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Short communication

High dose of an n-3 polyunsaturated fatty acid diet lowers activity of C57BL/6 mice

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ABSTRACT

n-3 Polyunsaturated fatty acids (PUFA) are increasingly consumed as food additives and supplements; however, the side effects of these fatty acids, especially at high doses, remain unclear. We previously discovered a high fat n-3 PUFA diet made of fish/flaxseed oils promoted significant weight gain in C57BL/6 mice, relative to a control, without changes in food consumption. Therefore, here we tested the effects of feeding mice high fat (HF) and low fat (LF) n-3 PUFA diets, relative to a purified control diet (CD), on locomotor activity using metabolic cages. Relative to CD, the HF n-3 PUFA diet, but not the LF n-3 PUFA diet, dramatically reduced ambulatory, rearing, and running wheel activities. Furthermore, the HF n-3 PUFA diet lowered the respiratory exchange ratio. The data suggest mixed fish/flaxseed oil diets at high doses could exert some negative side effects and likely have limited therapeutic applications.

1. Introduction

n-3 Polyunsaturated fatty acids (PUFAs), the bioactive components of fish and flaxseed oil, are routinely consumed by the public as food additives or supplements. They are also recognized to have utility for treating metabolic and inflammatory diseases [1–5]. Generally, dietary studies evaluating n-3 PUFA efficacy in differing model systems have relied on low doses of n-3 PUFAs. With animal studies, investigators have commonly used ~5% (weight/weight) fish or flaxseed oil as intervention, corresponding to approximately 2–6% of total energy as n-3 PUFAs [6]. This dose, especially of fish oil, is often selected to model n-3 PUFA intake of Greenland Eskimos that consume n-3 PUFAs in the range of 1–6% of total energy [7,8]. A few studies have tested the effects of fish or flaxseed oils at higher doses with mixed results on functional endpoints [9–11]. Overall, very little is known about the effects of high doses of n-3 PUFAs, which could have a unique therapeutic niche or could exert negative side effects, raising potential safety issues for the general public.

We previously reported long-term administration of high levels of a mixed fish/flaxseed oil diet to C57BL/6 mice promoted significant body weight gain [12]. These findings suggested high doses of n-3 PUFAs increased body weight by lowering activity since we ruled out changes in food consumption [12]. Therefore, the objective of this study was to determine if short-term dietary consumption of a high dose of n-3 PUFAs could lower energy expenditure prior to any large differences in body weight. Studies were conducted in comparison to a low fat (LF) purified mouse control diet (CD) and a LF n-3 PUFA diet.

2. Experimental methods

2.1. Mice and diets

All experiments with mice fulfilled guidelines established by the East Carolina University Brody School of Medicine for euthanasia and humane treatment. Male C57BL/6 mice (~4–6 weeks old) were placed for 3 weeks on two experimental diets, developed in collaboration with Harlan-Laboratories (Madison, WI). Mice were administered either a purified control diet (CD), 5% fat by weight, a LF n-3 PUFA diet, or a HF n-3 PUFA diet, 20% fat by weight, as previously described [13]. The composition of the diets is listed in Table 1.

2.2. Metabolic cage studies

Mice were placed in fully automated metabolic cages (TSE Systems) for 4 days to monitor activity during week 2. Mice were placed one per cage and were acclimated for 48 h followed by data collection for 48 h. Airflow through the cages was held constant at 0.5 L/min. 12 h light and dark cycles were maintained with ad libitum access to food and water. Locomotor activity in

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the metabolic cages was measured by the breaking of 32 infrared laser beams that span each cage in the *xy* and *yz* planes. TSE LabMaster software recorded in 20 min intervals each time a series of laser beams were broken by ambulatory, rearing, and running wheel activity. Metabolic activity was measured via indirect calorimetry recording maximal O_2 consumption (VO_2) and CO_2 production (VCO_2). VO_2 and VCO_2 values were normalized by the software to body weight in kilograms and are reported as ml/h/kg [14]. Respiratory exchange ratio (RER) was calculated as VCO_2/VO_2. The data were analyzed for average light and dark activity [14]. Significance was established using a two-way ANOVA followed by a Bonferroni Multiple Comparison t test using GraphPad Prism [14]. *P* values less than 0.05 were considered significant.

Table 1
Composition of control, low fat, and high fat n-3 PUFA diets.

Formula (g/Kg)	CD	LF n-3 PUFA	HF n-3 PUFA
Casein	185	185	220
L-Cystine	2.5	2.5	3.0
Corn Starch	370	370	174
Mineral Mix, AIN-93M-MX (94049)	35	35	42
Vitamin Mix, AIN-93-VX (94047)	15	15	18
Choline Bitartrate	2.5	2.5	3.0
TBHQ, antioxidant	0.02	0.02	0.06
Maltodextrin	140	140	140
Sucrose	150	150	150
Cellulose	50	50	50
Soybean Oil	50	3.8	15
Flaxseed Oil	-	23.1	92.5
Fish Oil	-	23.1	92.5

3. Results

3.1. Body weight and food consumption

Mice weighing 16–19 g were placed on diets. Final body weight gain, confirmed with measurements of adipose mass with Echo-MRI, was identical between the CD and the experimental diets (data not shown). Mice on the HF n-3 PUFA diet consumed less food relative to CD; however, there was no difference in the total kcal consumed between the differing diets relative to CD (data not shown).

3.2. Metabolic cage studies

The time course of ambulatory, rearing, and running wheel activities are shown in Fig. 1A–C. These data were used to calculate the average activity for the entire light and dark cycles (Fig. 1D–F). There was a significant increase in activity during the dark cycle with all of the diets relative to the light cycle for all measurements (Fig. 1D–F).

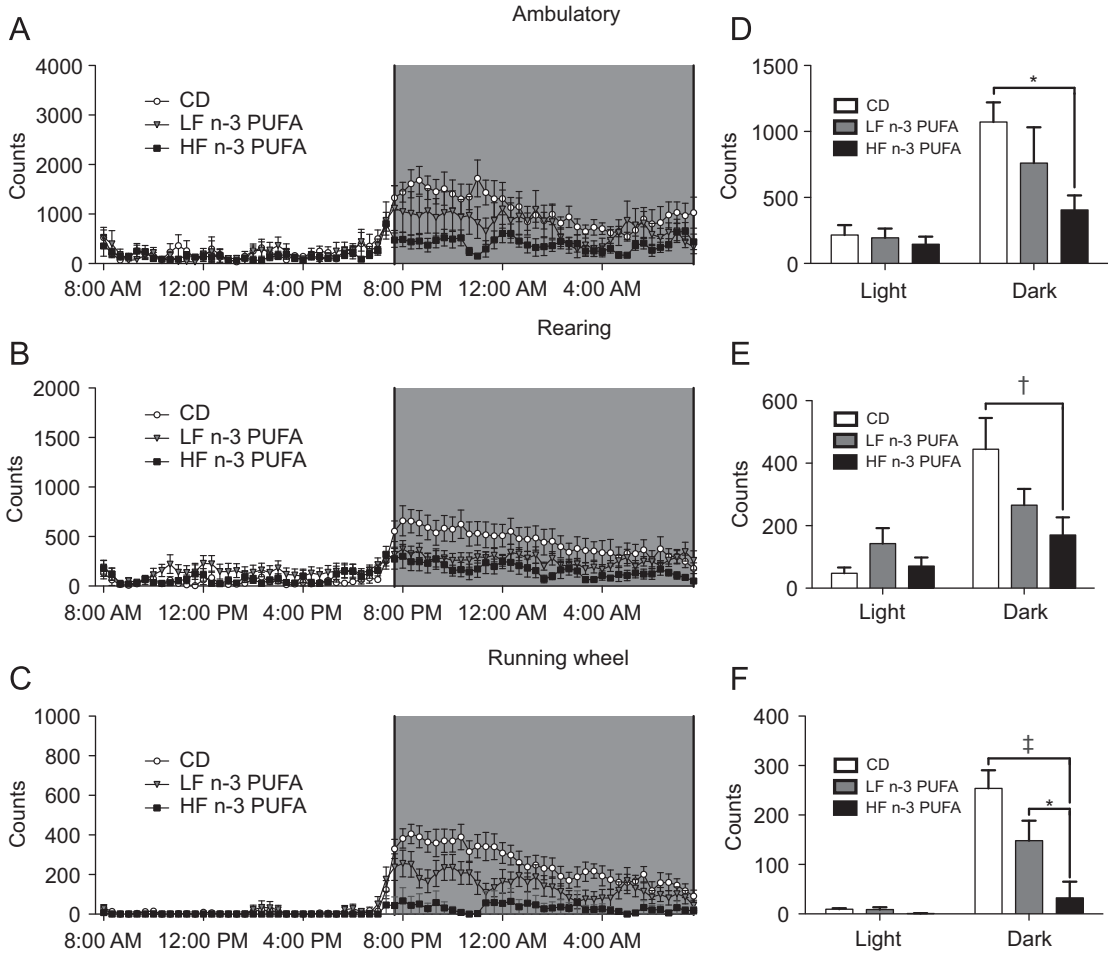

Fig. 1. HF n-3 PUFA diet lowered activity. (A) Ambulatory, (B) rearing, and (C) running wheel activities as a function of time for mice fed differing diets. Corresponding average light and dark cycle (D) ambulatory, (E) rearing and (F) running wheel activities. Counts represent the number of laser beams broken due to movement in the cage. Data are mean ± SEM, $n=7$ mice per diet. Significance during the dark cycle compared to CD is indicated by *$P<0.05$, †$P<0.01$, ‡$P<0.001$.