologic, in which the proportion of each macronutrient has been shown to influence subsequent EI (4,7) to metabolic feeding studies in which the poor satiating efficiency of fat is implied by the overconsumption of energy on a high-fat relative to a high-CHO diet (13,27,37,40) to nutrient infusion studies in which there is some evidence that the oxidative hierarchy of the macronutrients may feedback to satiety (18,19,25). Alcohol is most readily oxidised, fat least readily oxidised, and protein and CHO fall between the two (1,15,34,43). However, in all of these studies, it is not only the macronutrient content of the diet but also the energy content that may be affecting food intake. Both van Stratum (42) and Stubbs (36) have previously shown that the EI, and hence the satiating effects of fat and CHO, were very similar on high-fat and high-CHO diets when the energy density was maintained constant. The absence of hyperphagia when provided with a high-fat diet and yet eating ad lib. implies that the energy content of the diet and hence its nutrient density may be as important as the macronutrient composition per se.

Disassociating the macronutrients from their energy content by giving equal amounts of energy for each substrate allows the specific effects of the macronutrients to be investigated. Studies in which the diets are iso-energetic, differing only in macronutrient composition, however, vary in their conclusions. Protein has often been shown to be more satiating than, for example, CHO (2,5,22,23,35), although other studies fail to show this effect (11). When equal amounts of energy are given as either fat or CHO, fat may be less (6,21,26,32) or equally as satiating as CHO (11,16,17,35). Less data are available for alcohol, but it appears to be poorly regulated (40), failing to increase satiety or decrease EI in response to ingestion (29).

One possibility for these conflicting data may be the wide variety of study protocols and subjects involved in the different studies, including the comparison of only two macronutrients (commonly either protein with CHO or fat with CHO), different

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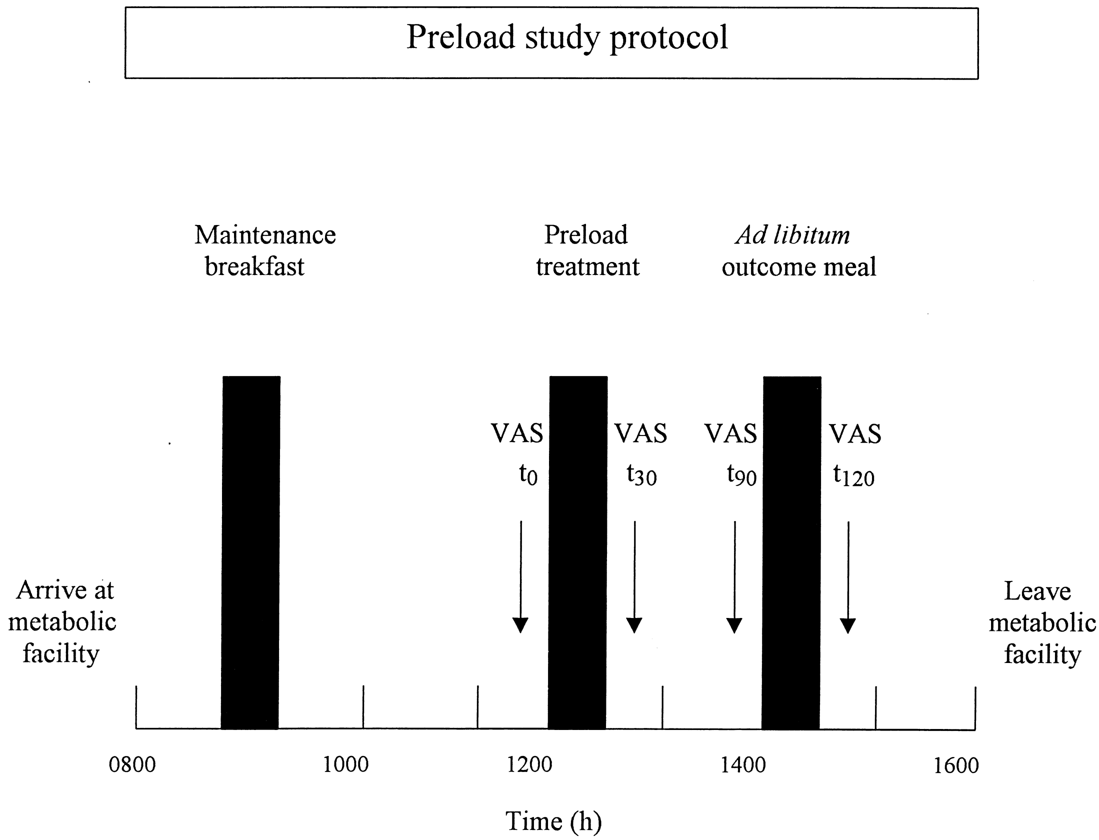

FIG. 1. Preload protocol. VAS, visual analogue scores.

preload doses, and different protocol timings. No studies have compared the relative satiating effects of all four macronutrients given in iso-energetic amounts to subjects following an identical protocol on all occasions and few have even compared the three major macronutrient components, protein, CHO, and fat, within one study. Our current study aimed to assess the relative short-term satiating effects of fat, CHO, protein, and alcohol per se in a group of subjects each following an identical protocol by giving iso-energetic doses of the individual macronutrients on four separate occasions.

METHODS

Subjects

Twelve female volunteers participated in the study. All subjects were lean [body mass index (BMI) < 25 kg/m^2) and aged between 20 and 60 years. Subjects were blind to the manipulation of macronutrient composition but were informed that on at least one occasion they would be required to consume an alcoholic beverage. The study was approved by the Dunn Nutrition Unit's ethics committee.

Study Design

Subjects came to the unit on four separate occasions. All pre-menopausal women were asked to attend during days 7–14 of their menstrual cycle. They arrived fasted at the metabolic facility at 0830 hours each day and remained sedentary within the confines of the unit until 1600 hours (Fig. 1). On each morning, a randomly assigned preload drink and small meal was given followed 90 min later by an ad lib. lunch meal, which was provided in excess and designed to allow the subjects to eat as much or as little as they chose. Immediately prior to and at 30, 90, and 120 min following the preload treatment, 100-mm visual analogue scales (VAS) were completed by the subjects rating subjective feelings of hunger, satiety and food preference, asking the questions "How hungry are you?," "How full are you?," (anchored at each end with "not at all" and "as hungry/full as I have ever been") and "How pleasant did you find the meal?," "How satisfying did you find the meal?," (anchored at each end with "very pleasant/satisfying" and "very unpleasant/not satisfying").

Protocol

On arrival at the unit at 0900 hours a breakfast of cornflakes, toast, scrambled eggs, and orange juice was provided (25 en% fat, 65 en% CHO, 15 en% protein), which was intended to provide 25% of daily intake in order to maintain energy balance and provide a constant level of appetite prior to presentation of the preload. No other food was available to the subjects throughout the morning. Energy balance was calculated as 1.4 times (33) predicted basal metabolic rate (BMR). Predicted BMR was in turn calculated from the weight, height, age, and sex of each individual subject. The preload was given at 1200 hours and consumed within 20 min by subjects in isolation in an attempt to minimize any social interaction effects that may influence the amount of food eaten (8). Subsequent EI was assessed from a single meal offered to the subjects 90 min later, which comprised a main meal of

TABLE 1

COMPOSITION OF THE FOUR MACRONUTRIENT PRELOADS, GIVEN AS A FISH AND POTATO PIE PLUS A GIN AND TONIC DRINK

Diet	Energy (MJ)	ED (kJ/g)	CHO (MJ)	Fat (MJ)	Protein (MJ)	Alcohol (MJ)
High CHO (+64 g CHO)*	2.2	3.1	1.50	0.42	0.25	0
High fat (+27 g fat)*	2.1	2.9	0.44	1.44	0.23	0
High protein (+60 g protein)*	1.9	2.4	0.38	0.37	1.13	0
High alcohol (+34 g alcohol)	2.1	2.8	0.45	0.42	0.24	0.98

* Alcohol placebo replaced alcoholic beverage.

chicken risotto and a dessert of fruit loaf, both of which were given in excess and from which subjects could eat ad lib. Both courses had a composition of 40 en% fat, 48 en% CHO, 12 en% protein, representing a typical lunchtime meal. The meal was designed to be of homogeneous composition to ensure that an even macronutrient consumption was maintained irrespective of the quantity of food consumed. Both the rissotto and fruit loaf were covertly weighed before and after the meal to allow calculation of energy and macronutrient intake.

Preloads

The four preload treatments comprised a 1MJ baseline meal to which an additional 1MJ of each macronutrient was added in turn. The baseline meal was designed to be 40 en% fat, 48 en% CHO, 12 en% protein, and comprised a fish and potato pie plus a drink flavoured to mimic a common alcoholic beverage, gin and tonic. Thus, to each preload in random sequence was added either 1) + 1MJ (64 g) CHO; 2) + 1MJ (27 g) fat; 3) + 1MJ (60 g) protein; or 4) + 1MJ (34 g) alcohol. All preloads were "covert," designed to look and taste as similar as possible, with emphasis placed upon producing meals of similar total volume (Table 1). Covert manipulation of the alcohol content was by the addition of a gin placebo (gin flavouring from International Flavour and Fragrance Ltd., Haverhill, UK) to the nonalcoholic drinks and distilled alcohol (Beefeaters™ gin, James Burrough, London, UK) to the alcohol treatment. All drinks were made up using 300 g of slimline tonic water (Schweppes™ Beverages Ltd, Uxbridge, UK). Although it was possible to produce a nonalcoholic beverage that entirely mimicked the taste of the alcoholic equivalent by using specific gin flavouring, the effects of ethanol upon a wide range of human behaviors, including eating behavior, cannot be replicated.

Analysis

Data on self-reported hunger ratings and EI during the outcome meal were analyzed using Student's paired *t*-test and one-way ANOVA including both subject and preload treatment effects. Analyses are identified in the text by t and F statistics, respectively. Scheffe's post hoc test was used to further identify individual treatment effects using linear model methodology within Macintosh Data Desk, version 4, statistical program (Odesta Corporation, Northbrook, IL, USA).

RESULTS

Hunger and Satiety Ratings

There was no significant difference between levels of hunger ($F = 0.04$; $p > 0.05$) and satiety ($F = 0.68$; $p > 0.05$) prior to the mid-morning preload at t_0 on any of the 4 treatment days, although the between subject variability was highly significant for both measures ($p < 0.05$). This ensured that all subjects were in a similar state of appetite before being presented with the test. When all treatments were combined there was a highly significant decrease in self-reported hunger ($t = 6.26$; $p < 0.001$) and an increase in self-reported satiety ($t = 6.23$; $p < 0.001$) immediately following the preloads. When analyzed individually, while hunger decreased between the baseline (t_0), and following the preload (t_{30}) for all macronutrients, this decrease was significant only following the high-protein ($t = 5.77$; $p < 0.001$) and high-CHO ($t = 2.63$; $p < 0.05$) treatments. Satiety also increased immediately following both protein and CHO preloads ($p < 0.05$).

When analyzed individually throughout the length of the test, between baseline and t_{120}, macronutrient composition had a significant, albeit very weak, effect on ratings of hunger ($F = 3.19$; $p < 0.05$) and satiety ($F = 3.35$; $p < 0.05$). Protein primarily contributed to this differential; subjects were consistently less hungry and more satiated throughout the test following the protein preload (Fig. 2). Self-reported hunger was slightly lower and satiety slightly higher following the CHO relative to the fat preload at t_{30}, t_{90}, and t_{120}, although the wide between-subject variability prevented this from reaching significance ($p > 0.05$). Neither were significantly different from reports of hunger or satiety on the alcohol treatment. Under these specific conditions of a single, oral 1-MJ preload, when assessed over a time frame of 120 min, there was no evidence of differential effects on cognitive appetite ratings between fuel sources other than for the macronutrient protein.

When asked to assess the pleasantness of each preload immediately following its consumption, subjects showed no significant preference for any of the four treatments ($F = 2.79$; $p > 0.05$), although the high protein preload was rated the least pleasant and high fat the most pleasant (protein 52.1 mm, 39.2 SD; alcohol 32.2 mm, 25.7 SD; CHO 35.6 mm, 27.5 SD; fat 26.7 mm, 31.2 SD). There was also no significant difference in satisfaction of each preload immediately upon consumption ($F = 0.301$, $p > 0.05$: protein 30.6 mm, 15.6 SD; alcohol 24.5 mm, 14.2 SD; CHO 20.9 mm, 18.7 SD; fat 27.8 mm, 19.2 SD).

EI

When analyzed for both treatment and subject effects, significantly less energy was consumed at the ad lib. meal following the protein preload ($F = 3.11$; $p < 0.05$; Fig. 3) relative to the other macronutrients. Subjects translated the cognitive feeling of lower hunger and greater satiety into a reduced intake of energy at the lunch meal 120 min after the preload had been administered. There was no significant difference in energy consumed at the lunch meal between either the CHO or the fat preloads ($p > 0.05$) despite subjects reporting to be slightly more hungry and less satiated following the addition of fat into the preload. Subjects consumed the greatest amount of energy following the alcohol preload, although the effect did not reach significance ($p > 0.05$). EI at the outcome meal was 2195 (880 SD) kJ following the protein preload, 2772 (1191 SD) kJ following the alcohol preload, 2502 (681 SD) kJ following the CHO preload, and 2558 (1050 SD) kJ following the fat preload. Intake at lunch was 20.8% ($p < 0.05$), 12.3% ($p > 0.05$), and 14.2% ($p > 0.05$) lower following the protein preload than following the individual alcohol, CHO, and fat preloads, respectively. Between subject effects were highly significant ($F = 9.72$; $p < 0.001$) for all treatments. Subjects consumed widely varying amounts of energy across all treatments (range: protein, 786-3676 kJ/meal; alcohol, 1173–5056 kJ/meal; CHO, 1633–3592 kJ/meal; fat, 1452–4281 kJ/meal) despite reporting similar levels

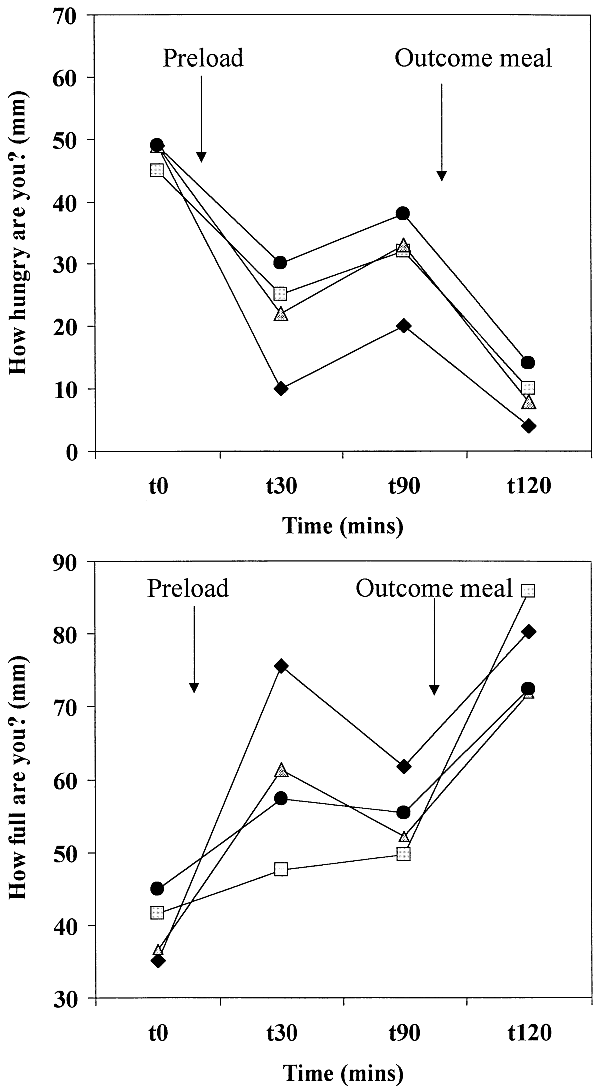

FIG. 2. Self-reported ratings of hunger and satiety following iso-energetic (+1 MJ) supplements of protein (◆), alcohol (■), carbohydrate (▲), and fat (●).

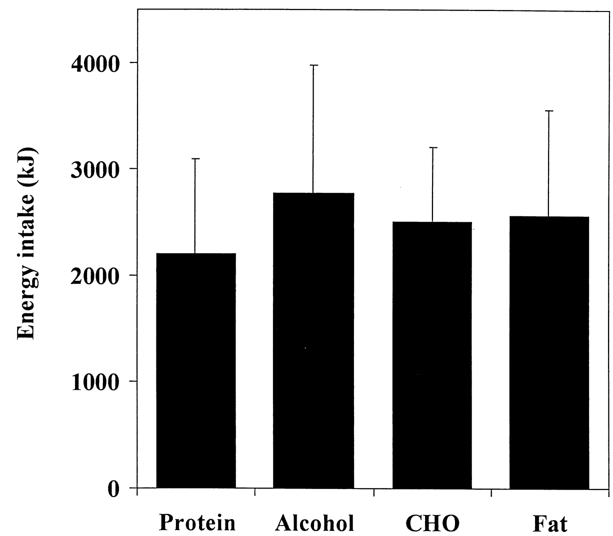

FIG. 3. Energy intake at an ad lib. lunch 120 min after ingestion of 1-MJ preload supplements of protein, carbohydrate (CHO) and fat (mean ± sd) alcohol.

of hunger and satiety at the start (t_0) of the study, presumably a consequence of habitual eating habits.

DISCUSSION

This study shows that when individual macronutrients are incorporated into the diet at levels of energy up to 1 MJ, and assessed over a short time frame, only protein has a significant differential short-term satiating effect, resulting in reduced self-reported hunger, increased self-reported satiety and a reduced intake of energy in the next meal. It must be acknowledged, however, that preload studies are limited by the nature of their short-term effects, the effect upon subjects asked to remain sedentary within the confines of a metabolic facility and the ability of both self-reported hunger and satiety and an ad lib meal presented shortly after the preload to detect the differential effects. While alterations in the energy content of a preload may be readily detected by the individual and in turn influence appetite regulation, this study shows that, under relatively iso-energetic conditions, the macronutrient composition per se does not strongly influence short-term EI when the energy content of each macronutrient supplement is not greater than 1 MJ. The maximum energy content of the preloads was confined by the alcohol treatment since the amount of alcohol that can be consumed as a single bolus cannot be increased above 1 MJ without considerable toxic effect, including considerable disinhibition, disorientation, confusion, and nausea.

There are a number of other shortcomings in this preload protocol that should also be acknowledged. While we aimed to maintain variables such as energy content, energy density, weight, taste, and subjective preference for the preload constant across treatments, it is actually a very difficult thing to achieve. The energy content of the preloads was maintained between 1.9 and 2.2 MJ, but the difficulties in also matching weight resulted in a considerable difference in energy density. The density of the protein treatment was only 82% of the average density of fat, protein, and alcohol combined. It is possible that the significantly increased satiety and decreased hunger and EI may be due, at least in part, to this differential. It might also be noted that the relatively small number of subjects taking part in this study may also have served to reduce the statistical power and hence the ability to discriminate between macronutrient preloads.

The considerable satiating effect of protein has been observed in several previous studies, including individuals self-recording free-living EI over a period of 7 days (7,9) and over four periods of 4 consecutive days (4). In iso-energetic designs within a metabolic facility, EI fell by 22% (22,23) and 12% (2) following a high-protein relative to a high-CHO meal and by 26% in studies in which a high-protein meal was compared with an almost protein-free meal (5). In our current study, the high-protein preload reduced intake by between 12.3% to 20.8% relative to high alcohol, fat, and CHO preloads. Stubbs et al. (35) recently showed a long-term suppression of hunger on an iso-energetic high protein diet, although no effect on EI relative to either CHO or fat at a meal 5 h after preload ingestion. Interestingly, de Graaf (11) has also failed to show an effect of a protein preload on voluntary EI measured over an entire day when giving up to 1.67 MJ as a bolus. This study however was not strictly iso-energetic because 99% of energy was given as CHO (=1.65 MJ), 92% as fat (=1.53 MJ), and only 77% as protein (=1.29 MJ).

There was no evidence from this study of a hierarchy in the satiating efficiency of the individual macronutrients when given in iso-energetic, relatively small quantities. While being less satiating than protein, fat and CHO had an almost identical effect on subsequent EI, with only a 2% difference in the consumption of energy from the ad lib. lunch time meal. Self-reported hunger was consistently lower and satiety consistently higher following the CHO preload throughout the 120 min following ingestion, but these differences did not reach significance. Stubbs and coworkers (35) failed to show significant differential effect of fat and CHO on short-term EI, although ratings of hunger showed CHO to be transiently more satiating than fat, and de Graaf (11) also found fat to be as satiating as CHO when the differential effects of energy content were removed. Our results, however, fail to concur with a number of other iso-energetic studies that have shown fat to be less satiating than CHO (6,21,26,32) and where it has been suggested that "a fat supplement generates a weaker or non-existent action of satiety when compared with CHO" (26). Interestingly, in several of these studies (6,21,26) although the energy content of the preloads has been matched, the energy density has not. This may explain the lower satiating effect of fat in these preload studies, as it may the greater satiating effect of protein in our current protocol. The similarity of energy density of the fat and CHO preloads (2.9 and 3.1 kJ/g, respectively) which differed by less than 7% may in turn account for the similarity in self-reported ratings and also EI in this current study.

The differential effects of fat and CHO undoubtedly occur when, as in the natural situation, the energy density of the macronutrients differ radically and the introduction of fat into the diet results in a parallel introduction of energy, lower satiety per MJ of energy consumed, and a subsequent higher intake and hyperphagia on a high-fat diet. For example, in carefully controlled ad lib. feeding studies previously carried out in our laboratory (30), women were satiated on an intake of 8.1 MJ/day when given a high CHO (CHO: 62%; fat: 25% of energy) diet yet only reached satiation and stopped eating after the consumption of 10.5 MJ/day when given a high fat diet (CHO: 37%; fat: 50% of energy). EI would have been 23% lower if satiation on the high fat diet occurred as it did on the high CHO diet, after the consumption of only 8.1 MJ/day. It is this hyperphagia that may largely be responsible for the correlation between a high fat diet and both a high BMI and percentage body fat of obese subjects (12,20,28,39,41).

The inability of alcohol to decrease subsequent EI is unsurprising, because considerable evidence now shows that alcohol-derived energy is poorly regulated. Alcohol consumed during a meal is commonly not compensated for by an equivalent decrease in nonalcohol-derived energy in both metabolic (29,40) and community survey studies (3,10,14,24,31). A previous study carried out in this laboratory (29) showed no differential effects on subsequent appetite when 1-MJ preloads of alcohol and CHO, both in liquid form, were given to women. It was concluded following that study that neither alcohol-derived or CHO-derived energy is recognized by any short-term system regulating appetite control when given as a drink in doses of less than 1 MJ. It would appear that differential effects still cannot be detected when the CHO source is of solid rather than liquid origin.

The mechanisms regulating appetite and EI in humans remain to be elucidated. The interaction between behavioral and physiological control are complex and may help to explain the inability of this short preload study to elucidate differences between the individual metabolic fuels. Eating behavior is very strongly entrenched, and strong physiological signals are required to drive the individual away from habitual, learned activities such as the amount of energy consumed during a single meal. It appears that throughout this series of preload treatments, with the exception of the protein arm, any physiological signals generated by the ma-

APPENDIX 1

FISH AND POTATO PIE; GIN & TONIC-PRELOADS

	g	Energy (kJ)	Fat (g)	CHO (g)	Protein (g)	Alcohol (g)
			(+1 MJ fat)			
Boiled potatoes	150	452	0.2	25.5	2.7	0
Eggs	18	111	1.9	0	2.3	0
Whole milk	10	28	0.4	0.5	0.3	0
Raw onions	30	45	0.1	2.4	0.4	0
Mature cheddar	10	172	3.4	0	2.6	0
Butter	39	1208	32	0	0.2	0
Smoked haddock	25	106	0.3	0	5.8	0
Slimline tonic	450	30	0	0	0.2	0
Gin	0	0	0	0	0	0
Sum	*732*	*2152*	*38.3*	*28.4*	*14.5*	*0*
% energy			67.0	20.7	11.3	0.0
			(+1MJ CHO)			
Boiled potatoes	170	512	0.2	28.9	3.1	0
Eggs	18	111	1.9	0	2.3	0
Whole milk	10	28	0.4	0.5	0.3	0
Raw onions	30	45	0.1	2.4	0.4	0
Mature cheddar	10	172	3.4	0	2.6	0
Smoked haddock	25	106	0.3	0	5.8	0
Margarine	6	186	4.9	0	0	0
CHO supplement	43	684	0	40.6	0	0
Tonic water	400	365	0	20.4	0	0
Gin	0	0	0	0	0	0
Sum	*712*	*2209*	*11.2*	*92.8*	*14.5*	*0*
% energy			19.1	65.9	11.0	0.0
			(+1MJ protein)			
Boiled potatoes	115	346	0.2	19.6	2.1	0
Eggs	20	123	2.2	0	2.5	0
Skimmed milk	20	28	0.02	1	0.7	0
Raw onions	30	45	0.1	2.4	0.4	0
Low-fat cheddar	30	352	4.9	0.5	9.9	0
Smoked haddock	150	634	1.4	0	35	0
Protein supplement	21	345	1.3	0.9	16.8	0
Slimline tonic	400	30	0	0	0.2	0
Gin	0	0	0	0	0	0
Sum	*786*	*1903*	*10.12*	*24.4*	*67.6*	*0*
% energy			20.0	20.1	59.5	0.0
			(+MJ alcohol)			
Boiled potatoes	150	452	0.2	25.5	2.7	0
Eggs	18	111	1.9	0	2.3	0
Whole milk	10	27.6	0.4	0.5	0.3	0
Raw onions	30	45.2	0.06	2.4	0.4	0
Mature cheddar	10	172.4	3.4	0.01	2.6	0
Smoked haddock	25	105.6	0.3	0	5.8	0
Margarine	6	185.5	4.9	0.06	0.01	0
Slimline tonic	400	30.1	0	0	0.2	0
Gin	100	966.5	0	0	0	33.4
Sum	*749*	*2095.9*	*11.16*	*28.47*	*14.31*	*33.4*
% energy			20.1	21.3	11.4	46.7

cronutrients were of insufficient magnitude to elicit a specific satiety response when given as a single 1-MJ dose. We might hypothesise that, while an increase in the energy content of the preloads, an increase in the duration of the studies or an increase in the variety of foods offered at the outcome meal enhance any differential effects, the specific effects of individual macronutrients when dissociated from their encumbent energy content is relatively small.

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