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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.

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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 arachidonic 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 (Cayman 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,	0	0	80
hydrogenated			
Safflower oil,	0	0	45
high oleic			
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

2.3. Indirect Calorimetry and Physical Activity Measurements

An open-ended indirect calorimetry system was used to measure oxygen consumption (O_2) and carbon dioxide (CO_2) production in mice for the calculation of metabolic rate and respiratory quotient (RQ) [29]. Animals at the end of feeding periods were placed in 4 metabolic chambers for measurements taken over three days with free access to food and water. The differential O_2 and CO_2 concentrations, flow rate, RQ and metabolic rate (Weir equation) were calculated and stored in a computer configured with data acquisition hardware (Analogic, Wakefield, MA) and software (Labtech, Wilmington, MA). Average daily food intake was also determined in the indirect calorimetry settings. In addition, measurements of physical activity were carried out using the Columbus Instruments Opto M3, a multi-channel activity monitor that utilizes infrared beams to monitor an animal's movement in the X, Y and Z axis. The total physical activity during a three day calorimetry experiment was determined by adding all the ambulatory counts in the X direction.

2.4. Quantitative Real-Time PCR

Tissue was collected into RNeasy (Qiagen) from anesthetized mice after a 4 h fast and stored at 4 °C. Total RNA was extracted and reverse transcribed as previously described [28]. Quantitative PCR was performed using primer sets for genes of interest, two reference genes (Gapdh and Ubc) and iQ Supermix or iQ SYBR Supermix (Bio-Rad) following the manufacturer's protocols.

2.5. Lipidomic Analyses of Brain Tissues

The detailed method of lipidomic analyses has been described previously [28]. Briefly, whole brains from various groups of mice were freshly harvested and quick-frozen in 2-methylbutane at –40 °C and then stored at –80 °C until further processing. The hypothalamus was punched from frozen brain, which was then weighed and homogenized in methanol containing the following internal standards: d_8 -arachidonic acid, d_8 -2-arachidonoyl glycerol (Cayman Chemical), diheptadecanoin, trionadecanoin (Nu-Chek Prep). Lipids were analyzed as previously described [28].

2.6. Statistical Analyses

Results are presented as mean $\pm$ SEM. One way repeated measure ANOVA was performed for body weight and cumulative food intake data. Two tail, unequal variance t-tests were performed for all the other statistical analysis using the Data Analysis Tool pack in Excel 2010 (Microsoft). A $p < 0.05$ was considered significant.

3. Results

3.1. Obesity Development in NEXLPL $^{-/-}$ Mice is Not Exacerbated by High Fat Diet

NEXLPL $^{-/-}$ mice and littermate controls were individually caged at 10 weeks and fed either a synthetic 45% high fat diet (HF) or a

synthetic high carbohydrate diet (HC) (Table 1). After 42 weeks of HC diet feeding, a significant increase in body weight was observed in NEXLPL $^{-/-}$ mice (Fig. 1A) as previously observed on chow [28]. As expected, HF feeding produced diet induced obesity in WT mice (Fig. 1B and C), but no significant difference was observed for body weight, fat mass or body composition in HF-fed NEXLPL $^{-/-}$ mice compared to HC-fed NEXLPL $^{-/-}$ mice (Fig. 1D). Accumulated food intake throughout the feeding period for HC-fed NEXLPL $^{-/-}$ mice was increased as compared to HC-fed WT control mice (Fig. 1E and F, $p < 0.05$), but no difference was observed for accumulated food intake for HF-fed NEXLPL $^{-/-}$ vs. HF-fed WT mice. This is likely due to the fact that HF-fed WT mice showed increased accumulated food intake compared to their HC fed WT counterparts (Fig. 1E, $p < 0.05$), however, there was no change in accumulated food intake between NEXLPL $^{-/-}$ mice fed either a HF or HC diet (Fig. 1F). Of additional interest, HF feeding usually decreases the respiratory quotient (RQ) in WT mice, but in contrast, there was a borderline increase in RQ for NEXLPL $^{-/-}$ mice ($p = 0.08$) (Table 2). Additionally, HF feeding increased the metabolic rate (MR) for both WT ($p = 0.06$) and NEXLPL $^{-/-}$ mice ($p = 0.01$) with a borderline decrease in physical activity for both groups of mice (Table 2). Noticeably, HF-fed NEXLPL $^{-/-}$ mice had similar lean body mass and total body mass compared to HC-fed NEXLPL $^{-/-}$ mice (Fig. 1D); thus, the increase in MR for NEXLPL $^{-/-}$ mice by HF feeding was not due to changes in lean or total body mass. When the MR was compared between NEXLPL $^{-/-}$ and WT mice, we noticed that NEXLPL $^{-/-}$ mice displayed a trend to higher MR compared to WT mice on both HC and HF diets (Table 2), but this difference did not reach statistical significance. We further calculated the feeding efficiency (calculated by change of body weight from baseline at each week divided by accumulated food intake in gram for that week) and compared NEXLPL $^{-/-}$ and WT mice on either HC diet (Fig. 1G) or HF diet (Fig. 1H). No significant difference was observed between NEXLPL $^{-/-}$ and WT mice on either diet. In summary, unlike WT mice, obesity development in NEXLPL $^{-/-}$ mice was not exacerbated by HF feeding.

3.2. Obesity Development in NEXLPL $^{-/-}$ Mice is Not Prevented by n-3 PUFA Supplementation

Because we previously demonstrated that NEXLPL $^{-/-}$ mice had reductions in the n-3 PUFA content in the hypothalamus prior to obesity development, we wanted to examine whether dietary supplementation of n-3 PUFAs would modify or even prevent obesity development in NEXLPL $^{-/-}$ mice. For this purpose, we designed diets with added DHA and EPA (Lovaza®, GSK) to enrich the n-3 PUFA content without changing the total fat percentage and total PUFA content. The HC-n-3 diet (D10072901, Research Diets) had an n-3/n-6 ratio of 3/1 compared to n-3/n-6 ratio of 1/7.18 in the standard synthetic HC diet with all other macronutrient components and total caloric contents well matched (Table 1).

Obesity was not prevented in NEXLPL $^{-/-}$ vs. WT mice fed the n-3 PUFA-enriched HC diet (HC-n3) (Fig. 2A and B). In fact, the HC-n-3 diet seemed to increase the body weight slightly ($p = 0.15$) with no change in % body fat compared to those on the HC control diets (Fig. 2C and D). Supplementation with n-3 PUFAs had no effect on cumulative food intake (Fig. 2E and F). The n-3 PUFA supplementation reduced the RQ modestly in

Fig. 1 – HF diet did not exacerbate obesity development in NEXLPL^{-/-} mice. **A.** Weekly body weight of HC-fed NEXLPL^{-/-} and WT mice ($n = 8$ for each group). **B.** Weekly body weight of HF-fed NEXLPL^{-/-} and WT mice ($n = 8$ for each group). **C.** Body weight and body composition of HF or HC-fed WT control mice (* $p < 0.05$). **D.** Body weight and body composition of HF or HC-fed NEXLPL^{-/-} mice. **E.** Accumulated food intake of HF or HC-fed WT mice. **F.** Accumulated food intake of HF or HC-fed NEXLPL^{-/-} mice. **G.** Feeding efficiency (changes of body weight from baseline per week divided by accumulated food intake for that week) for HC-fed WT and NEXLPL^{-/-} mice. **H.** Feeding efficiency for HF-fed WT and NEXLPL^{-/-} mice.

WT mice (0.872 on HC-n-3 vs. 0.921 on HC diet, $p = 0.09$), while resulting in a modest increase in RQ in NEXLPL^{-/-} mice (0.893 on HC-n-3 vs. 0.844 on HC diet, $p = 0.10$) (Table 2). The change

in MR with n-3 PUFA also represented a borderline inverse relationship in WT mice (0.01075 on HC-n-3 vs. 0.0097 on HC diet, decrease in MR with n-3 PUFA supplementation), vs.

Table 2 – Indirect calorimetry on NEXLPL^{-/-} and WT mice on various diets.

	Diets	RQ	MR	Physical activity
WT (n = 4)	HF	0.875 ± 0.035	0.0140 ± 0.013	10,235 ± 2141
			p = 0.06 vs. HC	p = 0.09 vs. HC
NEXLPL ^{-/-} (n = 4)	HF	0.918 ± 0.005	0.0156 ± 0.0001	11,229 ± 2123
		p = 0.08 vs. HC	*p = 0.01 vs. HC	p = 0.10 vs. HC
WT (n = 5)	HC	0.921 ± 0.025	0.0097 ± 0.0003	21,178 ± 5060
NEXLPL ^{-/-} (n = 4)	HC	0.844 ± 0.022	0.0107 ± 0.0010	17,747 ± 6676
WT (n = 4)	HCn-3	0.872 ± 0.022	0.0108 ± 0.0007	15,064 ± 2466
		p = 0.09 vs. HC		
NEXLPL ^{-/-} (n = 4)	HCn-3	0.893 ± 0.026	0.0100 ± 0.0004	17,040 ± 3176
		p = 0.10 vs. HC		

NEXLPL^{-/-} mice (0.0100 on HC-n3 vs. 0.0107 on HC diet, increase in MR with n-3 PUFA supplementation) (Table 2). None of these changes in RQ or MR were statistically significant. There was also no effect of n-3 PUFA supplementation on physical activity (Table 2). We also calculated the feeding efficiency for both WT and NEXLPL^{-/-} mice on HC or HCn-3 diets vs. diet, but no significant differences were observed (data not shown).

3.3. Dietary Responses in Plasma Biomarkers in NEXLPL^{-/-} vs. WT Control Mice

HF feeding increased the fasting plasma insulin (Fig. 3A) and leptin (Fig. 3B) in WT control mice as expected, with no changes in fasting glucose, FFA, TG or adiponectin (Table 3). Interestingly,

Fig. 2 – n-3 PUFA supplementation did not prevent obesity development in NEXLPL^{-/-} mice. A. Weekly body weight of HC and HCn-3-fed NEXLPL^{-/-} mice (n = 8 for each group). **B.** Weekly body weight of HC and HCn-3-fed WT mice (n = 8 for each group). **C.** Body weight and body composition of HC and HCn-3-fed NEXLPL^{-/-} mice. **D.** Body weight and body composition of HC and HCn-3-fed WT mice. **E.** Accumulated food intake of HC and HCn-3-fed NEXLPL^{-/-} mice. **F.** Accumulated food intake of HC and HCn-3-fed WT mice.

HF feeding did increase fasting plasma leptin in NEXLPL^{-/-} mice (Fig. 3B), but no change in insulin was observed (Fig. 3A). Although n-3 PUFA-enriched diets did not prevent obesity in NEXLPL^{-/-} mice, reductions in fasting FFA only occurred in HCn-3-fed NEXLPL^{-/-} mice (2.0 fold, $p = 0.02$, Fig. 3D), but not in WT mice (Fig. 3C, Table 3). Fasting plasma adiponectin was increased in both HCn-3-fed NEXLPL^{-/-} and WT control mice (Fig. 3D). Of note, we observed some unexpected effects of n-3 supplementation that had not been previously reported:

plasma TG (Fig. 3E) and leptin (Fig. 3F) were both increased in NEXLPL^{-/-} and WT mice on HCn-3 diet (Table 3).

3.4. Diet-Induced Changes in Hypothalamic Gene Expression in WT vs. NEXLPL^{-/-} Mice

Previously, we found that AgRP and NPY gene expression were elevated in 3 and 6 mo chow-fed NEXLPL^{-/-} mice compared to WT littermate controls [28]. Although a reduc-

Fig. 3 – Plasma metabolites of WT and NEXLPL^{-/-} mice in different diet feeding groups. **A.** Fasting plasma insulin for HC and HF-fed WT and NEXLPL^{-/-} mice. **B.** Fasting plasma leptin for HC and HF-fed WT and NEXLPL^{-/-} mice. **C.** Fasting plasma FFA for HC and HCn-3-fed WT and NEXLPL^{-/-} mice. **D.** Fasting plasma adiponectin for HC and HCn-3-fed WT and NEXLPL^{-/-} mice. **E.** Fasting plasma TG for HC and HCn-3-fed WT and NEXLPL^{-/-} mice. **F.** Fasting plasma leptin for HC and HCn-3-fed WT and NEXLPL^{-/-} mice ($n = 4$ for HF-fed WT mice, $n = 5$ for HF-fed NEXLPL^{-/-} mice, $n = 7$ for HC-fed WT mice, $n = 6$ for HC-fed NEXLPL^{-/-} mice, $n = 8$ for HCn-3-fed WT mice, $n = 5$ for HCn-3-fed NEXLPL^{-/-} mice).

Table 3 – Plasma metabolic biomarkers for various diet-fed NEXLPL^{−/−} and WT mice.

	Diets	Insulin	Glucose	FFA	TG	Leptin	Adiponectin
WT (n = 4)	HF	4.37 ± 0.40 *p < 0.05 vs. HC	172 ± 7	0.64 ± 0.04	0.33 ± 0.02	16,792 ± 702 *p < 0.05 vs. HC	1.88 ± 0.39
NEXLPL ^{−/−} (n = 5)	HF	2.37 ± 0.10	152 ± 5	0.62 ± 0.08	0.45 ± 0.17	17,275 ± 20 *p < 0.05 vs. HC	1.67 ± 0.34
WT (n = 7)	HC	2.26 ± 0.37	156 ± 7	0.55 ± 0.05	0.30 ± 0.07	7672 ± 3310	1.84 ± 0.20
NEXLPL ^{−/−} (n = 6)	HC	2.02 ± 0.23	158 ± 4	0.71 ± 0.09	0.35 ± 0.05	5230 ± 1921	1.91 ± 0.52
WT (n = 8)	HCn-3	2.55 ± 0.67	136 ± 10	0.53 ± 0.07	0.48 ± 0.13 *p = 0.05 vs. HC	11,395 ± 3590 p = 0.07 vs. HC	3.09 ± 0.49 *p < 0.05 vs. HC
NEXLPL ^{−/−} (n = 5)	HCn-3	2.09 ± 0.94	151 ± 6	0.35 ± 0.05 *p < 0.05 vs. HC	0.45 ± 0.04 *p = 0.05 vs. HC	10,066 ± 4553 *p = 0.05 vs. HC	3.34 ± 1.10 *p < 0.05 vs. HC

tion in n-3 PUFA content was detected prior to obesity development at 3 mo in chow-fed NEXLPL^{−/−} mice, significant increases in PUFA synthetic pathway enzyme gene expression, Fads1 (Δ -5 desaturase), Elovl 2 (elongase 2), and stearoyl-CoA desaturase 1 (SCD-1) were observed in 6 mo NEXLPL^{−/−} mice [28]. Here, we show that in 12 mo HC-fed NEXLPL^{−/−} mice AgRP gene expression remained elevated compared to WT mice (*p < 0.05), with no differences in NPY and POMC gene expression (Fig. 4A). This effect was similar to our previous observations at both 3 mo and 6 mo with chow-fed mice. Fads1 and SCD-1 gene expression was not increased in 12 mo HC-fed NEXLPL^{−/−} mice, unlike 6 mo NEXLPL^{−/−} chow-fed mice, but both Elovl2 and Elovl5 mRNA remained elevated compared to HC fed WT mice (*p < 0.05) (Fig. 4C).

HF feeding did not significantly alter gene expression in WT controls (Fig. 4A and C). Importantly, the increase in AgRP gene expression we observed in the HC fed NEXLPL^{−/−} mice was also observed after HF feeding (Fig. 4A, *p < 0.05), with increases in NPY mRNA noted as well (Fig. 4A, *p < 0.05 and **p < 0.05). HF feeding did not affect the gene expression of the enzymes involved in the PUFA synthetic pathway in WT mice (Fig. 4C), but significantly reduced Fads2, SCD1, and Acox1 gene expression in HF-fed NEXLPL^{−/−} mice compared to WT mice (Fig. 4C, **p < 0.05) or to HC-fed NEXLPL^{−/−} mice (Fig. 4C, *p < 0.05). HF feeding also significantly reduced Fads1 and Elovl2 gene expression in HF-fed NEXLPL^{−/−} mice compared to HC-fed NEXLPL^{−/−} mice (Fig. 4C, **p < 0.05).

In WT control mice fed an HC diet supplemented with n-3 PUFAs, we observed an increase in both orexigenic neuropeptides AgRP and NPY, and anorexigenic neuropeptide POMC gene expression (Fig. 4B, **p < 0.05). None of the PUFA synthetic pathway enzyme gene expression was affected by n-3 PUFA supplementation in WT mice (Fig. 4D). In NEXLPL^{−/−} mice, the effect of n-3 PUFA supplementation on gene expression was reversed compared to WT mice: with AgRP, Elovl2, and Elovl5 gene expressions all reduced (Fig. 4D, **p < 0.05). Taken together, n-3 PUFA supplementation appears to have led to differential changes in the expression of neuropeptide and PUFA synthetic enzymes in NEXLPL^{−/−} mice vs WT controls. However, it was interesting to note that n-3 PUFA supplementation increased SCD1 gene expression in both WT and NEXLPL^{−/−} mice (Fig. 4D, **p < 0.05).

Last, we examined the LPL gene expression in the hypothalamus of HC, HCn-3 and HF-fed WT mice (Fig. 4E). n-3 PUFA supplementation did not alter the levels of LPL mRNA in hypothalamus in WT mice, however, of interest HF feeding

significantly increased the level of LPL mRNA in WT mice (Fig. 4E, *p = 0.05 vs. HC diet).

3.5. Alterations of Hypothalamic PUFA Content by Dietary Modifications

Since gene expression of PUFA synthetic pathway enzymes were modified differentially in NEXLPL^{−/−} mice compared to WT mice by either HF feeding or n-3 PUFA supplementation, we next examined how hypothalamic PUFA content might be modified by dietary modifications. On HC diet, 12 mo NEXLPL^{−/−} mice failed to display a significant reduction in total fatty acids, saturated fatty acids and total n-6 PUFA compared to WT mice. When looking at the individual PUFA species, only a trend toward reduced 20:5 n-3, 22:5 n-3, 18:2 n-6, 20:4 n-6 and 22:4 n-6 PUFA was observed in HC-fed NEXLPL^{−/−} compared to WT mice (Fig. 5A and B); however, none of these changes were statistically significant. Our findings with 12 mo HC-fed mice are somewhat different from the previous finding with chow-fed 12 mo NEXLPL^{−/−} mice, in which n-3 PUFA deficiency was observed without changes in total FFA and n-6 PUFA content. Further lipidomic analysis revealed that in addition to the differences in total fat content (10% total fat in HC vs. 18% in chow), there were major differences in the fatty acid composition in the synthetic HC diet vs. chow [30]. In brief, the synthetic HC diet not only contained much less total SFA and monounsaturated fatty acids (MUFA), due to higher total fat content in the chow diet, but also contained disproportionally lower amounts of both n-3 and n-6 PUFA. Thus, the differential result of hypothalamic lipid content here might well be a result of long term (42 weeks) feeding of a synthetic diet that was PUFA deficient. Despite the caveats of having the control HC diet being PUFA deficient, the subsequent lipidomic analysis of hypothalamic tissue from HC n-3-fed mice still revealed some interesting insights into the effect of PUFA supplementation on brain lipid contents.

Analysis of the individual fatty acid species, revealed that n-3 PUFA enrichment increased the hypothalamic n-3 PUFA content of all very long chain species 20:5 n-3, 22:5 n-3, and 22:6 n-3 in WT mice (p < 0.001), but failed to modify the shorter chain 18:3 n-3 species (Fig. 5A). The source of n-3 PUFA in our HCn-3 diet was from EPA (20:5 n-3) and DHA (22:6 n-3). Thus, it was interesting to observe that the major changes in brain PUFA occurred in only in very long chain species. Because we did not supplement n-6 PUFAs in the diet, no change in the

Fig. 4 – Gene expression levels in the hypothalamus of various diet feeding groups of WT and NEXLPL-/- mice (n = 4 for each group, for A-D: *p < 0.05 NEXLPL-/- vs. WT, **p < 0.05 HF or HCn-3 vs. HC). A. Gene expression levels of various neuropeptides in HC and HF-fed WT and NEXLPL-/- mice. **B.** Gene expression levels of various neuropeptides in HC and HCn-3-fed WT and NEXLPL-/- mice. **C.** Gene expression levels of PUFA synthetic enzymes in HC and HF-fed WT and NEXLPL-/- mice. **D.** Gene expression levels of PUFA synthetic enzymes in HC and HCn-3-fed WT and NEXLPL-/- mice. **E.** Gene expression levels of LPL in HC, HCn-3 and HF-fed WT mice (*p = 0.05 HF vs. HC).

hypothalamic content of any of the n-6 PUFA species was detected as expected (Fig. 5B). Furthermore, although n-3 PUFA enrichment increased the hypothalamic n-3 PUFA content for both WT and NEXLPL-/- mice for all three of the very long chain PUFA species, the fold increase for 20:5 n-3

was significantly higher for NEXLPL-/- mice (~19 fold) compared to WT controls (~6 fold, p < 0.05, Fig. 5A), implying a potential difference in very long chain PUFA uptake from the diet into the hypothalamus in NEXLPL-/- mice. Additionally, n-3 PUFA enrichment increased the total n-3 PUFA in

Fig. 5 – Lipidomic analysis of hypothalamic neutral lipid content in HC and HC n-3-fed WT and NEXLPL^{-/-} mice (n = 4 for each group). A. Individual n-3 PUFA species concentrations in HC and HCn-3-fed WT and NEXLPL^{-/-} mice (* $p < 0.05$). B. Individual n-6 PUFA species concentrations in HC and HCn-3-fed WT and NEXLPL^{-/-} mice. C. Total hypothalamic lipid content of different class of lipids in HC and HCn-3-fed WT mice (* $p < 0.05$). D. Total hypothalamic lipid content of different class of lipids in HC and HCn-3-fed NEXLPL^{-/-} mice (* $p < 0.05$). E. 16:1/16:0 and 18:1/18:0 ratios in HC and HCn-3 fed WT and NEXLPL^{-/-} mice.

hypothalamus ($p < 0.05$), but total SFA, total MUFA, and total n-6 PUFA were all unchanged in WT mice (Fig. 5C). On the contrary, n-3 PUFA enrichment seemed to increase the hypothalamic lipid content in every class of lipids including a significant increase in total MUFA ($p < 0.05$) and total n-3 PUFA ($p < 0.01$) (Fig. 5D). Lastly, it was interesting to observe that the 18:1/18:0 ratio only trended higher in HCn-3-fed WT mice compared to HC-fed WT mice, but was significantly increased in HCn-3-fed NEXLPL^{-/-} mice vs. HC-fed NEXLPL^{-/-} mice ($p < 0.01$) (Fig. 5E).

4. Discussion

In mice with neuron-specific LPL deficiency (NEXLPL^{-/-}), obesity developed on a synthetic HC diet, consistent with our previous observations using chow diet. Importantly, HF diets did not exacerbate the development of obesity in NEXLPL^{-/-} mice, and while WT mice gained significantly more weight following high fat feeding NEXLPL^{-/-} mice did not. Moreover, the expected fall in RQ with high-fat feeding

was not seen in NEXLPL^{-/-} mice. It is interesting to note that the HF feeding was able to increase LPL gene expression in the hypothalamus of WT mice (Fig. 4E). With neuronal LPL deficiency, NEXLPL^{-/-} mice cannot respond to HF feeding in the same manner, and are unable to up-regulate hypothalamic LPL levels. These findings imply that up-regulated LPL may be an adaptive mechanism underlying diet-induced obesity. Since this mechanism is lacking in the NEXLPL^{-/-} mice, this may explain why these mice do not show an increase in body weight and fat mass during HF feeding. Thus, it is plausible to suggest that neuronal LPL deficient mice are less sensitive to the total fat content in the diet compared to WT controls. Overall, these results highlight potentially important but yet to be elucidated LPL-dependent mechanisms by which the brain lipid regulates energy homeostasis.

Because we have previously shown that NEXLPL^{-/-} mice were deficient in n-3 PUFAs, we reasoned that obesity development in chow-fed NEXLPL^{-/-} mice [28] might be prevented with n-3 PUFA supplementation. However, this was clearly not the case and obesity developed similarly for NEXLPL^{-/-} on either HC or HCN-3 diets. Previous work has demonstrated in mice that a 45% HF diet supplemented with 0.2% (wt/wt) purified EPA + DHA ethyl esters had no effect on body weight [31]. In addition, dietary supplementation of very long-chain n-3 fatty acids decreased whole body lipid utilization in rats [32]. Another study demonstrated a reduction in weight gain in previously obese mice fed 3% DHA + EPA wt/wt in the setting of a 36% fat diet for 9 weeks [33]. Our diets were twice as high in n-3 PUFA (6.9% of total fat), and while a beneficial effect on fasting plasma FFA was observed in the NEXLPL^{-/-} mice, obesity still developed.

When considering the results from the HF diet and n-3 PUFA supplementation on HC diet experiments, it appears that with neuron-specific LPL deficiency, NEXLPL^{-/-} mice lose the normal metabolic response to increased fat content in the diet (HF feeding) and dietary composition (n-3 PUFA supplementation). It is quite possible that the role of LPL in the brain is more than just lipid sensing but to preferentially control the uptake of dietary long chain n-3 fatty acids, or even essential fatty acid precursors such as linolenic acid to provide substrate for additional chain length elongation and desaturation in the hypothalamus. In fact we have previously demonstrated up-regulation of several of these enzymes in the hypothalamus of NEXLPL^{-/-} mice, but not liver [28].

Although dietary supplementation did not prevent obesity in NEXLPL^{-/-} mice, there were a number of metabolic benefits of n-3 PUFA supplementation including lower levels of fasting plasma FFA, and increased plasma adiponectin levels. In the studies of Rossmesl et al. [33], there were also beneficial effects on parameters of insulin action, however, the benefit was superior when EPA + DHA were given as phospholipids rather than triglycerides. A recent review has nicely summarized studies demonstrating the prophylactic, and to a lesser extent therapeutic value, of n-3 PUFA on insulin sensitivity and glucose homeostasis in mice [34]. There is also evidence that omega-3 fatty acids induce adiponectin in high fat fed mice [35]. In our own experiments, metabolically favorable effects of n-3 PUFA were in general more pronounced in WT than NEXLPL^{-/-} mice (Fig. 3C, Table 3), suggesting that a deficient response to nutrient composition in NEXLPL^{-/-} mice is associated with

metabolic effects systemically. Taken together, the fact that n-3 PUFAs can have systemic effects on various metabolic parameters despite obesity development, implies that long chain PUFAs are involved in peripheral metabolic regulation that is independent of the LPL function in the CNS, while the obesity development in NEXLPL^{-/-} mice is driven by neuron-specific modification of LPL gene expression and cannot be rescued simply by n-3 PUFA enrichment in diet.

The asynchronous peripheral vs. central response to nutrient composition in NEXLPL^{-/-} mice is highlighted by the fact that the expression of important orexigenic peptides involved in metabolic regulation in hypothalamus such as AgRP and NPY is often modified differentially in NEXLPL^{-/-} mice compared to WT mice in response to dietary modification. For example, when HF feeding failed to exacerbate obesity development in NEXLPL^{-/-} mice, AgRP gene expression remained elevated, but the expression of NPY and certain PUFA synthetic enzymes were differentially modified in NEXLPL^{-/-} vs. WT mice. When n-3 PUFA enrichment failed to prevent obesity in NEXLPL^{-/-} mice, the increases in AgRP, NPY and POMC in HC n-3 fed WT mice were not observed in HC n-3 fed NEXLPL^{-/-} mice, and the dietary modifications on certain PUFA enzyme gene expression in WT controls were all reversed in NEXLPL^{-/-} mice. All of these data suggest that the hypothalamus of neuronal LPL deficient mice is defective in response to dietary macronutrient modifications.

With the caveat that the control HC diet was PUFA deficient, the lipidomic analysis still revealed that dietary modification to various classes of hypothalamic neutral lipid species was quite different in NEXLPL^{-/-} (increases in lipids) vs. WT mice (decreases in lipids except for n-3 PUFA). The increases in all classes of lipids including n-3 PUFA in NEXLPL^{-/-} mice correlate well with the reductions in PUFA synthetic enzyme gene expression in NEXLPL^{-/-} on n-3 PUFA supplemented diets. It is interesting to note that NEXLPL^{-/-} mice seem to have a stronger response to EPA (20:5 n-3) supplementation than DHA (22:6 n-3) supplementation. This might imply the differential regulation of LPL at different steps of the PUFA synthetic pathways in the hypothalamus. Most importantly, the brain lipidomic analysis suggests that increasing n-3 PUFA content alone in the hypothalamus by dietary modification is not sufficient to prevent obesity in NEXLPL^{-/-} mice, suggesting a more essential role of LPL in CNS body weight regulation beyond the potential role in regulating hypothalamic lipid intake.

In summary, when CNS neurons are deficient in LPL (NEXLPL^{-/-}), HF diets fail to increase more weight gain than seen in WT mice. In NEXLPL^{-/-} mice HF feeding also failed to increase fat oxidation systemically. Moreover, despite the deficiency of n-3 PUFAs in chow-fed NEXLPL^{-/-} mice, n-3 PUFA supplementation failed to correct the obesity phenotype in mice fed synthetic diets high in carbohydrate or fat despite the reduction in AgRP neuropeptide expression and increases in hypothalamic n-3 PUFA contents. These new findings from dietary modification experiments still support our original hypothesis that neuronal LPL is important in providing signals from lipoprotein-derived lipids that control body weight and body composition, but highlight the importance of identifying mechanism underlying LPL-dependent regulation of energy homeostasis in the CNS.

Disclosures

All authors have no conflict of interest to declare.

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