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n-3 Essential fatty acids decrease weight gain in genetically obese mice

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1. Lean (*ln/ln*) and obese (*ob/ob*) mice were given diets containing a fat source of 100 g evening primrose (*Oenothera biennis*) oil (fatty acids 18:2n-6, 18:3n-6; EPO) or 100 g cod liver oil (20:5n-3, 22:6n-3; CLO)/kg diet.

2. Weight gain was lower in the *ob/ob* mice fed on CLO, an effect unrelated to food intake.

3. In the *ob/ob* mice fed on CLO, thromboxane synthesis by clotting platelets was reduced compared with that in *ob/ob* mice fed on EPO.

4. The *ob/ob* CLO-fed mice had lower arachidonic acid but higher levels of n-3 fatty acids in liver, brown adipose tissue and white adipose tissue.

5. The n-3 fatty acids in CLO therefore replaced the n-6 fatty acids in tissue lipids and reduced synthesis of '2 series' prostaglandins in addition to causing lower weight gain in the CLO-fed *ob/ob* mice.

The essential fatty acid (EFA) composition of tissues of the obese (*ob/ob*) mouse has previously been reported (York *et al.* 1982; French *et al.* 1983; Cunnane *et al.* 1985). The consistent observation in these reports has been that, proportionally, liver levels of linoleic acid (18:2n-6) are decreased and arachidonic acid (20:4n-6) is increased. Consistent with the differences in fatty acid composition, synthesis of 20:4n-6 from 18:2n-6 has recently been reported to be increased in the liver microsomes of *ob/ob* compared with lean (*ln/ln*) mice (Hughes & York, 1985). In comparison with the increase in 18:2n-6 metabolites in tissues of *ob/ob* mice, the n-3 EFA were proportionally decreased (Cunnane *et al.* 1985). Comparing the *ob/ob* mouse and obese Zucker rat, however, it is apparent that the lower 18:2n-6/20:4n-6 value in the *ob/ob* mouse is not a general phenomenon in obese rodents. Whereas the *ob/ob* mouse has higher tissue levels of 20:4n-6, the obese Zucker rat has a higher 18:2n-6/20:4n-6 value suggestive of impaired rather than increased 18:2n-6 metabolism to 20:4n-6 (Wahle & Radcliffe, 1977; Wahle *et al.* 1984).

The observation that diets supplemented with n-6 EFA were associated with significantly increased weight gain in *ob/ob* mice (Cunnane *et al.* 1985) suggests that the elevated levels of fatty acids derived from 18:2n-6 (dihomo-γ-linolenic acid (20:3n-6) and 20:4n-6) might be associated with obesity in this strain of mice, a possibility supported by the stimulatory effect of 20:4n-6 on blood glucose levels in *ob/ob* mice (Pratt, 1984). Furthermore, in view of the lower n-3 EFA in the liver of *ob/ob* mice, it has also been considered possible that supplemental n-6 EFA may have been associated with increased weight gain partly by increasing 20:3n-6 and 20:4n-6, but also by further reducing the n-3 EFA by competitive replacement (Cunnane *et al.* 1985).

20:3n-6 is the precursor of the '1 series' prostaglandins (PG) and 20:4n-6 is the precursor of the '2 series' PG. PG have been shown to have bimodal effects on lipolysis; the lower, more physiological concentrations inhibiting lipolysis and the higher concentrations stimulating lipolysis (Kather & Zimmer, 1983). The net effect in vivo is considered to be antilipolytic with PG excess considered to exist in metabolic obesity (Curtis-Prior, 1975). The higher proportions of 20:3n-6 and 20:4n-6 in the liver phospholipids (PL) of *ob/ob* mice suggest a possible origin of excess '2 series' PG which might contribute to obesity in *ob/ob* mice.

In the present study we have therefore addressed the issue of competitive interactions of dietary n-6 and n-3 EFA on the development of obesity in *ob/ob* mice. The effect of dietary EFA manipulation on PG synthesis was assessed by the generation of thromboxane (TX) A_2 by platelets in freshly clotted blood. In order to control accurately the EFA intake of the *ln/ln* and *ob/ob* mice, semi-synthetic diets were given in which the fat component was an oil, the EFA composition of which was primarily n-6 or n-3 fatty acids. These oils were evening primrose (*Oenothera biennis*) oil (720 g 18:2n-6 and 90 g γ-linolenic acid (18:3n-6)/kg oil; EPO), and cod liver oil (80 g eicosapentaenoic acid (20:5n-3) and 80 g docosahexaenoic acid (22:6n-3)/kg oil; CLO).

Our present results support the previous hypothesis that an imbalance in metabolism of n-6 and n-3 EFA exists in *ob/ob* mice (Cunnane *et al.* 1985). Supplemental n-3 EFA partly offset this abnormality whereas supplemental n-6 EFA contributed to it. Significantly lower production of TXB_2 by platelets in clotting blood from the *ob/ob* mice fed on supplemental n-3 EFA was also observed.

MATERIALS AND METHODS

Animals and diets

Male *ob/ob* and *ln/ln* mice (C57BL/6J) at 6 weeks of age were obtained from Jackson Laboratory, Bar Harbour, ME, USA. They were housed in groups of six in polypropylene cages (Nalgene) and were fed on a semi-synthetic diet (Teklad Test Diets, Madison, WI, USA) and distilled water *ad lib*. The diet contained (g/kg): 200 casein, 600 sucrose, 100 fat source, 55 cellulose, 35 minerals, 10 vitamins in accordance with values recommended by the (US) National Research Council (1978). The fat source was either EPO or CLO. The groups were designated: *ln/ln* (EPO), *ln/ln* (CLO), *ob/ob* (EPO) and *ob/ob* (CLO).

A small group (*n* 2) of *ob/ob* and *ln/ln* mice were fed on a basal diet (Purina rodent chow no. 5001) throughout the study. They were used as a reference for weight gain and liver PL fatty acid analysis since a true 'control' group was not possible in the present study.

The diets were given for 16 weeks. The mice were then anaesthetized with diethyl ether and blood was drawn from the heart. The clotting blood was incubated at 37° for 30 min. Serum was then removed and frozen at −70°. This procedure was designed to maximize TXA_2 synthesis by the clotting platelets. Liver, brown adipose tissue (BAT) and white adipose tissue (epididymal fat; WAT) were dissected, weighed and frozen for analysis.

Analtyical methods

TXB_2

Serum TXB_2 (the stable metabolite of TXA_2) was measured by radioimmunosassay (Soma *et al.* 1985) with antibody purchased from L'Institut Pasteur (Paris).

Total lipids

Serum triacylglycerol (TG) was measured by automated centrifugal analysis using an enzyme assay (Cobas BIO; Roche). Total PL in liver, BAT and WAT were measured by the spectrophotometric method of Stewart (1980) which employs ammonium ferrothiocyanate as the colour reagent. Total TG in liver, BAT and WAT were measured by internal standardization using gas–liquid chromatography. Triheptadecanoin (17:0) was the internal standard and was added to the samples at the stage of total lipid extraction.

Fatty acids

Fatty acid analysis was carried out as previously described (Cunnane *et al.* 1985). Total lipids of liver, BAT and WAT were extracted with chloroform–methanol and the TG and

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Table 1. *Final body- and tissue weights, weight gain and food intake of lean* (ln/ln) *and obese* (ob/ob) *mice fed on diets containing 100 g evening primrose* (Oenothera biennis) *oil* (*EPO*) *or 100 g cod liver oil* (*CLO*)/*kg for 16 weeks*

(Values are means and standard deviations of six samples. Final body-weight and weight gain values only have been compared with the reference groups (REF) of *ln/ln* (*n* 2) and *ob/ob* (*n* 2) mice fed on a stock diet)

	EPO		CLO		REF
	Mean	SD	Mean	SD	mean
Final body-wt (g)					
ln/ln	36	4	29	5	29
ob/ob	64**	4	56**††	3	65
Change in body-wt (g)					
ln/ln	14	4	7	4	7
ob/ob	28**	4	21**	3	29
Change in body-wt (%)					
ln/ln	61	—	29	—	29
ob/ob	76**	—	55**	—	77
Liver wt (g)					
ln/ln	1·7	0·3	1·4	0·4	—
ob/ob	5·0**	0·6	4·7**	0·8	—
Liver (g/kg body-wt)					
ln/ln	47	4	48	1	—
ob/ob	81**	13	78**	20	—
Brown adipose tissue‡					
ln/ln	170	50	130	40	—
ob/ob	740**	90	610**	170	—
Brown adipose tissue (g/kg body-wt)					
ln/ln	5	1	4	1	—
ob/ob	12**	1	11**	3	—
White adipose tissue (g)					
ln/ln	0·6	0·2	0·4	0·1	—
ob/ob	1·2**	0·4	1·1**	0·3	—
White adipose tissue (g/kg body-wt)					
ln/ln	17	4	17	3	—
ob/ob	19	4	20	4	—
Food intake (g/d)					
ln/ln	4·0	1·2	3·7	0·8	4·1
ob/ob	5·5	0·7	6·4	1·6	6·5

Mean values were significantly different from those for *ln/ln* mice (ANOVA): ** $P < 0.01$.
Mean value was significantly different from that for EPO (Student's *t* test): †† $P < 0.01$.
‡ Values rounded to nearest 10 mg due to inaccuracy of dissection.

total PL fractions separated by thin-layer chromatography as previously described (Cunnane *et al.* 1985). Fatty acids in these fractions were transmethylated with boron trifluoride–methanol and analysed by gas–liquid chromatography using a Hewlett-Packard 5880 with automated sample delivery and integration of fatty acid peaks.

Statistics

Statistical analyses between groups were done using two-way analysis of variance (ANOVA) and Student's *t* test where applicable.

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Table 2. *Tissue total phospholipids (PL) and triacylglycerol (TG) in lean* (ln/ln) *and obese* (ob/ob) *mice fed on diets containing 100 g evening primrose* (Oenothera biennis) *oil (EPO) or 100 g cod liver oil (CLO)/kg for 16 weeks*

(Values are means and standard deviations for six animals/group)

	EPO		CLO	
	Mean	SD	Mean	SD
Serum				
TG (mg/l)				
ln/ln	1000	280	890	270
ob/ob	750	140	970	120
Liver				
PL (mg/g)				
ln/ln	11·8	1·1	12·5	1·2
ob/ob	11·4	1·9	9·0††	1·9
TG (mg/g)				
ln/ln	5·0	4·1	5·2	3·4
ob/ob	25·3**	9·0	17·2**	7·5
Brown adipose tissue				
PL (mg/g)				
ln/ln	3·1	1·4	4·1	1·1
ob/ob	0·8**	0·2	1·1**††	0·1
Total PL (μg)				
ln/ln	470	65	478	83
ob/ob	470	108	558	112
TG (mg/g)				
ln/ln	751	91	690	108
ob/ob	859**	57	825**	60
White adipose tissue				
PL (mg/g)				
ln/ln	0·8	0·4	1·6	0·9
ob/ob	0·8	0·1	0·6	0·2
Total PL (μg)				
ln/ln	480	220	640	330
ob/ob	960	190	660††	260
TG (mg/g)				
ln/ln	669	56	619	74
ob/ob	815**	72	825**	40

Mean values were significantly different fom those for *ln/ln* mice (ANOVA): ** $P < 0{\cdot}01$.
Mean values were significantly different from those for EPO (Student's *t* test): †† $P < 0{\cdot}01$.

RESULTS

General

Body-weight in the *ob/ob* (CLO) mice was significantly lower than that in the *ob/ob* (EPO) group after 10 weeks ($P < 0{\cdot}01$) and continued so until the end of the study (difference 14% at 16 weeks, $P < 0{\cdot}01$, Table 1). Differences in final body-weight of the *ob/ob* (CLO) and *ob/ob* (EPO) mice were reflected by differences in weight gained over the 16-week period (Table 1). Values for the reference groups of *ob/ob* and *ln/ln* mice fed on a basal diet are also shown for comparison. The growth curve of the reference *ob/ob* group was not significantly different from that of the *ob/ob* (EPO) group but was significantly higher than that of the *ob/ob* (CLO) group. Growth curves in the *ln/ln* groups were not significantly different from each other but weight gained by the *ln/ln* (CLO) mice was significantly less than that gained by the *ln/ln* (EPO) mice.

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Table 3. *Proportional composition of fatty acids (mg/g total fatty acids) from liver total phospholipids of lean* (ln/ln) *and obese* (ob/ob) *mice fed on diets containing 100 g evening primrose* (Oenothera biennis) *oil (EPO) or 100 g cod liver oil (CLO)/kg for 16 weeks*

(Values for the *ob/ob* (EPO) and *ob/ob* (CLO) mice are the means and standard deviations of six samples and are compared with those of the reference *ln/ln* and *ob/ob* mice (REF) fed on a stock diet (*n* 2))

Fatty acid		EPO		CLO		REF
		Mean	SD	Mean	SD	Mean
16:0	*ln/ln*	177	37	228††	18	192
	ob/ob	150**	7	163**†	12	151
16:1n-7	*ln/ln*	10	2	21††	4	11
	ob/ob	28**	3	38**††	5	38
18:0	*ln/ln*	193	11	166††	12	156
	ob/ob	142**	13	136**	21	130
18:1n-9	*ln/ln*	77	22	153††	14	122
	ob/ob	186**	20	231**††	36	217
18:2n-6	*ln/ln*	157	7	37††	7	150
	ob/ob	141**	10	45**††	7	109
20:3n-6	*ln/ln*	22	4	6††	3	17
	ob/ob	18	2	9††	2	26
20:4n-6	*ln/ln*	267	11	56††	4	116
	ob/ob	178**	103	44**††	5	175
22:5n-6	*ln/ln*	42	5	nd††		6
	ob/ob	52	10	nd††		nd
20:5n-3	*ln/ln*	nd		69††	10	6
	ob/ob	nd		101††	21	7
22:6n-3	*ln/ln*	21	2	216††	18	148
	ob/ob	32**	4	152**††	72	124

nd, not detected (< 1 mg/g).
Mean values for *ob/ob* mice were significantly different from those for *ln/ln* mice (ANOVA): ** $P < 0.01$.
Mean values were significantly different from those for EPO (Student's *t* test): † $P < 0.05$, †† $P < 0.01$.

Body and tissue weights at the time of killing the mice are shown in Table 1. Weights of liver, BAT and WAT, both absolute and relative to final body-weight, were greater in the *ob/ob* groups but were not significantly affected by differences in dietary fat. Food intake was comparable in all the groups of *ob/ob* mice (EPO, CLO and the reference group) but was greater in the *ob/ob* than in the *ln/ln* mice (Table 1).

TXB_2

TXB_2 values in serum from freshly clotted blood were similar in all groups (*ob/ob* (EPO), *ln/ln* (EPO) and *ln/ln* (CLO)) except the *ob/ob* (CLO) mice in which the mean values were significantly lower (39 (SD 22) *v.* 6 (SD 3) ng/ml, $P < 0.01$).

Total lipids

Serum TG levels were not different between groups (Table 2). Liver total PL was lower in the *ob/ob* (CLO) group (significant compared only with the *ln/ln* (CLO) group, $P < 0.01$). Liver TG was higher in the *ob/ob* mice but were not significantly different between the *ob/ob* (CLO) and *ob/ob* (EPO) mice. BAT total PL was significantly higher in the *ln/ln* mice (mg/g) but, based on the total weight of the dissected BAT, was equivalent in the *ln/ln* and *ob/ob* mice. BAT total TG (mg/g) was not significantly different between the *ln/ln* and *ob/ob* mice. WAT total PL (mg/g) was not significantly different between groups. WAT total TG (mg/g)

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Table 4. *Proportional composition of fatty acids (mg/g total fatty acids) from brown adipose tissue phospholipids from lean* (ln/ln) *and obese* (ob/ob) *mice fed on diets containing 100 g evening primrose* (Oenothera biennis) *oil (EPO) or 100 g cod liver oil (CLO) for 16 weeks*

(Values are means and standard deviations for six samples)

Fatty acid		EPO		CLO	
		Mean	SD	Mean	SD
16:0	*ln/ln*	159	37	181	18
	ob/ob	160	13	197††	13
16:1n-7	*ln/ln*	60	5	65	18
	ob/ob	62	33	45	8
18:0	*ln/ln*	64	22	77†	21
	ob/ob	105**	47	117**	11
18:1n-9	*ln/ln*	291	32	372††	40
	ob/ob	245**	105	230**	14
18:2n-6	*ln/ln*	278	45	54††	24
	ob/ob	227	61	91††	15
20:3n-6	*ln/ln*	7	1	2††	0·2
	ob/ob	6	3	4	1
20:4n-6	*ln/ln*	47	8	34††	4
	ob/ob	102**	9	41**††	4
20:5n-3	*ln/ln*	nd		63††	17
	ob/ob	4	1	64††	7
22:6n-3	*ln/ln*	40	24	142††	52
	ob/ob	21	6	182††	24

nd, not detected (< 1 mg/g).
Mean values were significantly different from those for *ln/ln* mice (ANOVA): ** $P < 0.01$.
Mean values were significantly different from those for EPO (Student's *t* test): † $P < 0.05$, †† $P < 0.01$.

was higher in both groups of *ob/ob* mice than in the *ln/ln* mice but was not significantly affected by differences in dietary fatty acids.

Fatty acids

The fatty acid composition of the total PL from liver, BAT and WAT is shown in Tables 3–5. The values are of proportional composition (total number of fatty acids integrated equal 100% but since some fatty acids have not been included in the tables, values do not add up to 100%). Quantitative fatty acid levels will also be discussed and are based on the values for total PL and TG (Table 2). In liver and BAT, the TG fatty acids reflected similar differences between groups as the PL fatty acids, hence the TG fatty acids have not been shown.

EPO-fed mice (*ln/ln* and *ob/ob*) had proportionally higher levels of all n-6 EFA compared with those fed on CLO. Conversely, CLO-fed mice had proportionally higher n-3 EFA and decreased n-6 EFA. These differences were consistent regardless of tissue or lipid fractions measured. Therefore, only changes in fatty acid composition peculiar to a specific tissue or lipid fraction, or inconsistent with previous observations, will be elaborated on further.

In the total PL of the liver, saturated fatty acids (16:0, 18:0) were lower but monounsaturated fatty acids (16:1n-7 and 18:1n-9) were higher in the *ob/ob* than in *ln/ln* groups. In liver total PL, 20:4n-6 was lower in the *ob/ob* (EPO) and *ob/ob* (CLO) mice than in the *ln/ln* (EPO) or *ln/ln* (CLO) mice. However, in the reference group fed on the basal diet, 20:4n-6 was higher in the liver total PL of the *ob/ob* than the *ln/ln* mice (Table 3). In BAT total PL, one particular change in fatty acid composition was notable; 20:4n-6 was

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Table 5. *Proportional fatty acid composition (mg/g total fatty acids) of phospholipids and triacylglycerols of white adipose tissue from lean* (ln/ln) *and obese* (ob/ob) *mice fed on diets containing 100 g evening primrose* (Oenothera biennis) *oil (EPO) or 100 g cod liver oil (CLO)/kg for 16 weeks*

(Values are means and standard deviations for six samples)

Fatty acid		Phospholipids				Triacylglycerols			
		EPO		CLO		EPO		CLO	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
16:0	*ln/ln*	112	27	164††	9	142	21	169	16
	ob/ob	108	18	202††	11	157	11	208††	8
16:1n-7	*ln/ln*	67	10	108††	18	55	9	106††	12
	ob/ob	71	8	40	5	77**	7	133**††	13
18:0	*ln/ln*	35	12	55	11	12	1	12	1
	ob/ob	63**	11	165**††	13	12	1	11	1
18:1n-9	*ln/ln*	194	12	363††	47	201	25	465††	68
	ob/ob	348**	49	231**††	14	366	22	471††	27
18:2n-6	*ln/ln*	430	89	78††	24	498	27	72††	27
	ob/ob	279**	52	128**††	21	351**	30	105**††	13
20:3n-6	*ln/ln*	7	1	1	1	—	—	—	—
	ob/ob	5	3	7	0·2	—	—	—	—
20:4n-6	*ln/ln*	22	6	26	6	—	—	—	—
	ob/ob	38**	18	67**††	8	—	—	—	—
18:3n-3	*ln/ln*	9	1	55††	8	7	1	74††	10
	ob/ob	14**	6	10**††	3	3**	1	29**††	2
22:6n-3	*ln/ln*	27	8	55	6	nd		24††	12
	ob/ob	11	5	107††	11	nd		10††	2

nd, not detected (< 1 mg/g).
Mean values were significantly different from those for *ln/ln* mice (ANOVA): ** $P < 0.01$.
Mean values were significantly different from those for EPO (Student's *t* test): † $P < 0.05$, †† $P < 0.01$.

significantly (proportionally and quantitatively) higher in the *ob/ob* (EPO) group than in the *ob/ob* (CLO) group or in the *ln/ln* mice (Table 4). In WAT total PL from the *ob/ob* mice, levels of 18:2n-6 and 18:1n-9 were inversely proportional to those of 20:4n-6 and 22:6n-3; in the *ob/ob* (EPO), 18:1n-9 and 18:2n-6 were proportionally higher whereas in the *ob/ob* (CLO) mice, 20:4n-6 and 22:6n-3 were increased (Table 5). The long-chain non-EFA (16:0, 16:1n-7, 18:1n-9) were significantly higher but 18:2n-6 was significantly lower in the WAT TG of the *ob/ob* (CLO) compared with *ob/ob* (EPO) mice.

DISCUSSION

The premise for the present study was that n-6 EFA are proportionally increased and n-3 EFA are proportionally decreased in livers of *ob/ob* mice. Since n-6 EFA have been shown to increase weight gain in these mice (Cunnane *et al.* 1985), the dietary ratio of these families of EFA might be related to weight gain in *ob/ob* mice. Thus, the hypothesis was that dietary supplementation with n-3 EFA might reduce weight gain in *ob/ob* mice. Our present results provide evidence to support a role of n-3 EFA in reducing weight gain in *ob/ob* mice and suggest that this effect may be associated with (a) reduced n-6 EFA in tissues and (b) reduced 20:4n-6 conversion to '2 series' PG (TXB_2). Energy intake was not statistically different between the *ob/ob* (CLO) mice and the *ob/ob* (EPO) mice, indicating that reduced weight

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gain in the former group was not related to reduced energy intake. Rather, replacement of n-6 EFA by n-3 EFA (both proportionally and quantitatively) in liver and BAT total PL occurred in the *ob/ob* (CLO) mice, in conjunction with a decrease in total lipids in liver (Table 3).

A central role for 20:4n-6 in the comparative effect of EPO and CLO on weight gain in *ob/ob* mice seems apparent on the following basis: levels of 20:4n-6 (proportional and quantitative) in liver and BAT total PL from *ob/ob* (CLO) mice and '2 series' PG, as assayed by TXB_2 synthesized by freshly clotted blood, were decreased in the *ob/ob* (CLO) mice. Assay of TXB_2 in freshly clotted blood is an accurate estimate of the PG-synthesizing capacity of platelets (Soma *et al.* 1985). Dietary manipulation of platelent TXB_2 synthesis is reproducible but not necessarily representative of effects in other tissues. Nevertheless, n-3 EFA are well-known competitive inhibitors of 20:4n-6 utilization by the platelet cyclooxygenase (Hornstra, 1981). Our findings therefore provide preliminary evidence that reduced weight gain in CLO-fed *ob/ob* mice occurs concurrently with reduced synthesis of '2 series' PG. The fact that the *ln/ln* mice had similar TXB_2 levels to the *ob/ob* (EPO) mice yet weighed significantly less suggests that the effective PG concentration causing lipolysis in the *ob/ob* and *ln/ln* mice is different.

In our previous report (Cunnane *et al.* 1985) and in reports of others (French *et al.* 1983; York *et al.* 1982), 20:4n-6 has been shown to be higher in liver PL of *ob/ob* mice than *ln/ln* mice. In the reference group of the present study, 20:4n-6 was also higher in the liver PL in the *ob/ob* mice than in the *ln/ln* mice. However, in the *ob/ob* (EPO) and *ob/ob* (CLO) mice fed on the semi-synthetic diets, 20:4n-6 was lower than in the respective *ln/ln* groups (Table 3). The likely explanation for this descrepancy must lie in the different composition of the reference and semi-synthetic diets. The *ob/ob* (EPO) and *ob/ob* (CLO) mice in the present study were fed on a semi-synthetic diet in order that the total fat intake could be manipulated to maximize differences in n-6 and n-3 EFA intake. The reference group was fed on a basal diet (Purina chow) which was also used previously.

20:4n-6 was significantly higher in the BAT total PL of the *ob/ob* (EPO) group (proportionally and quantitatively), suggesting that dietary manipulation of EFA composition in BAT may be a significant factor associated with weight gain in *ob/ob* mice. This is supported by the high degree of incorporation of labelled 18:2n-6 into BAT in EFA-deficient rats (Becker, 1984). EFA (mixed n-6 and n-3) have been shown to have a stimulatory effect on BAT thermogenesis concomitant with reduced weight gain in rats (Nedergaard *et al.* 1983).

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