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Basic Science

Obesity development in neuron-specific lipoprotein lipase deficient mice is not responsive to increased dietary fat content or change in fat composition☆

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ABSTRACT

We have previously reported that mice with neuron-specific LPL deficiency (NEXLPL–/–) become obese by 16 weeks of age on chow. Moreover, these mice had reduced uptake of triglyceride (TG)-rich lipoprotein-derived fatty acids and lower levels of n-3 long chain polyunsaturated fatty acids (n-3 PUFAs) in the hypothalamus. Here, we asked whether increased dietary fat content or altered dietary composition could modulate obesity development in NEXLPL–/– mice. Male NEXLPL–/– mice and littermate controls (WT) were randomly assigned one of three synthetic diets; a high carbohydrate diet (HC, 10% fat), a high-fat diet (HF, 45% fat), or a HC diet supplemented with n-3 PUFAs (HCn-3, 10% fat, Lovaza, GSK®). After 42 weeks of HC feeding, body weight and fat mass were increased in the NEXLPL–/– mice compared to WT. WT mice fed a HF diet displayed typical diet-induced obesity, but weight gain was only marginal in HF-fed NEXLPL–/– mice, with no significant difference in body composition. Dietary n-3 PUFA supplementation did not prevent obesity in NEXLPL–/– mice, but was associated with differential modifications in hypothalamic gene expression and PUFA concentration compared to WT mice. Our findings suggest that neuronal LPL is involved in the regulation of body weight and composition in response to either the change in quantity (HF feeding) or quality (n-3 PUFA-enriched) of dietary fat. The precise role of LPL in lipid sensing in the brain requires further investigation.

1. Introduction

Lipids are a major constituent of the brain; and most lipids in the brain exist in phospholipid pools as essential structural components of cell membranes that play important roles in the developing and adult brain. The majority of brain phospholipids are long-chain polyunsaturated fatty acids (PUFAs), such as docosahexaenic acid (DHA) and arachadonic acid (AA) [1]. Numerous reports have shown that a deficiency in brain DHA can have detrimental cognitive effects such as

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learning and memory, and anxiety [2–8]. While saturated and monounsaturated fatty acids are synthesized *de novo* within the brain [8], PUFAs are mostly obtained directly from the diet, or synthesized in the liver then supplied to brain via the blood [8,9]. Precisely how the brain maintains its unique fatty acid composition is still under debate.

In recent years, lipids (such as non-esterified fatty acids, cholesterol) and lipid derivatives (such as endocannabinoids) have been shown to play important roles in information processing and the regulation of energy homeostasis [10–14]. For example, fatty acid availability in the hypothalamus appears to have a profound effect on the regulation of energy balance [15–17]. In addition, the importance of long chain fatty acid delivery during brain development has been repeatedly implicated [18,19]. Infusion of free fatty acids into the third ventricle of rodents [15,17,20–23], and brain-specific modification of enzymes involved in lipid metabolism, i.e. CPT1 [17] and CPT1c [20,21], and fatty acid synthase (FAS) [24] by direct injection, have shown that fatty acid metabolism in the CNS plays an important role in the regulation of food intake and body weight. Despite evidence supporting the essential role of lipid metabolism in the brain [7,25], it remains unclear how the *de novo* synthesis vs. the transport of various classes of fatty acids into the brain is regulated.

We hypothesized that lipoprotein lipase (LPL), a rate-limiting enzyme in the hydrolysis of triglyceride (TG), and tissue uptake of fatty acids from circulating TG-rich lipoproteins [26], could cleave fatty acids from lipoproteins, facilitating the entry of these TG-rich lipoprotein-derived lipids into the brain via either passive diffusion or protein-mediated uptake [27]. Historically, the delivery of fatty acids to the brain, particularly in the form of triglyceride (TG)–rich lipoproteins, has not been extensively examined. We recently created mice with a neuron-specific LPL deficiency (NEXLPL−/−), which became obese on a chow diet by 16 weeks of age, and showed reduced uptake of TG-rich lipoprotein-derived fatty acids and lower levels of n-3 long chain polyunsaturated fatty acids (n-3 PUFAs) in the hypothalamus [28]. This mouse was the first physiological model where disruption of lipid metabolism in the brain was achieved by genetic modification and resulted in obesity. We reasoned that altered dietary composition could modify the obesity phenotype in NEXLPL−/− mice. Thus, we have conducted experiments to test if obesity development is affected by either high-fat feeding or rescued by dietary supplementation with n-3 PUFAs.

2. Methods

2.1. Mice, Diet Composition, and Feeding

NEXLPL−/− mice and littermate controls were generated as described [28]. At 10 weeks of age, male mice were individually caged for a week before being fed with the experimental diets. To test the effect of altered dietary fat content on obesity development mice were fed either a protein matched (20% kcal) high fat diet (HF, 45% fat, D12451 Research Diets) or a high carbohydrate diet (HC, 10% fat, D12450B Research Diets) for 42 weeks. For the n-3 PUFA supplementation experiments, a high carbohydrate diet was supplemented with calculated amounts of Lovaza (GSK), containing n-3 PUFA in the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with other nutrient composition including identical amounts of saturated fatty acids and monounsaturated fatty acids as for the control diet (HCn-3, 10% fat). The caloric content of HCn-3 was maintained the same as HC (Table 1). All mice were started at 10 weeks of age, and fed for 42 weeks, and were 52 weeks of age at the time of the terminal experiments.

2.2. Measurement of Body Weight, Body Composition, and Plasma Metabolic Parameters

Body weight and food intake were monitored on a weekly basis. Body composition was measured on anesthetized mice by dual-energy x-ray absorptiometry using a mouse densitometer (PIXImus2, Lunar Corp., Madison, WI) at the end of feeding when terminal blood and tissues were collected for analysis. Blood was collected by cardiac puncture, and plasma was stored at −20 °C until further analysis. Plasma glucose was measured using the Cayman Glucose Colorimetric Assay Kit (Caymen Chemical, San Diego CA). TG and FFA were measured using enzymatic, colorimetric assays (Sigma, St. Louis, MO and Wako Chemicals USA, Richmond, VA, respectively), and insulin was measured using a RIA kit (Linco Research, St. Charles, MO). Plasma leptin and adiponectin were measured using specific enzyme-immunoassay kits (ELISA) designed for quantitative determination of mouse plasma samples (Alpco Diagnostics, Salem, NH).

Table 1 – Nutrient composition of diets.

Product	D12451	D12450B	D12450B + Lovaza
%	HF kcal	HC kcal	HC-n3 kcal
Protein	20	20	20
Carbohydrate	35	70	70
Fat	45	10	10
Total	100	100	100
Ingredient (selected)	kcal	kcal	kcal
Corn starch	691	1260	1260
Maltodextrin 10	400	140	140
Sucrose	291	1400	1400
Soybean oil	225	225	0
Lard	1598	180	0
Safflower oil	0	0	23
Olive oil	0	0	117
Coconut oil, hydrogenated	0	0	80
Safflower oil, high oleic	0	0	45
Lovaza	0	0	131
Saturated fatty acids, %	36.3	25.1	25.0
Monounsaturated fatty acids, %	45.3	34.7	30.4
Polyunsaturated fatty acids, %	18.5	40.2	44.6
n-6:n-3 ratio	8.5:1	7:1	1:3