Effect of qualitative differences in dietary fat on dexfenfluramine mediated depression of food intake and serotonin metabolism

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Previously, we showed that rats fed greater amounts of dietary saturated fat select more protein and less carbohydrate than rats fed diets with lesser amounts. Four experiments were designed to determine if dietary fat-induced differences in selection behavior were mediated by the serotonergic system. All diets were isoenergetic with 20% (w/w) fat. In experiment 1, rats were fed diets (24% protein, 40% carbohydrate, 20% fat) containing 9%, 8%, or 3% saturated fat. Dexfenfluramine (0, 0.6 and 1.2 mg/kg) resulted in a dose-dependent decrease in food intake in all groups at 1, 2, and 16 hours after food presentation ($P < 0.0006$). In addition, there was a significant diet by drug interaction at each time interval ($P < 0.05$), indicating the effect was not independent of dietary fat composition. When rats selected from high-protein low-carbohydrate and low-protein high-carbohydrate diets containing 9% or 3% saturated fat (total fat content of all diets remained 20% (w/w)), dexfenfluramine resulted in decreased intake of food, protein, and carbohydrate in both groups ($P < 0.05$). However, there was no significant diet by drug interaction at any time interval. Consumption of the low-protein high-carbohydrate diet was decreased more than consumption from the high-protein low-carbohydrate diet regardless of diet fat treatment after 1 or 2 hours of feeding. Serotonin turnover was assessed in experiments 3 and 4 using pargyline to block monoamine oxidase. There was no dietary fat by treatment effect on hypothalamic indoleamines or tryptophan when rats were fed single diets with a fixed protein/carbohydrate ratio, or when rats consumed selection diets. Thus, turnover rates were similar across diet fat treatments. The results of these experiments suggest that qualitative differences in dietary fat composition may influence the anorectic response to dexfenfluramine when rats are fed single diets with fixed protein/carbohydrate ratios, but that changes in steady-state serotonin metabolism or in serotonin turnover are not involved in the mechanism.

Keywords: saturated fat; *d*-fenfluramine; macronutrient intake; protein selection; carbohydrate selection; feeding behavior

Introduction

Our previous studies have shown that qualitative differences in dietary fat influences feeding behavior in rats.[1-3] Macronutrient selection, but not total food intake, differed in rats fed diets with different fatty acid composition and allowed to select for protein or carbohydrate. For example, rats fed 20% (w/w) fat diets containing either beef tallow or lard as dietary fat selected more protein and less carbohydrate than rats fed soybean oil-based diets.[1,2] More recent work has indicated the effect is not due to differences in the relative or absolute amounts of the essential fatty acids, 18:2ω6 or 18:3ω3, but that there is a highly significant relationship between dietary saturated fat (SFA) and the proportion of energy selected as protein or carbohydrate.[3]

The mechanism by which differences in dietary fat composition influence protein and carbohydrate selection is unknown. However, the effect is not mediated by an unconditioned response to sensory properties of

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diets.[2] Thus, physiological alterations may account for the observed differences in macronutrient selection. Changes in both peripheral and central nervous system metabolism arising from differences in dietary fat composition may be important. The role of the central nervous system in the regulation of food intake behavior has undergone extensive examination.[4-5] Many investigations have focused upon the importance of amino acids and neurotransmitter systems in the regulation of food intake.[6] In particular, the role of the serotonergic system as a mediator of both total food intake and macronutrient selection has received much attention.[7] Recent work, including studies with serotonergic agonists such as dexfenfluramine, has shown that serotonin (5-hydroxytryptamine, 5-HT) may play an integral role in macronutrient specific appetites.[8-10] Since 5-HT plays a role in feeding behavior, it is logical to ask whether differences in dietary fat composition mediate its effect through alterations in 5-HT. To address this question, we examined the effect of differences in dietary fat composition on both levels and turnover of 5-HT. In addition, we looked for differences in the food intake response following pharmacologic perturbation since this may be more sensitive to other aspects of 5-HT neurotransmission, including release or post-synaptic response to 5-HT. The purpose of these studies was to determine if alterations in dietary fat composition (i.e., the amount of SFA) which affect feeding behavior are mediated by changes in 5-HT metabolism or neurotransmission.

Materials and methods

Animals and diets

Male Wistar rats (Charles River, St. Constant, Quebec) were singly housed in galvanized wire mesh cages in a temperature-controlled environment (24 ± 1°C) with a 12-hour light cycle (04:00 h–16:00 h). Initial body weights were 70–90 g. For each experiment, rats were assigned randomly to diets that varied only in their fatty acid composition. Diets were nutritionally adequate, purified granular mixtures with 5% non-nutritive fiber, 2.5% vitamin mix, 5.1% mineral mix, and 0.25% L-methionine. A detailed description has been previously published.[2] All diets were isoenergetic with 20% (w/w) fat. Dietary fatty acid profiles were obtained by blending fat sources,[3] and fatty acid composition was determined by gas chromatography as previously described.[2] Fatty acid profiles are provided in *Table 1*. Protein content was verified with the micro-kjeldhal technique.[11] Water was available ad libitum but food was provided only during the dark cycle (16:00 h–04:00 h), and during the first 4 hours of the light cycle. Thus, rats had no access to food from 08:00 h–16:00 h so they would become accustomed to eating a meal at the commencement of the dark cycle.

Protein and carbohydrate consumption were obtained from food intake data. For both macronutrients, the Atwater value of 17 kJ/g (4 kcal/g) was used to calculate protein and carbohydrate energy. Energy density of diets was 19 kJ/g (4.48 kcal/g).

Table 1 Fatty acid composition of experimental diets[a]

	% of total diet (w/w)				
Fatty acid	9% SFA	8% SFA	7% SFA	3.5% SFA	3% SFA
16:0	5.60	5.49	4.52	2.80	2.24
16:1ω7	0.65	0.84	0.42	0.24	0.02
18:0	3.44	2.40	2.34	0.78	0.77
18:1ω9	8.02	7.70	8.00	14.00	4.78
18:2ω6	2.11	3.20	4.44	1.97	10.90
18:3ω3	0.19	0.12	0.28	0.24	1.27
ΣSFA	9.04	7.89	6.86	3.58	3.01
ΣMUFA	8.67	8.54	8.42	14.24	4.80
ΣPUFA	2.30	3.32	4.72	2.21	12.17
ω6:ω3	11.30	26.67	15.86	8.21	8.58

[a] Fat was extracted from premixed diets and the fatty acid profile determined by gas chromatography.

Dexfenfluramine studies

Experiment 1. The purpose was to determine if the effect of dexfenfluramine on total food intake is independent of dietary fat composition. Rats (N = 7 or 8/diet fat treatment) consumed 20% (w/w) fat diets (9%, 8%, or 3% SFA, w/w total diet) (*Table 1*) containing 24% protein and 40% carbohydrate. Rats consumed diets for 6 weeks prior to testing with dexfenfluramine. Each day, beginning 6 days before studies with dexfenfluramine commenced, rats were administered 0.9% saline (1 ml/kg body weight) intraperitoneally 15 minutes before the beginning of the dark cycle. Food intake was measured at 1, 2, and 16 hours for the last 4 days of the saline-injection period to ensure that the effect of saline injections on food intake had stabilized.

Dexfenfluramine (Servier Amerique, France) was administered in 3 dosages (0, 0.6, and 1.2 mg/kg body weight) in 0.9% saline vehicle in a completely randomized design. The maximum dose was 1.2 mg/kg since dexfenfluramine has been reported to affect feeding behavior at doses up to 1.25 mg/kg.[12] Saline (0.9%) was used as the control treatment. Dosages were administered with one day between treatments. Food intake was measured at 1, 2, and 16 hours after the beginning of the dark cycle.

Experiment 2. The purpose was to determine if the effect of dexfenfluramine on food consumption and the selection of protein and carbohydrate is independent of dietary fat composition. As in experiment 1, diets varied only in their fatty acid composition (9% or 3% SFA) (*Table 1*). Rats (N = 8 or 9/diet fat treatment) consumed 20% (w/w) fat diets containing 24% protein and 40% carbohydrate. After 2 weeks, rats were presented with selection diets. Rats selected from 2 diets with the same fat composition previously fed, but varying in their protein and carbohydrate composition (55% protein, 4% carbohydrate and 5% protein, 61% carbohydrate). Rats consumed selection diets for 3 weeks prior to testing with dexfenfluramine. Saline in-

jections were administered to rats daily for 5 days prior to testing, and food intake was monitored at 1, 2, and 16 hours for the final 3 days of saline injections.

Since a dose-effect in experiment 1 was observed with 1.2 mg/kg, dexfenfluramine was administered only at 2 dosages (0 and 1.2 mg/kg body weight). Within each diet fat treatment, one-half of rats were assigned randomly to either dexfenfluramine or saline (0.9%) treatments. Two days later, the sequence was reversed; rats that received drug on day 1 were injected with saline, and vice versa. In the control treatment (0 dose), rats received saline on both days. After one complete sequence, the procedure was repeated.

Serotonin turnover studies

In each experiment, rats were assigned randomly by diet groups to intraperitoneal administration of pargyline (N-methyl-N-2-propynylbenzylamine, hydrochloride crystalline, Sigma, St. Louis, MO) (75 mg free base/kg body weight) or 0.9% saline (1 ml/kg body weight). Injection solutions were prepared immediately prior to treatment. Rats were administered pargyline or saline at 15:15 h and decapitated 45 minutes after administration of drug or vehicle. This corresponded with the time rats received their food during the course of the experiment. Brains were dissected rapidly from the skull, the hypothalamus removed, and immediately frozen on dry ice. Hypothalami were stored at −70°C until analysis for tryptophan and 5-hydroxyindoles.

Experiment 3. The purpose was to determine the effect of differences in dietary fat composition on 5-HT metabolism when rats consumed single diets with a fixed protein/carbohydrate ratio. Rats (N = 7 or 8/diet fat treatment) were maintained on 20% (w/w) fat diets containing either 9% or 3% SFA (w/w, % of total diet) for 8 weeks. Rats were challenged previously with the macronutrient selection paradigm and were treated with dexfenfluramine (experiment 2). The last treatment with dexfenfluramine was 7 days prior to assessing 5-HT turnover, during which time rats consumed diets with a fixed protein/carbohydrate ratio (24% protein, 40% carbohydrate).

Experiment 4. The purpose was to determine the effect of dietary fat composition on 5-HT metabolism when rats selected from 2 diets that varied in the proportion of protein and carbohydrate. Rats (N = 10 or 11/diet fat treatment) were assigned to 20% (w/w) fat diets with 9%, 7%, or 3.5% SFA (w/w, % of total diet) (*Table 1*). Rats consumed diets with 24% protein and 40% carbohydrate for 2 weeks, then were challenged with the selection paradigm as described above (experiment 2). Rats consumed selection diets for 2 weeks prior to assessing 5-HT turnover.

Analysis of hypothalamus tryptophan and 5-hydroxyindoles

Levels of tryptophan, 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in hypothalami were determined according to the method of Mefford.[13] Briefly, hypothalami were sonicated (Sonifer Cell Disruptor, Heat System Co., Melville, NY) for 10 seconds in 0.5 ml of 0.2 M perchloric acid solution with 1% sodium metabisulfite and 0.01% EDTA. Samples were centrifuged at 11,205g (Microfuge II Centrifuge, Beckman Instruments, Inc., Palo Alto, CA) for 10 minutes, followed by filtration of the supernatant (HA-0.45 μm filter, Millipore Inc., Bedford, MA). A 100-μL sample of the filtered supernatant was injected in a HPLC-ECD system.

The mobile phase consisted of 0.1 M sodium acetate, 1.09×10^{-3} M citric acid and 1.71×10^{-3} M EDTA in water:methanol (94:6) at pH 3.7 ± 0.1. The flow rate was 1.0 ml/minute. The column was a reverse-phase μBondapak C_{18} column (Beckman Ultrasphere ODS 4.6 mm × 25 cm). The TL-5 glassy carbon electrode was coupled to a LC-4B amperometric detector with a +0.85 volt oxidizing potential (Bioanalytical Systems Inc., West Lafayette, IN). Tryptophan and 5-hydroxyindoles were quantified by linear regression of sample peak heights to equations generated by standard solutions. 5-HT turnover was calculated according to the method of Brodie et al.[14]

Statistical analyses

Statistical analyses were conducted with SAS 6.03 (SAS Institute, Inc., Cary, NC) for the microcomputer. The acceptable level of significance (i.e., Type I error) was $P \leq 0.05$ for all analysis. For dexfenfluramine studies, statistical analysis was performed on the mean difference score (MDS) (intake with control treatment minus intake after dexfenfluramine). Differences for each time period were cumulative. Data were analyzed by repeated measures two-way analysis of variance (ANOVA) with dietary fat composition and drug treatment as main effects. Comparison of group means was accomplished with Student Newman-Keul's test for multiple comparisons.[15] In experiment 1, drug treatment was the repeated factor; and in experiment 2, drug treatment and replicate were repeated factors. As there was no significant effect of replicate for any mean difference score, data are presented as the mean value for the two replicates. Data from 5-HT turnover studies were analyzed by two-way ANOVA with dietary fat composition and drug treatment as main effects.

Results

Dexfenfluramine studies

Experiment 1. The purpose was to determine if the effect of dexfenfluramine on food intake is independent of dietary fat composition (i.e., amount of SFA). Mean body weights were similar to all diet fat groups prior to treatment with dexfenfluramine (366 ± 14, 376 ± 11, and 394 ± 15 g, mean ± SEM, 9%, 8%, and 3% SFA, respectively). There were no significant differences in absolute food intake between diet fat groups following the control treatment. Average food intake

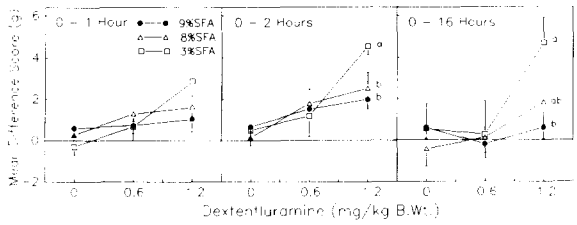

Figure 1 Rats were fed 20% (w/w) diets with a fixed protein/carbohydrate ratio (24% protein, 40% carbohydrate) for 6 weeks. Access to food was permitted only between 16:00 h and 08:00 h. Dexfenfluramine was administered intraperitoneally in a completely random design. Food intake was measured at 1, 2, and 16 hours after the presentation of food. MDS was calculated from cumulative food intake for each time period. Values are mean ± SEM. $N = 7$ or 8/diet fat treatment. Significant diet by drug interaction was present at each interval ($P < 0.05$). Points within a time interval not sharing a common superscripted letter are significantly different based on Student Newman-Keul's test for multiple comparisons ($P < 0.05$).

during each interval was 4.1 ± 0.2, 6.0 ± 0.3, and 21.4 ± 0.7 g (mean ± SEM, hours 0–1, 0–2, and 0–16, respectively).

The effect of dexfenfluramine on food intake is expressed as MDS (intake with the control treatment minus intake with dexfenfluramine such that the greater the MDS, the greater the effect of dexfenfluramine on food intake). For each diet fat treatment, dexfenfluramine resulted in a dose-dependent depression in total food intake when examined at 1, 2, and 16 hours after treatment ($P < 0.0006$) (*Figure 1*). In addition, there was a significant diet by drug interaction at each time interval ($P < 0.05$). Comparison of group means revealed significant differences between diet fat treatments with the 1.2 mg/kg dose after 2 or 16 hours of feeding. After 2 hours of feeding, the MDS was greater in rats fed diets with 3% SFA compared to those fed either 7.5% or 8% SFA. After 16 hours, the MDS remained significantly greater for rats fed 3% SFA compared to those fed diets with 8% SFA. In contrast to the 2-hour data, however, there was no significant difference in the MDS between rats fed 3% and 7.5% SFA (*Figure 1*).

Experiment 2. The purpose was to determine if the effect of dexfenfluramine on food consumption and the selection of protein and carbohydrate is independent of dietary fat composition. Prior to commencement of studies with dexfenfluramine, body weights were similar (365 ± 7 and 381 ± 7 g, mean ± SEM, 9% and 3% SFA, respectively).

During the 5 or 6 weeks that preceded studies with dexfenfluramine, there were no significant differences in food intake when rats consumed diets with a fixed protein/carbohydrate ratio (data not shown) or when selection diets were consumed (*Table 2*). Rats consuming diets with greater amounts of SFA selected more protein and less carbohydrate than rats fed diets with lesser amounts of SFA (*Table 2*). However, selection patterns were disrupted at a time coincident with the initiation of saline injections, such that analysis of the cumulative intake data for that period (6 days) indicated that selection of protein and carbohydrate was no longer significantly different between dietary fat treatments (*Table 2*).

Treatment with dexfenfluramine (1.2 mg/kg body weight) resulted in decreased intake of food, protein, and carbohydrate as indicated by an increased MDS (*Figure 2*). This effect was evident across time for each nutrient except carbohydrate, which was not significantly different from the control treatment (0 dose) at 16 hours.

When absolute food intake was examined, each diet fat group showed a significant decrease in response to dexfenfluramine at the end of each time interval (*Table 3*). In addition, consumption of carbohydrate was decreased more than protein after 1 or 2 hours of feeding (*Figure 2*). Despite differences in the effect of dexfenfluramine on the consumption of the respective diets, however, the percent energy selected as protein or carbohydrate was not affected by drug treatment except after 16 hours of feeding, when there was a slight but significant effect of drug treatment on percent protein energy (*Table 3*). In contrast to experiment 1, there was no significant dietary fat composition by drug treatment interaction when the data were ex-

Table 2 Food intake and macronutrient selection prior to the treatment with dexfenfluramine[1]

	Days 1–15					Days 16–21				
	Food g/d	PRO[3] g/d	% PRO Energy[4]	CHO[5] g/d	% CHO Energy[6]	Food g/d	PRO g/d	% PRO Energy	CHO g/d	% CHO Energy
9% SFA[2]	21 ± 0.4	5.6 ± 0.3[a]	24 ± 1[a]	7.4 ± 0.4[a]	32 ± 2[a]	17 ± 0.6	4.2 ± 0.5	22 ± 2	6.8 ± 0.7	34 ± 3
3% SFA	22 ± 0.6	4.2 ± 0.4[b]	17 ± 2[b]	9.7 ± 0.7[b]	40 ± 2[b]	17 ± 0.8	3.9 ± 0.4	20 ± 2	7.1 ± 0.5	37 ± 2

[1] Rats were fed single diets with a fixed protein/carbohydrate ratio (24% protein, 40% carbohydrate) for 2 weeks followed by a 3-week selection paradigm. Rats consumed diets with the same dietary fat as in the single diets, but differing in their protein and carbohydrate composition (55% protein, 4% carbohydrate and 5% protein, 61% carbohydrate). Rats had access to food only during the dark cycle. Selection days 16–21 are distinguished from days 1–15 only by the commencement of sham intraperitoneal injections. Values are mean ± SEM. $N = 8$ or 9/diet fat treatment. Values that do not share a common superscripted letter are significantly different ($P < 0.05$).
[2] Saturated fatty acids, % of total diet (w/w).
[3] Protein.
[4] Percent energy consumed as protein.
[5] Carbohydrate.
[6] Percent energy consumed as carbohydrate.

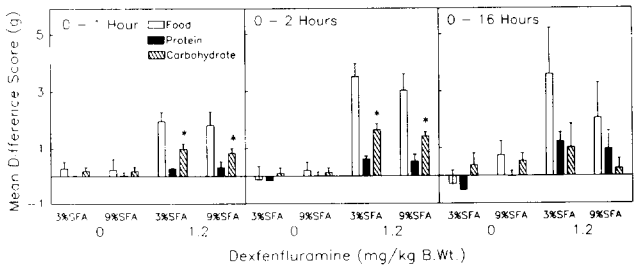

Figure 2 Rats were fed single diets with 20% (w/w) fat and a fixed protein/carbohydrate ratio (24% protein, 40% carbohydrate) for 2 weeks followed by a 4-week selection period (55% protein, 4% carbohydrate and 5% protein, 61% carbohydrate). Dietary fat composition was identical in single diets and selection diets. Access to food was permitted only between 16:00 h and 08:00 h. Dexfenfluramine was administered intraperitoneally. Each rat was tested at each dose. Food intake was measured at 1, 2, and 16 hours after the presentation of food. Carbohydrate and protein intake were calculated from food intake data. MDS for food, protein, and carbohydrate are cumulative for each time period. Values are mean ± SEM. N = 8 or 9/diet fat treatment. Significant effect of drug for food ($P < 0.0003$), protein ($P < 0.05$), and carbohydrate ($P < 0.0001$) after 1 or 2 hours of feeding. After 16 hours, significant effect of drug only for food ($P < 0.05$) and protein ($P < 0.008$). No significant diet by drug interaction was present at any interval ($P > 0.05$). An asterisk indicates that MDS was significantly greater for carbohydrate than for protein ($P < 0.003$).

pressed as MDS or as absolute intakes of food and macronutrients.

Serotonin turnover studies

Experiment 3. The purpose was to determine the effect of differences in dietary fat composition on 5-HT metabolism when rats consumed single diets with a fixed protein/carbohydrate ratio. Both dietary fat treatments had similar body weights when the turnover study was conducted (415 ± 8 and 435 ± 10 g, mean ± SEM, 9% and 3% SFA, respectively). Dietary fat treatment had no effect on levels of hypothalamic indoleamines, or tryptophan (*Figure 3*). Treatment with pargyline resulted in increased levels of 5-HT and decreased levels of 5-HIAA compared to saline treated rats (*Figure 3*). Pargyline had no effect on hypothalamic tryptophan levels. Turnover rates were also similar regardless of dietary fat composition. (*Table 4*).

Experiment 4. The purpose was to determine the effect of dietary fat composition on serotonin metabolism when rats selected from 2 diets that varied in their proportion of protein and carbohydrate.

Before commencement of the selection paradigm, mean body weights were similar between groups (180 ± 3, 189 ± 3, and 184 ± 4 g, mean ± SEM, 9%, 7%, and 3.5% SFA, respectively). After consuming selection diets for 2 weeks, however, rats fed diets with 7% SFA had a significantly greater mean body weight than those fed diets with 9% or 3.5% SFA (296 ± 5, 314, ± 6, and 285 ± 7 g, mean ± SEM, 9%, 7%, and 3.5% SFA, respectively).

Food intake was similar for all groups when rats consumed diets with a fixed protein/carbohydrate ratio (data not shown) and during the selection period (*Table 5*). Consistent with our previous studies, rats fed diets with 9% SFA selected significantly more protein and less carbohydrate than rats fed diets with 7% or 3.5% SFA (*Table 5*). In addition, simple linear regression showed a highly significant relationship between the amount of dietary SFA and percent protein or percent carbohydrate energy (PE = 0.56 SFA + 3.02, where PE = percent protein energy, $r^2 = 0.47$, $P < 0.001$).

Table 3 Food intake and macronutrient selection following treatment with dexfenfluramine[1]

Diets	Dexfenfluramine (mg/kg)	Food (g/interval)	PRO (g/interval)	% PRO Energy	CHO (g/interval)	% CHO Energy
			Hour 0–1			
3% SFA	0	3.3 ± 0.3[a]	0.56 ± 0.06[a]	16 ± 2	1.56 ± 0.18[a]	41 ± 3
	1.2	1.3 ± 0.1[b]	0.27 ± 0.06[b]	18 ± 3	0.58 ± 0.04[b]	38 ± 4
9% SFA	0	3.6 ± 0.3[a]	0.78 ± 0.16[a]	19 ± 3	1.49 ± 0.15[a]	35 ± 4
	1.2	1.8 ± 0.2[b]	0.44 ± 0.10[b]	23 ± 4	0.66 ± 0.10[b]	34 ± 3
			Hours 0–2			
3% SFA	0	5.7 ± 0.4[a]	1.01 ± 0.16[a]	15 ± 1	2.66 ± 0.18[a]	43 ± 3
	1.2	2.2 ± 0.1[b]	0.39 ± 0.07[b]	17 ± 2	1.02 ± 0.10[b]	41 ± 3
9% SFA	0	5.7 ± 0.5[a]	1.16 ± 0.20[a]	18 ± 2	2.47 ± 0.21[a]	37 ± 3
	1.2	2.7 ± 0.4[b]	0.64 ± 0.14[b]	20 ± 2	1.04 ± 0.17[b]	36 ± 3
			Hours 0–16			
3% SFA	0	24.1 ± 1.1[a]	5.36 ± 0.40[a]	20 ± 2[a]	10.06 ± 0.84	38 ± 2
	1.2	20.0 ± 0.9[b]	4.14 ± 0.39[b]	18 ± 2[b]	9.06 ± 0.57	39 ± 2
9% SFA	0	23.2 ± 0.8[a]	5.84 ± 0.40[a]	23 ± 2[a]	8.76 ± 0.68	34 ± 3
	1.2	21.0 ± 0.7[b]	4.88 ± 0.54[b]	21 ± 2[b]	8.49 ± 0.75	36 ± 3

[1] Rats were fed single diets with 20% (w/w) fat and a fixed protein/carbohydrate ration for 2 weeks followed by a 4-week selection period (55% protein, 4% carbohydrate and 5% protein, 61% carbohydrate). Dietary fat composition was identical in single and selection diets. Access to food was permitted only between 16:00 h and 08:00 h. Dexfenfluramine (0 and 1.2 mg/kg body weight) was administered intraperitoneally. Each rat was tested at each dose. Food intake was measured at 1, 2, and 16 hours after the presentation of food. Carbohydrate and protein intake were calculated from food intake data. Values are mean ± SEM. N = 8 or 9/diet fat treatment. Values within a single diet treatment and time interval not sharing a common superscripted letter are significantly different ($P < 0.05$). There was no significant dietary fat composition by drug treatment interaction for any parameter ($P > 0.05$).

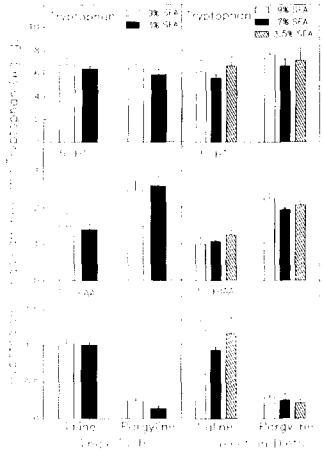

Figure 3 Single diets: Dietary regimen is described in *Figure 2*, except that after studies with dexfenfluramine rats consumed diets with a fixed protein/carbohydrate profile for one week before assessing 5-HT turnover. Values are mean ± SEM. $N = 8$ or 9/diet fat treatment. Effect of pargyline on steady-state hypothalamic indoleamines ($P < 0.05$). No significant diet fat by drug treatment interaction ($P > 0.05$). Selection Diets: Rats consumed 20% (w/w) fat diets with 24% protein and 40% carbohydrate for 2 weeks, followed by a 2-week selection period. Dietary fat composition was identical in single diets and selection diets, but rats selected from 2 diets that varied in their protein and carbohydrate composition (55% protein, 4% carbohydrate and 5% protein, 61% carbohydrate). 5-HT turnover was assessed after rats consumed selection diets for 2 weeks. Effect of pargyline on steady-state hypothalamic indoleamines ($P < 0.05$). No significant dietary fat composition by drug treatment interaction ($P > 0.05$).

Levels of 5-HT were increased and 5-HIAA decreased in rats treated with pargyline relative to levels in animals treated with saline (*Figure 3*). Differences in dietary fat composition had no significant effect on hypothalamic indoleamines or tryptophan levels (*Figure 3*). Consequently, turnover rates were similar regardless of dietary fat composition (*Table 5*).

Discussion

We previously demonstrated alterations in macronutrient selection in response to qualitative differences in dietary fatty acids.[1,2] Rats consuming greater amounts of dietary SFA select more protein and less carbohydrate than rats fed diets with lesser amounts of SFA.[3] The mechanism that mediates this effect has not been elucidated. However, we hypothesize that the effect of dietary fat on macronutrient selection is mediated via physicochemical changes in central nervous tissue metabolism. The serotonergic system has been implicated as a regulator of macronutrient appetites,[8-10] and thus presents itself as a highly compelling area of investigation.

We used dexfenfluramine, a 5-HT agonist,[12,16] to examine aspects of 5-HT neuotransmission among rats consuming diets that differed in their fatty acid composition. As expected, all diet fat treatments demonstrated a significant dose-response to dexfenfluramine as indicted by an increased MDS (*Figures 1* and *2*), where MDS represents the absolute amount of food intake suppression caused by each dose of dexfenfluramine. In both experiments, the greatest response to dexfenfluramine was observed generally within the first 2 hours after administration of the 5-HT agonist. Moreover, the 0–16 hour data show that rats had not compensated for suppression of food and/or protein intake within that time period.

When rats were fed diets with a fixed protein/carbohydrate ratio, the magnitude of the depression in food intake following dexfenfluramine was dependent upon dietary fat composition (*Figure 1*), with the greatest decrease occurring among rats fed the 3% SFA. In contrast, when rats selected from high-protein low-carbohydrate and low-protein high-carbohydrate diets, the reduction of food intake and macronutrient

Table 4 Effect of dietary fat on serotonin turnover[1]

	5-HT[5] Steady-State (μg/g)	5-HT Pargyline (μg/g)	Rate Constant[6] (h^{-1})	Turnover Time[7] (h)	Turnover Rate[8] (μg/g · h^{-1})
Single diets[2]					
9% SFA[3]	1.49 ± 0.34	2.46 ± 0.26	1.29	0.78	1.92
3% SFA	1.40 ± 0.14	2.59 ± 0.27	1.59	0.63	2.23
Selection diets[4]					
9% SFA	1.36 ± 0.18	2.26 ± 0.14	1.20	0.83	1.63
7% SFA	1.07 ± 0.03	1.94 ± 0.05	1.16	0.86	1.24
3.5% SFA	1.25 ± 0.13	2.07 ± 0.10	1.09	0.92	1.36

[1] Pargyline or saline were administered intraperitoneally 45 minutes before the beginning of the dark cycle. Rats were sacrificed 45 minutes later. Values are mean ± SEM. Turnover was calculated by the method of Brodie et al.[14]
[2] Rats were previously fed selection diets for 7 weeks. Rats consumed single diets with a fixed protein/carbohydrate ratio (24% protein, 40% carbohydrate) for 7 days prior to measuring turnover. $N = 8$ or 9/diet fat treatment.
[3] Saturated fatty acid, % of total diet (w/w).
[4] Rats were fed single diets (24% protein, 40% carbohydrates) for 2 weeks followed by selection diets (55% protein, 4% carbohydrate and 5% protein, 61% carbohydrate) for 2 weeks. $N = 10$ or 11/diet fat treatment.
[5] 5-hydroxytryptamine.
[6] Rate Constant = 5-HT (pargyline) − 5-HT (steady-state) × 60/t (minutes), where t = time lapsed between pargyline or saline injection and sacrifice.
[7] Turnover Time = 1/Rate Constant.
[8] Turnover Rate = 5-HT (steady state) × Rate Constant.

Table 5 Food intake and macronutrient selection prior to determination of serotonin turnover[1]

Diets	Food g/d	PRO g/d	% PRO Energy[3]	CHO g/d	% CHO Energy[4]
9% SFA[2]	21.6 ± 0.6	7.7 ± 0.7[a]	31 ± 2[a]	5.7 ± 0.5[a]	24 ± 3[a]
7% SFA	22.4 ± 0.7	4.8 ± 0.6[b]	20 ± 2[b]	9.4 ± 0.9[b]	37 ± 3[b]
3.5% SFA	20.6 ± 0.8	3.2 ± 0.3[b]	14 ± 2[b]	10.0 ± 0.7[b]	43 ± 2[b]

[1] Rats were fed single diets with a fixed protein/carbohydrate ratio (24% protein, 40% carbohydrate) for 2 weeks followed by a 2-week selection period. Rats selected from diets with the same dietary fat as in the single diets, but differing in their protein and carbohydrate composition (55% protein, 4% carbohydrate and 5% protein, 61% carbohydrate). Values are mean ± SEM. $N = 10$ or 11/diet fat treatment. Values that do not share a common superscripted letter are significantly different ($P < 0.05$).
[2] Saturated fatty acids, % of total diet (w/w).
[3] Percent energy consumed as protein.
[4] Percent energy consumed as carbohydrate.

intake by dexfenfluramine was comparable regardless of dietary fat composition (*Figure 2*).

The reason for the discrepancy in the results of experiments 1 and 2 is not readily apparent. Housing conditions were similar for each experiment. Each trial with dexfenfluramine was preceded by 5 or 6 days of saline injections so rats would become accustomed to handling and intraperitoneal injections. In addition, each set of animals had comparable mean body weights at the time of treatment with dexfenfluramine. The only apparent systematic difference in the two experiments was the dietary regimen.

When rats consumed selection diets prior to treatment with dexfenfluramine, rats fed diets with 9% SFA selected more protein and less carbohydrate than those fed diets containing 3% SFA during the first 15 days of the selection period (*Table 2*). A similar pattern of macronutrient selection was evident throughout the feeding trial in the 5-HT turnover study (*Table 5*). These results are consistent with our previously reported findings.[1-3] Differences in macronutrient selection associated with differences in the amount of dietary SFA disappeared, however, during the period that corresponded with daily saline injections (*Table 2*) and throughout the studies with dexfenfluramine (*Table 3*). The reason for the change in selection patterns is unknown. Our previous studies did not follow selection patterns beyond 14 days, so the absence of differences in protein and carbohydrate selection may reflect habituation to diets that occurs with time, or a generalized stress response to the initiation of saline injections. In addition, it has been reported that dietary chloride affects protein selection,[17] so selection patterns may have been altered in response to the additional chloride delivered in the saline injections The data do not argue consistently for this hypothesis since selection patterns were not altered in the same direction in both groups (*Table 2*).

Although not consistently reported,[18-20] a selective reduction in the intake of high-carbohydrate low-protein diets when rats are presented with a choice has been reported following administration of dexfenfluramine.[10,21] Our data are consistent with this observation. Whether the data are analyzed based on consumption from the respective food cups (data not shown), or as the selection for protein and carbohydrate (*Table 3, Figure 2*), the effect of dexfenfluramine on the carbohydrate component was greater at the end of 1 or 2 hours of feeding. However, this selective effect on the intake of carbohydrate had a limited effect on macronutrient selection. Comparison of the effect of dexfenfluramine on the percent of energy consumed as protein and carbohydrate did not reveal any significant drug effect at the end of 1 or 2 hours of feeding (*Table 3*). Dexfenfluramine altered the percent energy selected as protein only after 16 hours, but even in this case the physiological importance of such a minor, albeit statistically significant, change is questionable.

In order to find an effect of dexfenfluramine, rats must consume a sufficient amount of food during an interval such that a decrease in consumption can be measured reliably. Only the intake of the high-protein low-carbohydrate diet was questionable in this respect. Therefore, the ability to detect differences in the response to dexfenfluramine as a function of diet fat may have been impeded by the low baseline intake from the high-protein low-carbohydrate diet. Others have circumvented this problem by designing diet pairs such that the baseline selection from each diet is similar.[21] We chose to use diets consistent with previous selection studies (55% protein, 4% carbohydrate and 5% protein, 61% carbohydrate) to ensure continuity between the behavioral studies and experiments designed to investigate whether dietary fat composition alters the effect of dexfenfluramine on macronutrient selection.

Dexfenfluramine has been shown to affect both peripheral and central metabolism. However, the available evidence suggests the effect of dexfenfluramine on food intake behavior is mediated by central 5-HT metabolism. Notably, the anorectic effect of dexfenfluramine is blocked by central 5-HT antagonists, but not by 5-HT antagonists whose effect is limited to peripheral organs because of an inability to cross the blood brain barrier.[22] In addition, it seems doubtful the effect of dexfenfluramine is mediated by delayed gastric emptying,[23] because vagotomy has a similar effect on gastric emptying whereas the combination of dexfenfluramine and vagotomy potentiates the anorectic effect of dexfenfluramine.[24]

Dexfenfluramine has been demonstrated to enhance

serotonergic function by stimulating 5-HT release.[12,16] However, the mechanism by which differences in dietary fat composition might alter the effect of dexfenfluramine on food intake is not known. One possibility is that steady-state 5-HT metabolism is responsive to dietary fat composition (i.e., amount of SFA in our studies) and that the pool of 5-HT available for release is altered. After feeding rats diets containing either lard or soybean oil for 28 days, differences in dietary fat composition resulted in greater activity of monoamine oxidase (V_{max} but not K_m) in rats fed lard-based diets compared to those fed soybean oil-based diets.[1] However, steady-state levels of brainstem 5-HT and its metabolite, 5-HIAA, were unchanged.[1] Data from experiments 3 and 4 provide complementary findings. To eliminate meal-induced alterations in 5-HT metabolites, rats were killed 8 hours after their last food ingestion. The results showed no differences in steady-state hypothalamic levels of tryptophan, 5-HT, or 5-HIAA as a function of dietary fat treatment. Furthermore, 5-HT turnover was unaltered by differences in dietary fat composition when rats consumed diets with a fixed protein/carbohydrate ratio, or when consuming selection diets. We used pargyline, a monoamine oxidase inhibitor,[25] to measure 5-HT turnover.[13] However, steady-state hypothalamic levels of 5-HIAA were not affected by dietary fat composition, suggesting that the changes previously noted in monoamine oxidase activity in response to dietary fat composition were unimportant. Taken together, these data suggest dietary fat composition has a minimal effect on steady-state 5-HT metabolism.

In conclusion, these experiments suggest qualitative differences in dietary fat may influence the anorectic response to dexfenfluramine when rats are fed single diets with fixed protein/carbohydrate ratios. This effect does not appear to be mediated by changes in steady-state 5-HT metabolism, however, as neither 5-HT levels nor turnover responded to differences in dietary fat.

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