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RAPID COMMUNICATION

Novel Approach to Diet Design for Determining Macronutrient Preference

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MULLEN, B. J. AND R. J. MARTIN. *Novel approach to diet design for determining macronutrient preference.* PHARMACOL BIOCHEM BEHAV **42**(2) 343–346, 1992.—Previous studies indicate that rats fed a saturated vs. unsaturated fat consume more of a high-protein/low-carbohydrate and less of a low-protein/high-carbohydrate diet. Present studies were conducted to determine whether saturated-fat-fed rats are preferring protein or avoiding carbohydrate. Three combinations of diets were prepared in which one macronutrient was held constant and two covaried: 1) high protein/low carbohydrate and low protein/high carbohydrate, 2) high fat/low carbohydrate and low fat/high carbohydrate, and 3) high fat/low protein and low fat/high protein. Male Sprague-Dawley rats (75–99 g) were fed either 34% tallow (n = 30) or corn oil (n = 30) diets for 4 days. These diets were removed and one third of each group was given one of the diet combinations. Rats previously fed tallow consumed more high-protein/low-carbohydrate and low-carbohydrate/high-fat diets and less high-carbohydrate/low-fat diet than controls previously fed corn oil. These data suggest that tallow-fed animals are avoiding carbohydrate rather than preferring protein.

Diet selection Macronutrient selection Dietary fat

THE ability of animals to self-select a nutritionally adequate diet from various food sources is widespread throughout the animal kingdom (5). Many methodological approaches have been developed to study this phenomenon. Among them are variations in test diets presented for selection (2). Two distinct approaches that have been employed are: a) the use of single sources of macronutrients, such as dextrose, casein, and lard, each fortified with vitamins and minerals (7,8); or b) the use of two or more mixed diets that contain all the necessary nutrients but in varying proportions (1,3). In either case, multiple diets are presented simultaneously and the animal is allowed to select freely. There are advantages and disadvantages to either design. In the first approach, that of single macronutrients, there are often marked textural or palatability differences between diets, factors that may influence selection. In addition, since fat is more calorically dense than either protein or carbohydrate, caloric density is variable. Addition of fiber to the fat diet may equalize the calories per gram, but then large differences in fiber content become a problem. An advantage, however, to using single macronutrient sources is that each macronutrient can be selected independently. In the second approach, that of mixed diets, texture and palatability are similar and caloric density can be controlled. However, with mixed diets one cannot select a macronutrient independently of the others, that is, the levels of two macronutrients covary. For example, a high-protein diet will contain low carbohydrate and, conversely, a low-protein diet will contain high carbohydrate (fiber, fat, and micronutrients can be identical between the diets). Thus, if an animal selects the high-protein diet is it selecting for protein or against carbohydrate? In the present experiment, we suggest an alternative approach for diet design. We used mixed diets and tested several combinations of these diets to tease out the specific selection behavior. While this approach does not remove all ambiguity, it does minimize textural differences between diets, controls for caloric density, and helps clarify particular nutrient preferences or aversions.

We used this approach to test the effect of dietary fat on diet selection. Our lab has shown previously that a diet containing 34% saturated fat (tallow) vs. one of unsaturated fat (corn oil) will promote subsequent consumption of a high-protein/low-carbohydrate (hi pro/lo cho) vs. a low-protein/high-carbohydrate (lo pro/hi cho) diet (4). This "fat effect" on dietary behavior can be seen after only 18 h exposure to the high-fat diets and persists for several days (4). To determine if tallow-fed rats are preferring protein or avoiding carbohy-

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TABLE 1

DIET COMPOSITION (g/100g DIET) FOR COMPARING TYPES OF FAT

Casein	20.0
Dextrose	0.0
Fat (tallow or corn oil)	34.1
Minerals*	4.0
Vitamins†	2.2
Celufil‡	39.4
DL-Methionine	0.4
kcal/100 g	386

*AIN Mineral Mixture 76 contains the following compounds (g/100 g): 50.0 calcium phosphate, dibasic, 7.4 sodium chloride, 22.0 potassium citrate monohydrate, 5.2 potassium sulfate, 2.4 magnesium oxide, 0.35 manganous carbonate, 0.6 ferric citrate, 0.16 zinc carbonate, 0.03 cupric carbonate, 0.001 potassium iodate,0.001 sodium selenite, 0.055 chromium potassium sulfate, 11.8 sucrose.

†AIN Vitamin Mixture 76 contains the following compounds (mg/100 g): 60 thiamine hydrochloride, 60 riboflavin, 70 pyridoxine hydrochloride, 300 nicotinic acid, 160 D-calcium pantothenate, 20 folic acid, 2 D-biotin, 0.1 cyanocobalamin, 80 retinyl palmitate, 2 DL-a-tocopheryl acetate, 0.25 cholecalciferol, 0.5 menaquionine, 97 sucrose.

‡A nonnutritive bulk of fine mesh cellulose.

drate, rats were fed tallow or corn oil and then allowed to select from diet pairs varying in: a) protein and carbohydrate, b) protein and fat, or c) carbohydrate and fat.

METHOD

Young, male Sprague-Dawley rats (Harlan-Sprague-Dawley, Indianapolis, IN) weighing between 75-99 g were used. Animals were housed in individual hanging wire cages with free access to food and water. Room temperature was maintained at 23 ± 2°C on a 12 L:12 D cycle. Initially, rats had free access to a nonpurified diet (Rat Chow 5012, Ralston Purina, St. Louis, MO). Experimental diets were used as specified. The diet components were purchased from United States Biochemical Corp. (Cleveland, OH). Beef tallow was purchased from Wiley Foods Inc. (Atlanta, GA).

To investigate the specific selection behavior rats display in response to dietary fat, multiple pairs of isoenergetic diets were formulated in which one of the macronutrients was held constant while the remaining two covaried. For 4 days, animals were fed diets (Table 1) containing either 34% corn oil or 34% tallow. These diets were then replaced with one of the following pairs of diets (Table 2): a) 60% protein/15% carbohydrate and 10% protein/65% carbohydrate (9.6% fat in both diets), b) 27% fat/15% carbohydrate and 4.8% fat/65% carbohydrate (21% protein in both diets), or c) 27% fat/10% protein and 4.8% fat/60% protein (26% carbohydrate in both diets). Food intakes were monitored daily.

Data were analyzed using Statistical Analysis Systems (SAS) (6). Significant differences in intake between the tallow and corn oil groups for a given diet were identified using Student's *t*-test.

RESULTS

Average intake of the fat diets prior to selection was slightly greater in the tallow groups that were subsequently assigned to the pro/fat and cho/fat diet pairs as compared to the corn oil groups (data not shown). Figure 1 shows consumption of various pairs of selection diets by rats previously fed 34% corn oil or 34% tallow for 4 days. Combined total intake of selected diets was not significantly influenced by previous consumption of either tallow or corn oil diets (data not shown). However, differences were observed in the diet selections between corn oil- and tallow-fed rats. For the protein/carbohydrate selection diets, the 34% tallow group ate less of the low pro/high cho than the 34% corn oil group. When diet pairs consisted of carbohydrate/fat, the 34% tallow group ate significantly more of the low-cho/hi-fat and

TABLE 2

DIET COMPOSITION (g/100 g DIET) FOR COMPARING CARBOHYDRATE/PROTEIN, FAT/CARBOHYDRATE, AND PROTEIN/FAT COMBINATIONS

	High Protein/ Low Carbohydrate	Low Protein/ High Carbohydrate	High Fat/ Low Carbohydrate	Low Fat/ High Carbohydrate	High Protein/ Low Fat	Low Protein/ High Fat
Casein	60.0	10.0	20.8	20.8	60.0	10.0
Dextrose	14.9	64.9	14.9	64.9	25.7	25.7
Fat						
Tallow	4.8	4.8	13.5	2.4	2.4	13.5
Corn Oil	4.8	4.8	13.5	2.4	2.4	13.5
Minerals*	4.0	4.0	4.0	4.0	4.0	4.0
Vitamins†	2.2	2.2	2.2	2.2	2.2	2.2
Celufil‡	8.9	8.9	30.7	2.9	2.9	30.7
DL-Methionine	0.4	0.4	0.4	0.4	0.4	0.4
kcal/100 g	386	386	386	386	386	386

*AIN Mineral Mixture 76 contains the following compounds (g/100 g): 50.0 calcium phosphate, dibasic,7.4 sodium chloride, 22.0 potassium citrate monohydrate, 5.2 potassium sulfate, 2.4 magnesium oxide, 0.35 manganous carbonate, 0.6 ferric citrate, 0.16 zinc carbonate, 0.03 cupric carbonate, 0.001 potassium iodate, 0.001 sodium selenite, 0.055 chromium potassium sulfate, 11.8 sucrose.

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*‡A nonnutritive bulk of fine mesh cellulose.

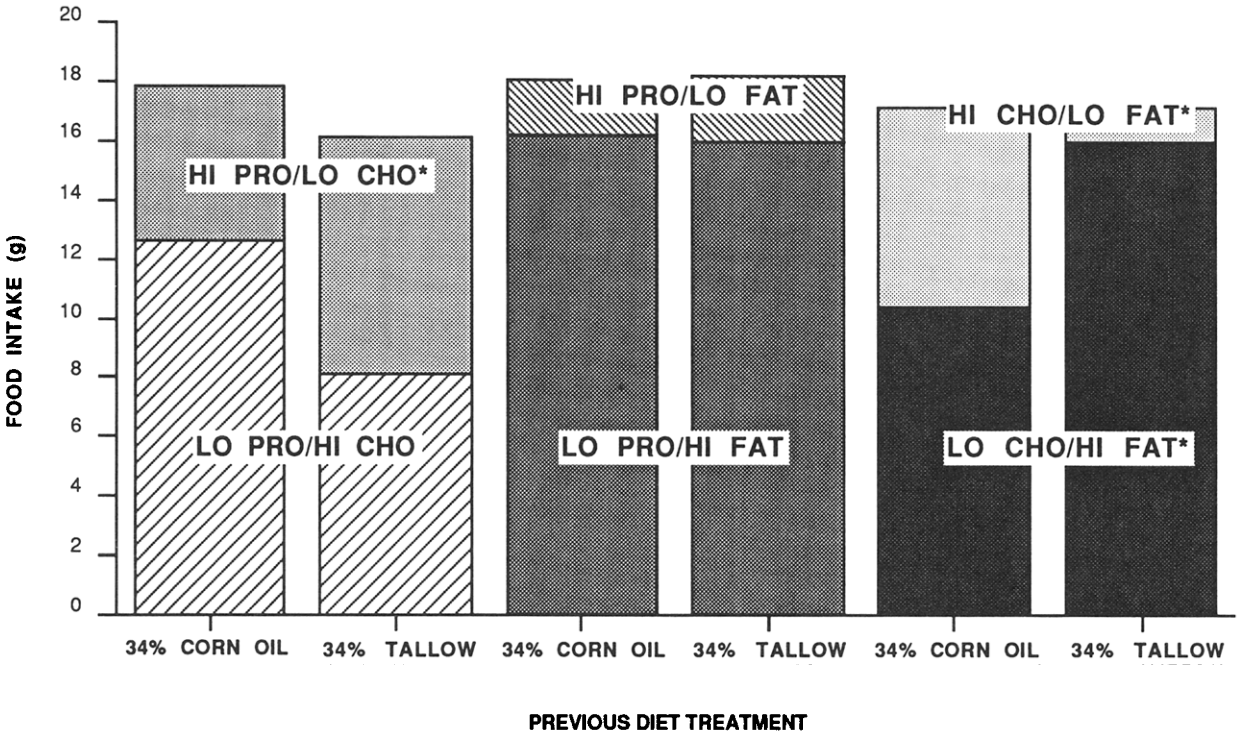

FIG. 1. Diet selection in rats previously fed 34% corn oil or tallow diets for 4 days. Rats were allowed to select between one of three diet pairs for 24 h. Bars represent the mean intake for a given diet. Mean intakes were compared for a given diet between corn oil and tallow groups. *Significant difference between means ($p < 0.05$).

less of the hi-cho/lo-fat diets than did the 34% corn oil groups. There were no differences between the fat-fed groups in consumption of the pro/fat selection diets; both groups ate significantly more of the lo-pro/hi-fat than of the hi-pro/lo-fat diets.

DISCUSSION

Data from our previous studies indicate that tallow-fed rats consume more hi-pro/lo-cho and less lo-pro/hi-cho diet than rats fed corn oil. The present experiment was designed to clarify whether or not tallow-fed rats are preferring protein or avoiding carbohydrate. It appears that when tallow-fed rats can avoid carbohydrate they do so. For example, as reported previously, in the protein/carbohydrate combination tallow-fed rats chose more of the hi-pro/lo-cho diet as compared to corn oil controls. In the fat/carbohydrate combination, tallow-fed rats consumed more of the hi fat/lo cho than controls. When carbohydrate is not varying, as in the protein/fat diet combination, tallow-fed rats chose similarly to that of the corn oil group. Both groups consumed more of the lo-pro/hi-fat than the hi-pro/lo-fat diet, another indication that they are not selecting for protein.

If one were to argue that the tallow group is selecting high protein, then rats would have selected the high-protein diet both times protein was varying, that is, in the carbohydrate/protein and fat/protein diet combinations. However, this did not occur as the data shows that tallow-fed rats ate more low-pro/hi-fat than hi-pro/lo-fat diet.

We recognize that with the use of mixed diets food intake may be affected by interactions between nutrients, for example, protein may be more palatable when paired with fat rather than with carbohydrate. The present study does not address this potential interaction; however, it is an area for further investigation.

This article describes a novel approach to studying diet selection behavior. The study was designed to discriminate between preferences for single nutrients with the use of mixed diets. While this approach does not remove all ambiguity in macronutrient preference, it does retain the advantage of using mixed diets and further clarifies diet selection behavior under these conditions.

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