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# Dietary Fat Source Influences Neuronal Mitochondrial Monoamine Oxidase Activity and Macronutrient Selection in Rats

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CRANE, S. B. AND C. E. GREENWOOD. *Dietary fat source influences neuronal mitochondrial monoamine oxidase activity and macronutrient selection in rats.* PHARMACOL BIOCHEM BEHAV 27(1) 1–6, 1987.—We previously reported that qualitative changes in dietary fat influence certain monoaminergic mediated behaviours such as pain sensitivity and thermoregulation in a cold environment after an amphetamine challenge. The purpose of this study was to further explore the behavioural consequences of alterations in dietary fat intake by examining another behaviour known to be mediated by the monoamines—food intake regulation—and to begin investigating a biochemical link between dietary fat composition and behaviour. Rats were stabilized to 20% (w/w) soybean oil (SBO) or lard diets for 10 days and then allowed to select for protein (PRO) and carbohydrate (CHO) intake. While total food intake was unchanged, rats fed the SBO diet selected lower PRO (3.1±0.6 vs. 4.9±0.6 g/day, SBO vs. lard, respectively) and higher CHO (9.6±0.7 vs. 7.8±1.2) intakes than those consuming the lard based diet. Comparable differences were seen in a second trial. Current evidence suggests that the regulation of PRO and CHO intake is under serotonergic control. Therefore to determine whether dietary fat is mediating its effect on macronutrient selection via alterations in serotonin (5HT) metabolism, brain stem concentrations of 5HT and its metabolite 5-hydroxyindole acetic acid (5HIAA) and whole brain (minus brain stem) mitochondrial monoamine oxidase (MAO) activity were measured in a separate set of animals fed the SBO or lard diets for 28 days. Vmax of MAO was decreased in rats fed the SBO diets (20.2±7.4 vs. 27.9±8.9 nmol/mg prot/20') compared to those fed the lard diets. Km was unaltered by dietary fat fed. The change in activity of MAO was insufficient to alter steady-state levels of 5HT or 5HIAA. We propose that changes in neuronal functioning, induced by altered dietary fat, contributed to the differences seen in PRO and CHO selection.

Dietary fat Protein intake Carbohydrate intake Food selection Feeding behaviour
Monoamine oxidase Serotonin Mitochondria Soybean oil Lard

WE have recently reported that the fatty acid composition of the diet, in the absence of essential fatty acid deficiency, affects behaviour [5,36]. To examine this relationship between dietary fat and behaviour, a variety of behaviours in rats fed diets containing either 20% lard or soybean oil (SBO) for four weeks were monitored. Rats fed SBO were more able to maintain body temperature in a cold environment after an amphetamine challenge [36], were less sensitive to pain [36], and showed enhanced performance in an environmentally-cued testing paradigm which is thought to reflect cognitive learning skills (Place Navigation Water Task) [5]. Since both the thermoregulatory behaviour [35] and pain sensitivity [20,27] are, in part, mediated by the monoaminergic neurotransmitters, this present study examined the effect of dietary fat on feeding behaviour which is known to be mediated by monoamines [16]. Specifically, the selection of dietary protein and carbohydrate was monitored in rats—a behaviour thought to be under serotonergic control [18].

Dietary fat intake has been previously shown to influence neuronal cell membrane composition [2, 4, 7, 25], with alterations in phospholipid polar head groups, fatty acid profiles and cholesterol content of the membrane being observed. Concomitant with these changes in membrane composition are alterations in membrane-bound protein function [8, 10, 29]. Therefore, to begin investigating a biochemical link between dietary fat composition and behaviour, the activity of monoamine oxidase (MAO), a mitochondrial membrane bound enzyme involved in the degradation of the monoamines, serotonin (5HT) and the catecholamines was measured. Previous studies using *in vitro* techniques to modify membrane composition provide evidence that MAO

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TABLE 1
DIET COMPOSITION

| Ingredient | Standard g/kg | High Protein g/kg | Low Protein g/kg |
|---|---|---|---|
| Casein (87% Protein) | 270 | 632 | 57.4 |
| Cornstarch | 401.5 | 39.5 | 614.1 |
| Cellulose | 50 | 50 | 50 |
| Vitamins* | 25 | 25 | 25 |
| Minerals† | 51 | 51 | 51 |
| L-Methionine | 2.5 | 2.5 | 2.5 |
| Fat Source (see below) | 200 | 200 | 200 |
| | Soybean Oil Diet | Lard Diet | |
| Soybean Oil g/kg‡ | 200 | 10 | |
| Sunflower Oil g/kg§ | — | 10 | |
| Lard g/kg¶ | — | 180 | |

*Complete TD67231, Teklad Test Diets [22].
†Bernhard-Tomarelli, Teklad Test Diets [1].
‡Hain Pure Food Co. Inc.
§Unico.
¶Tenderflake, Maple Leaf.

TABLE 2
FATTY ACID COMPOSITION OF DIETS

| Fatty Acid | Fatty Acid (%) Soybean Oil | Lard |
|---|---|---|
| C12:0 | 0.65 | 1.76 |
| C14:0 | 1.89 | 3.13 |
| C16:0 | 15.16 | 21.72 |
| C16:1 (ω-7) | 0.49 | 1.64 |
| C18:0 | 9.35 | 12.30 |
| C18:1 (ω-9) | 19.28 | 30.38 |
| C18:1 (other) | 3.24 | 3.97 |
| C18:2 (ω-6) | 40.13 | 18.76 |
| C18:3 (ω-3) | 6.13 | 1.35 |
| C20:2 | 2.17 | 2.96 |
| C20:3 (ω-6) | | 1.67 |
| C22:0 | 0.56 | 0.36 |
| C22:1 | 0.59 | |
| C24:0 | 0.35 | |
| Σ sats | 27.96 | 39.27 |
| Σ monounsats | 23.60 | 35.99 |
| Σ polyunsats | 48.43 | 24.74 |

Diets contained 20% (w/w) fat. The lard was supplemented with 5% soybean oil and sunflower oil prior to mixing the diet. Lipid was extracted from the premixed diets and analysed for fatty acid content by gas-liquid chromatography using flame ionization detection.

activity is influenced by the lipid composition of the bilayer [12, 15, 23, 31].

## METHOD

### *Animals and Diets*

Male Wistar rats (Charles River Laboratories) weighing 75 to 90 grams were housed individually in wire mesh cages and supplied diet and water ad lib. The semi-synthetic diet compositions listed in Table 1 consisted of isocaloric mixtures varying in the source of 20% (w/w) dietary fat. SBO and sunflower oil were added to the diet containing lard at a level of 1% each, to eliminate the possibility of an essential fatty acid deficiency. Fatty acid composition of the diets (Table 2) was measured after mixing the diets. Fatty acid methyl esters were prepared [21] following Folch extraction [6], separated on a carbowax fused silica column using a Varian GLC (Vista 6000) and quantified using flame ionization detection. To ensure that dietary fatty acid composition would be reflected in membrane fatty acid composition, animals were maintained on the diet for 28 days, a period in excess of that shown to be required in previous studies [7].

Animals were housed, and all procedures carried out, on a randomized basis to avoid 'positional' effects.

### *Experimental Design*

*Experiment I.* The purpose of this experiment was to explore the influence of dietary fat on feeding behaviour, including protein and carbohydrate selection in rats. Two separate trials were performed (n=9 and 10 for lard vs. SBO, respectively, in the first experiment, and n=8 and 7 in the second trial) in which animals were fed the 20% lard or SBO diets for an initial period of 10 or 17 days prior to selecting from diets with varying protein and carbohydrate concentrations and containing the same dietary fat previously fed. Protein and carbohydrate intakes were calculated as described below.

*Experiment II.* To investigate a biochemical link between dietary fat and behaviour, this experiment determined whether qualitative changes in dietary fat intake could alter neuronal membrane bound enzyme function by measuring whole brain mitochondrial MAO activity following dietary manipulation. Animals (four per group) were fed either SBO or lard diets for 28 days. After sacrifice, brain mitchondrial MAO activity was determined for each individual animal.

*Experiment III.* The purpose of this experiment was to explore the kinetic changes observed in MAO activity resulting from alterations in dietary fat composition and to determine if the extent of change in MAO activity was of a sufficient magnitude to alter 5HT metabolism. SBO or lard diets were fed to 19 and 10 animals, respectively, for 28 days prior to sacrifice. Mitochondrial MAO kinetics, from whole brain minus brain stem, were estimated for each animal individually. Brain stems (containing the raphe nuclei) from the same animals were assayed for indoleamine concentrations.

### *Preparation of Tissue*

All chemicals used for biochemical analysis were purchased from Fisher Scientific Company unless otherwise stated.

Animals were killed by decapitation and brains excised, dissected and stored in ice-cold normal saline until used for mitochondrial isolation (maximum time: 2 hours). Brain stems were frozen on dry ice followed by long term storage at −70°C.

TABLE 3

PROTEIN AND CARBOHYDRATE SELECTION IN RATS FED DIFFERENT DIETARY FATS*

| Diet Fat | Protein Intake (g/day) | CHO Intake (g/day) | Energy Intake (kcal/day) | Protein Energy (%) |
|---|---|---|---|---|
| Trial 1† | | | | |
| Soybean Oil | 3.1 ± 0.6‡ | 9.6 ± 0.7‡ | 84.3 ± 3.1 | 15.7 ± 2.8‡ |
| Lard | 4.9 ± 0.6 | 7.8 ± 1.2 | 84.9 ± 6.8 | 25.1 ± 3.5 |
| Trial 2† | | | | |
| Soybean Oil | 3.6 ± 0.7‡ | 9.0 ± 0.7‡ | 84.5 ± 4.6 | 18.2 ± 3.1‡ |
| Lard | 6.2 ± 1.2 | 6.8 ± 1.6 | 86.6 ± 2.8 | 31.0 ± 6.3 |

*Rats selected from diets containing 20% (w/w) fat and either 55% protein and 17% carbohydrate or 5% protein and 67% carbohydrate.

†In trial 1, rats were prefed the standard 20% fat diets 10 days prior to selecting for protein and carbohydrate for 18 days. In trial 2, prefeeding period was 17 days and rats selected for 10 days. Dietary fat source during selection period was identical to that fed during the stabilization period. Values expressed are daily mean intakes during the selection period.

‡Mean ± SD. In trial 1, n=10 and 9 for SBO and lard, respectively. In trial 2, n=7 and 8 for SBO and lard, respectively. Means for SBO and lard are significantly different, $p<0.01$.

*Preparation of Mitochondria*

Mitochondria were isolated by the method of Whittaker and Barker [32]. Brain samples were homogenized in 10 volumes of 0.32 M sucrose (Anala®, BDH Chemicals) pH 7.0 and centrifuged at 1000×g (3000 rpm) for 15 minutes at 0°C (Beckman J2-21 centrifuge). The supernatant was decanted and further centrifuged at 18,000×g (13,000 rpm) for 20 minutes at 0°C, yielding a crude mitochondrial pellet (P2 pellet). This pellet was resuspended in 0.32 M sucrose and applied to a discontinuous sucrose gradient (0.32 M, 0.8 M, 1.2 M) and centrifuged at 100,000×g (27,500 rpm) for 70 minutes (Beckman L8-55 Ultracentrifuge). The resulting pellet contained the mitochondrial fraction. Electron micrographic verification of this procedure had previously been performed [17].

To obtain the data in Fig. 1, the pellet was resuspended in 0.5 M potassium phosphate buffer and a sample removed for protein analysis by the method of Bradford [3]. For all other experiments, the pellet was resuspended in distilled water and an aliquot removed for protein determination by the method of Lowry *et al.* [19], and the remainder made up to 0.5 M potassium phosphate buffer pH 7.4. The final preparation was divided into aliquots and stored at −70°C for later analysis.

*Monoamine Oxidase Assay*

Mitochondrial samples were analyzed for MAO activity by the method of Wurtman and Axelrod [34] within one week of preparation. A comparison of enzyme activity in freshly prepared and frozen mitochondrial samples showed no decrease due to short term storage at −70°C. In addition, previous experiments using freshly prepared P2 pellets showed similar dietary fat induced differences in MAO activity (data not published) suggesting that differences in MAO activity measured were not secondary to changes induced by freezing the tissue preparation.

In experiment 2, duplicate aliquots of the mitochondrial preparation containing linearly increasing protein concentrations (eight points per animal) were incubated with a constant concentration of $^{14}$C-tryptamine (15,800 dpm, 43.4 mCi/mmole, NEN) in a total volume of 300 μl of 0.5 M potassium phosphate buffer at pH 7.4. After a 20 minute incubation at 37°C, the reaction was stopped by the addition of 200 μl of 2 N HCl. The product, $^{14}$C-indole acetic acid, was extracted into 6 ml toluene. The toluene phase was added to 10 ml of scintillant (Ready-Solv HP/b, Beckman) and total

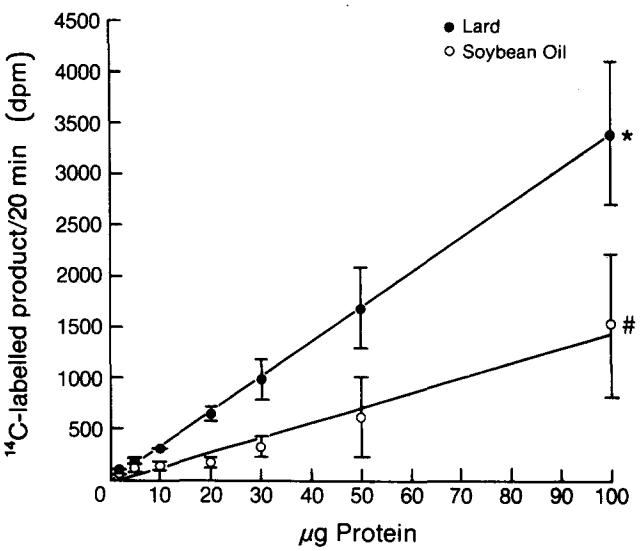


FIG. 1. Whole brain mitochondrial MAO activity measured 28 days after rats were fed diets containing either 20% (w/w) SBO or lard. Isolated mitochondria (0–100 μg protein) were incubated in the presence of $^{14}$C-tryptamine (15,800 dpm/assay tube) and MAO activity expressed as the amount of toluene soluble product generated per 20 minutes. *Mean±SD (n=4 rats per diet). #$p<0.001$.

TABLE 4

KINETIC PARAMETERS OF MAO OF ANIMALS FED DIFFERENT DIETARY FATS*

| Dietary Fat | MAO Activity Km ($\mu$M) | MAO Activity Vmax (nmol/mg prot/20') |
|---|---|---|
| Soybean Oil | 8.61 ± 1.51† | 20.23 ± 7.36‡ |
| Lard | 10.82 ± 3.54 | 27.93 ± 8.94 |

*Rats were fed 20% fat diets containing SBO or lard for 28 days. Whole brain (minus brain stem) mitochondrial MAO kinetics were determined using nine tryptamine levels (0.5–70 $\mu$M) per animal. Kinetic parameters were calculated by Lineweaver-Burke plots. In all samples examined, a linear relationship was observed at the substrate levels employed.

†Mean ± SD, n=19 and 10 for SBO and lard fed animals, respectively.

‡Means significantly different from one another, $p<0.03$.

radioactivity determined by counting in a liquid scintillation counter (Beckman LS 7800). The data were subjected to linear regression analysis, followed by a Student's *t*-test comparing slopes.

To establish the kinetic parameters of the MAO enzyme present in the samples, a protein concentration of 15 $\mu$g, on the linear portion of the activity curve, was used in subsequent experiments. The specific activity of the substrate ($^{14}$C-tryptamine, 30,000 dpm) was altered by the addition of unlabelled tryptamine (0.5 to 70 $\mu$M) and the assay performed in singleton at seven levels of substrate for each individual animal. The half-maximal substrate concentration (Km) and maximum velocity (Vmax) of the enzyme was determined by the Lineweaver-Burke method after linear regression of the data, as employed by others for calculating MAO kinetics [9, 11, 23]. Because MAO is a membrane bound enzyme, the use of Lineweaver-Burke method of analysis which assumes linearity could result in the generation of inaccurate values for the Km and Vmax. In all tissue samples examined, a linear relationship was observed at the substrate levels employed, hence Lineweaver-Burke analysis was used.

*Protein and Carbohydrate Selection*

For an initial stabilization period (10 and 17 days in trials 1 and 2, respectively), rats were fed semi-synthetic diets containing 23.5% protein, 44% carbohydrate and either 20% w/w lard or SBO. Two diets were then given to each animal, one containing 5% protein and 67% carbohydrate by weight, the other containing 55% protein and 17% carbohydrate (Table 1). The two diets both contained the same fat at the same concentration which had been fed to the animal during the initial period. Changes in body weight and food consumption were recorded every two to three days during the selection period (18 and 10 days in trials 1 and 2, respectively). Average daily protein and carbohydrate intakes were calculated (g/day) and converted to energy values using the Atwater value of 4 kcal/g for both macronutrients. The proportion of total calories consumed as protein was then determined. Energy density of the diet was 4.48 kcal/g.

*Serotonin and 5-Hydroxyindole Acetic Acid*

Brain stem levels of 5HT and its metabolite, 5-hydroxyindole acetic acid (5HIAA), were measured in 0.1 M perchloric acid supernatants by HPLC [24]. The mobile phase consisted of 0.1 M sodium acetate buffer (pH 4.7) containing 6% methanol, with a 2.0 ml/min flow rate. The electrochemical detector was set at 5 nA/V, with a potential of 0.7 volt. Isoproterenol was used as internal standard.

*Statistical Analysis*

Differences among group means were established using Student's *t*-test. In experiment III, where sample size differed, the *t* statistic was calculated by the method for unequal sample sizes and variances [28].

## RESULTS

General growth characteristics of the rats were not affected by the diets fed. In all instances, initial body weight, final body weight and weight gain (approximately 175 g/28 days) were similar in both groups of animals. Total food intake was also unaffected by the diets fed (data not shown).

*Protein and Carbohydrate Selection*

Although total caloric consumption was similar in both groups of animals, the SBO-fed rats selected significantly less protein and more carbohydrate than the lard-fed animals (Table 3). Differences in feeding behaviour were apparent after an initial stabilization period to the dietary fat of 10 days (trial 1). By extending the stabilization period to 17 days prior to allowing the animals to select for protein and carbohydrate (trial 2), the magnitude of change in behaviour was similar, suggesting that maximal adaptation to the dietary fats, in terms of behavioural response, occurs rapidly.

*Mitchondrial Monoamine Oxidase Activity*

After 28 days of feeding, MAO activity in brain mitochondria from rats consuming the SBO diet was significantly lower than from those fed the lard diet (Fig. 1). To further probe the effects of this change in enzyme activity, the kinetic parameters of MAO were established in a separate set of animals. The Km of MAO was not altered by diet. The Vmax, however, was significantly lower in the animals fed SBO than those fed lard (Table 4) as the dietary fat source. It appears, therefore, that the lower enzyme activity seen in mitochondria from animals fed the SBO diet is not likely the result of changes in the binding properties of the enzyme, but rather on the total number of enzyme molecules available for binding.

*Brain Indoleamine Concentration*

To determine whether this lower enzyme activity would be reflected in altered neurotransmitter metabolism, brain stem 5HT and 5HIAA were measured. No difference was observed in the absolute levels of either 5HT (561±100 vs. 568±75 ng/g wet weight; mean±S.D. for SBO vs. lard fed rats, respectively) or 5HIAA (580±115 vs. 562±110).

## DISCUSSION

The results of this study continue to add evidence in support of our hypothesis that animal behaviours are modified by the composition of dietary fat, even in the absence of essential fatty acid deficiency. Specifically, these studies

now demonstrate that fat composition of the diet influences macronutrient selection in rats. In addition the activity of a membrane bound enzyme, MAO, was altered, suggesting that the effect of dietary fat on feeding behaviour may be related to biochemical effects of dietary fat influencing neuronal function.

The effect of dietary fat on feeding behaviour appears to be specific for macronutrient selection since total caloric intake was unaffected. Rats consuming the diet high in polyunsaturated fatty acids (SBO) selected less protein and more carbohydrate, both as total quantity and as proportion of calories, than rats consuming the low polyunsaturated (lard) fat diet (Table 3). Given the design and results of the feeding trials, it is impossible to distinguish between specific appetites for protein and carbohydrate. In this feeding paradigm rats are able to modify the relative amounts of protein and carbohydrate consumed, but not able to selectively decrease their intake of either protein or carbohydrate unless they decrease total energy intake at the same time. Thus the results of these studies clearly demonstrate that dietary fat modifies the proportion of protein and carbohydrate consumed, but cannot be used to interpret independent appetites for either macronutrient.

Since macronutrient selection is mediated, in part, by serotonin [18], brain stem concentrations of the indoleamines were monitored 28 days after feeding the diets. It was postulated that dietary fat intake could influence indoleamine metabolism either via alterations in 5HT catabolism, since MAO activity is altered (Fig. 1, Table 4), or via alterations in the availability of the precursor amino acid tryptophan to the brain, mediated by changes in the plasma ratio of free to albumin-bound tryptophan [26]. No differences in steady-state concentrations of either 5HT or 5HIAA were observed, suggesting that dietary fat is not influencing macronutrient selection simply via alterations in 5HT metobolism.

While the present study does not directly elucidate the mechanism of action of dietary fat, the results of the enzyme (MAO) data (Fig. 1, Table 4) are consistent with other studies suggesting that neuronal membrane composition is influenced by dietary fat composition [2,7] and that these lipid bilayer changes appear to precipitate alterations in membrane protein function [8, 10, 29]. In addition, studies using *in vitro* techniques to modify membrane composition provide evidence that the activity of MAO is influenced by the lipid composition of the bilayer [12, 15, 23, 31]. Thus while the magnitude of change in MAO activity is probably not large enough to be physiologically significant in itself, as evidenced by the lack of effect on 5HT metabolism, the results do suggest that the neuron is responding biochemically to the dietary fat manipulation, probably at the level of the lipid bilayer. Further studies are required to elucidate the physiologically important effect of dietary fat.

The behavioural changes associated with dietary fat intake occur rapidly. Our earlier studies [5,36] only monitored alterations in pain sensitivity, thermoregulation and cognitive performance after 28 days of feeding the diets. Macronutrient selection, however, is now shown to be influenced after a 10 day stabilization period (Table 3, trial 1). By extending the stabilization period to 17 days (Table 3, trial 2), the magnitude of change in macronutrient selection observed was only slightly greater, suggesting that maximal adaptation to dietary fat intake, in terms of behavioural response, occurs quickly. The timing of these behavioural changes are consistent with earlier reports showing alterations in cardiac mitochondrial membrane composition and associated enzyme activity in growing rats within 11 days of feeding different dietary fat sources [13,14]. Even though it has been suggested that nervous tissue membrane composition may be more resistent to dietary fat induced change than peripheral tissues [30], these data suggest that neuronal function has been altered.

The physiological significance of the alteration in feeding behaviour becomes apparent when the magnitude of change in macronutrient selection is examined. Earlier studies elucidating the role of 5HT in the regulation of protein and carbohydrate intakes [18], administered serotonergic drugs or the precursor amino acid tryptophan at levels which caused large perturbations in 5HT metabolism in order to observe similar magnitudes of change in protein and carbohydrate selection. Thus while the mechanism of action of dietary fat does not appear to be mediated simply via alterations in 5HT metabolism, these behavioural results are consistent with rather significant changes in neuronal communication.

In conclusion, the results of these studies indicate that alterations in dietary fat intake influence neuronal function and feeding behaviour, specifically macronutrient selection, in rats. The possible ramifications of these studies, though highly speculative, are exciting. Previous studies have shown that animal membrane compositional changes in response to dietary fat are rapid and reversible [13], with the time course for membrane change paralleling that required to observe behavioural changes in our animals. Studies are also ongoing which promote the use of dietary intervention in the treatment of human neurologic disorders [33]. Since the fats employed in these studies were chosen to be reflective of those commonly consumed in the human diet, if dietary fat can indeed alter neuronal function, and thus behaviour, the application of this direction of research to degenerative neurologic disorders may be promising.

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