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Effect of Chronic Fluoxetine Treatment on Male and Female Rat Erythrocyte and Prefrontal Cortex Fatty Acid Composition

Robert K. McNamara^{1,*}, Jessica A. Able¹, Therese Rider², Patrick Tso², and Ronald Jandacek²

¹Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH 45267

²Department of Pathology, University of Cincinnati, Cincinnati OH 45237

Abstract

Omega-3 (*n*-3) polyunsaturated fatty acids (PUFA) and fluoxetine (FLX) have additive effects in the treatment of major depressive disorder, and FLX up-regulates genes that regulate fatty acid biosynthesis *in vitro*. Although these data suggest that FLX may augment *n*-3 fatty acid biosynthesis, the *in vivo* effects of FLX treatment on PUFA biosynthesis and peripheral and central membrane composition are not known. In the present study, male and female rats were treated with FLX (10 mg/kg/d) through their drinking water for 30 d (P60-P90). Plasma FLX and norfluoxetine (NFLX) concentrations were determined by liquid chromatography tandem mass spectrometry, and erythrocyte and prefrontal cortex (PFC) fatty acid composition determined by gas chromatography. To confirm central effects of FLX, serotonin turnover in the PFC was determined by high performance liquid chromatography. Chronic FLX treatment resulted in clinically-relevant plasma FLX concentrations in male and female rats, and significantly decreased serotonin turnover in the PFC. After correcting for multiple comparisons, chronic FLX treatment did not significantly alter erythrocyte fatty acid composition in male or female rats. Chronic FLX treatment significantly and selectively increased docosapentaenoic acid (22:5*n*-6) in the PFC of female rats, but not in male rats. This preclinical findings do not support the hypothesis that chronic FLX treatment increases *n*-3 fatty acid biosynthesis or membrane composition.

Keywords

Fluoxetine; Norfluoxetine; Prefrontal cortex; Serotonin; Erythrocyte; Omega-3 fatty acid; Docosahexaenoic acid; Arachidonic acid; Male; Female; Rat

1. Introduction

Several lines of evidence suggest that major depressive disorder (MDD) is associated with a dysregulation in polyunsaturated fatty acid (PUFA) homeostasis (McNamara, 2008). For

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*Corresponding Author: Robert K. McNamara, Ph.D. Department of Psychiatry University of Cincinnati College of Medicine 260 Stetson Street, Suite 3306 Cincinnati, OH 45219-0516 PH: 513-558-5601 FAX: 513-558-4805 robert.mcnamara@uc.edu.

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Although prior data suggest that FLX may augment *n*-3 fatty acid biosynthesis, the *in vivo* effects of FLX treatment on PUFA biosynthesis and peripheral and central membrane composition are not known. The present results do not support the hypothesis that chronic FLX treatment increases *n*-3 fatty acid biosynthesis or membrane composition.

example, cross-sectional studies have observed significant omega-3 (*n*-3) fatty acid deficits in erythrocytes (Edwards et al., 1998; McNamara et al., 2010; Peet et al., 1998) and postmortem prefrontal cortex (PFC) (McNamara et al., 2007) of MDD patients. Moreover, controlled intervention trials have found that dietary *n*-3 fatty acid supplementation has a significant advantage over placebo for reducing depression symptom severity in predominantly medicated MDD patients (Freeman et al., 2006; Lin & Su, 2007). A recent controlled trial found that combining *n*-3 fatty acids and fluoxetine (FLX) had additive effects for reducing depression symptom severity in MDD patients (Jazayeri et al., 2008). Moreover, preclinical studies have found that FLX and *n*-3 fatty acids both reduce behavioral indices of depression in rats (Carlezon et al., 2005). However, it is not known whether the antidepressant effects of FLX and long-chain *n*-3 fatty acids are mediated by common or divergent mechanisms.

One candidate mechanism by which FLX may exert therapeutic efficacy is through the augmentation of long-chain *n*-3 fatty acid biosynthesis and subsequent membrane composition. This is supported in part by an *in vitro* study finding that chronic exposure to antidepressant medications including FLX up-regulate sterol regulatory element-binding protein (SREBP) (Raeder et al., 2006), and SREBP positively regulates the expression of the principal genes that regulate PUFA biosynthesis, delta-5 desaturase (*FADS1*) and delta-6 desaturase (*FADS2*) (Matsuzaka et al., 2002). Moreover, antipsychotic medications also up-regulate SREBP and *FADS1* and *FADS2* expression (Fernø et al., 2005; Polymeropoulos et al., 2009), and significantly increased rat erythrocyte and PFC *n*-3 fatty acid composition (McNamara et al., 2009a). Chronic FLX treatment was also found to increase incorporation of the long-chain *n*-6 fatty acid [³H]arachidonic acid (AA, 20:4*n*-6) into rat cortical membranes (Qu et al., 2006), and to increase 5-HT_{2A/2C}-coupled cytosolic phospholipase A₂ (cPLA₂) AA turnover (Lee et al., 2007). Together, these data suggest that chronic FLX treatment may increase PUFA biosynthesis in addition to membrane turnover. However, the effects of chronic FLX treatment on peripheral and central membrane fatty acid composition have not been systematically investigated.

In the present study, we determined the effects of chronic FLX treatment on rat erythrocyte and PFC fatty acid composition. Because PUFA biosynthesis is regulated in part by ovarian hormones (Childs et al., 2010; Giltay et al., 2004; McNamara et al., 2009b) and there are gender differences in FLX metabolism (Amsterdam et al., 1997), we investigated both male and female rats. We have previously found that erythrocyte and PFC *n*-3 fatty acid composition does not change during different phases of the estrous cycle (McNamara et al., 2009b). Plasma FLX and norfluoxetine (NFLX) concentrations were determined by liquid chromatography tandem mass spectrometry. To confirm central effects of the FLX treatment regimen, we additionally determined serotonin turnover in the PFC by high performance liquid chromatography. Our primary hypothesis was that chronic FLX treatment would increase long-chain *n*-3 fatty acid biosynthesis from the dietary short-chain precursor α -linolenic acid (18:3*n*-3), and increase erythrocyte and PFC long-chain *n*-3 fatty acid composition in both male and female rats.

2. Methods

2.1. Animals

Immediately following weaning (Postnatal day: P21), male and female Long-Evans hooded rats (Harlan Farms, Indianapolis, IN), born in-house to nulliparous dams (*n*=20), were maintained on a customized research diet throughout gestation and the postnatal period (TD. 04285, Harlan-TEKLAD, Madison, WI). Determination of diet fatty acid composition by gas chromatography confirmed that the diet contained only short-chain α -linolenic acid (18:3*n*-3, 4.6%) and linoleic acid (18:2*n*-6, 23%), and did not contain long-chain *n*-3 or *n*-6 fatty acids (McNamara et al., 2008). Same-sex rats were housed 2 per cage with food and water available *ad libitum*, and maintained under standard vivarium conditions on a 12:12 h light:dark cycle.

Rats were sacrificed by decapitation on P90. Trunk blood was collected into EDTA-coated tubes, plasma isolated and erythrocytes washed 3x with 0.9% NaCl. Brains were extracted and immediately immersed in ice-cold 0.9% NaCl for 2 min. The brain was then dissected on ice to isolate bilateral PFC, from which the olfactory tubercle and residual striatal tissue were removed. PFC samples were individually placed in cryotubes and flash frozen in liquid nitrogen. All samples were stored at -80°C . All experimental procedures were approved by the University of Institutional Animal Care and Use Committee, and adhere to the guidelines set by the National Institutes of Health.

2.2. Chronic fluoxetine treatment

Male ($n=20$) and female ($n=20$) rats were randomly assigned to receive chronic FLX through their drinking water from P60 to P90, and male ($n=20$) and female ($n=20$) rats receiving only drinking water served as controls. Administration through drinking water obviates daily injection stress and surgical implantation of minipumps, mimics oral administration in human patient populations, and allows maintenance of drug dose in accordance with age-related increases in body weight. For three days prior to drug delivery, 24 h water consumption was determined for each cage using bottle weights (1 g water = 1 ml water), and ml water intake/kg body weight calculated. Based on daily ml/kg water consumption, FLX stock solution (4 mg/ml) (Mallinckrodt Inc., St. Louis MO) was added to drinking water at a concentration required to deliver a daily dose of 10 mg/kg/d. This dose was selected based on prior data demonstrating that it produces clinically-relevant plasma concentrations, reduces cortical serotonin turnover in rats, and reduces behavioral indices of depression in the forced swim test (R.K. McNamara, unpublished observations; Dhir & Kulkarni, 2008; Unceta et al., 2007). Fresh solutions were prepared and FLX concentration adjusted to body weight every 3 days. Red opaque drinking bottles were used to protect FLX from light degradation. Rats were maintained on FLX until being sacrificed on P90 (i.e., no withdrawal period). Plasma FLX and NFLX concentrations (ng/ml) were determined by liquid chromatography tandem mass spectrometry (Medtox Laboratories, Inc., St. Paul Minnesota).

2.3. Gas chromatography

The gas chromatography procedure used to determine membrane fatty acid composition has been described in detail previously (McNamara et al., 2008). Briefly, total fatty acid composition was determined with a Shimadzu GC-2010 (Shimadzu Scientific Instruments Inc., Columbia MD), and analysis of fatty acid methyl esters was based on area under the curve calculated with Shimadzu Class VP 4.3 software. Fatty acid identification was based on retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). All samples were processed by a technician blinded to treatment. We restricted our analysis of erythrocyte fatty acid composition to the principal saturated fatty acids (16:0, 18:0), monounsaturated fatty acids (18:1 n -9, 18:1 n -7), n -6 fatty acids (18:2 n -6, 20:3 n -6, 20:4 n -6, 22:4 n -6, 22:5 n -6), and n -3 fatty acids (20:5 n -3, 22:5 n -3, 22:6 n -3). We restricted our analysis of PFC fatty acid composition to the principal saturated fatty acids (16:0, 18:0), monounsaturated fatty acids (18:1 n -9, 18:1 n -7), n -6 fatty acids (20:4 n -6, 22:4 n -6, 22:5 n -6), and n -3 fatty acid (22:6 n -3). Fatty acid composition data are expressed as mg fatty acid/100 mg fatty acids (% total fatty acids).

2.4. HPLC-ECD

PFC 5-HT and 5-HIAA concentrations were determined by high performance liquid chromatography with electrochemical detection (HPLC-ECD) as previously described (McNamara et al., 2009c). Briefly, an antioxidant solution (0.4 N perchlorate, 1.34 mM ethylenediaminetetraacetic acid, and 0.53 mM sodium metabisulfite) was added to the samples followed by homogenization using an ultrasonic tissue homogenizer (Biologics, Gainesville,

VA). A fraction of the tissue homogenate was dissolved in 2% sodium dodecyl sulfate (SDS) (w/v) for protein determination (Pierce BCA Protein Reagent Kit, Rockford, IL). The remaining suspension was spun at $14,000 \times g$ for 20 min in a refrigerated centrifuge. The supernatant was reserved for HPLC. Samples were separated on a Microsorb MV C-18 column (5 μ m, 4.6 $\times$ 250 mm, Varian, Walnut Creek, CA) and simultaneously examined for 5-HT and 5-HIAA. Compounds were detected using a 12-channel coulometric array detector (CoulArray 5200, ESA, Chelmsford, MA) attached to a Waters 2695 Solvent Delivery System (Waters, Milford, MA).

2.5. Statistical analyses

Effects of chronic FLX treatment on erythrocyte and PFC fatty acid composition were determined with unpaired *t*-tests (2-tail), and Bonferroni-adjusted for multiple comparisons in erythrocytes ($\alpha=0.05/15 = 0.003$) and PFC ($\alpha=0.05/11 = 0.004$). Effects of chronic FLX treatment on body weight and serotonin turnover were determined with unpaired *t*-tests (2-tail, $\alpha=0.05$). Homogeneity of variance was determined using Bartlett's test. All analyses were performed with GB-STAT (V.10, Dynamic Microsystems, Inc., Silver Springs MD).

3. Results

3.1. Body weight

Consistent with prior studies (i.e., Thompson et al., 2004), the mean endpoint body weight of FLX-treated male rats ($n=20$)(376 $\pm$ 7.2 g) was significantly lower (-12%) than male controls ($n=20$)(428 $\pm$ 8.6 g)($p \leq 0.0001$). The mean body weight of FLX-treated female rats ($n=20$)(227 $\pm$ 5.8 g) was also significantly lower (-17%) than female controls ($n=20$)(275 $\pm$ 5.6 g) ($p \leq 0.0001$).

3.2. Plasma FLX and norfluoxetine (NFLX) concentrations

In FLX-treated male rats, mean plasma FLX (52.4 $\pm$ 9.4 ng/ml), NFLX (305 $\pm$ 36 ng/ml), and FLX+NFLX (358 $\pm$ 44 ng/ml) concentrations were found. In FLX-treated female rats, mean plasma FLX (96.4 $\pm$ 12.7 ng/ml), NFLX (544 $\pm$ 34 ng/ml), and FLX+NFLX (640 $\pm$ 43 ng/ml) concentrations were found. FLX and NFLX were not detected in plasma from control male and female controls not treated with FLX (<10 ng/ml).

3.3. PFC 5-HT turnover

Relative to controls, chronic FLX treatment did not significantly alter PFC 5-HT content (ng/mg protein) (CON: 4.0 $\pm$ 0.1 vs. FLX: 3.8 $\pm$ 0.1, $p=0.12$), and significantly decreased PFC 5-HIAA content (CON: 4.2 $\pm$ 0.1 vs. FLX: 3.3 $\pm$ 0.2, $p=0.001$) and the 5-HIAA/5-HT ratio (CON: 1.0 $\pm$ 0.02 vs. FLX: 0.8 $\pm$ 0.02, $p \leq 0.0001$).

3.4. Erythrocyte fatty acid composition

Comparison of erythrocyte fatty acid composition in control and FLX-treated male rats (Table 1) and control and FLX-treated female rats (Table 2) are presented. After correcting for multiple comparisons, neither male nor female FLX-treated rats exhibited significant alterations in any fatty acid relative to same-gender controls.

3.5. PFC fatty acid composition

Effects of chronic FLX treatment on PFC fatty acid composition in male rats (Table 3) and female rats (Table 4) are presented. After correcting for multiple comparisons, FLX-treated male rats did not exhibit any significant alterations in PFC fatty acid composition relative to male controls. FLX-treated female rats exhibited significantly greater docosapentaenoic acid

(22:5*n*-6) composition relative to controls ($p=0.001$), and there were no differences in other fatty acid compositions.

4. Discussion

The main finding of this study is that chronic FLX treatment, resulting in clinically-relevant plasma FLX concentrations and significant reductions in serotonin turnover in the PFC, did not significantly alter *n*-3 fatty acid composition in erythrocytes or PFC of male or female rats. An unexpected finding was that chronic FLX treatment significantly and selectively increased docosapentaenoic acid (22:5*n*-6) composition in the PFC of female rats. Therefore, these findings do not support our hypothesis that chronic FLX treatment increases *n*-3 fatty acid biosynthesis and erythrocyte and PFC long-chain *n*-3 fatty acid composition.

This study has three principal limitations. First, only one type and class of antidepressant was investigated, and other antidepressant medications may yield different results. Second, only one treatment duration (30 d) was investigated, and longer FLX exposure may have resulted in larger effects. However, a shorter FLX treatment duration (21 d) was found to increase [^3H]arachidonic acid incorporation into the rat brain (Qu et al., 2006). Third, only one dose of FLX was investigated, and a higher dose may have produced different results. However, this dose produced clinically-relevant plasma concentrations and reduced cortical serotonin turnover, and this dose and treatment regimen was found to reduce behavioral indices of depression in the forced swim test (R.K. McNamara, unpublished observations). Studies investigating the effects of different antidepressant medications at different doses and durations are warranted to evaluate the generalizability of these initial findings.

Although human plasma FLX and NFLX concentrations are poorly correlated with therapeutic response, plasma FLX (97 ± 51 ng/ml), NFLX (128 ± 49 ng/ml) and FLX+NFLX (243 ± 81 ng/ml) concentrations are observed in FLX-treated (20 mg/day) male and female human MDD patients (Amsterdam et al., 1997). Therefore, the observed mean rat plasma FLX concentrations are within the human plasma range, whereas mean rat plasma NFLX concentrations are ~4-fold greater than that observed in the plasma of MDD patients. Consistent with clinical studies (Amsterdam et al., 1997), females exhibited greater plasma FLX and NFLX concentrations relative to male rats.

Consistent with prior clinical (Barton et al., 2008; Sheline et al., 1997) and preclinical (Unceta et al., 2007) studies, chronic FLX treatment significantly decreased central 5-HIAA concentrations and serotonin turnover (5-HIAA/5-HT). This finding confirms that the FLX treatment regimen was sufficient to alter central serotonin neurotransmission. In view of prior data demonstrating that the 5-HT_{2A/2C} receptor can be coupled to PLA₂ and regulate membrane DHA and AA turnover (Garcia & Kim, 1997; Qu et al., 2003), reductions in serotonin turnover in the rat PFC in the present study may be anticipated to alter 5-HT_{2A/2C} receptor-mediated DHA and AA turnover in rat PFC. Nevertheless, the finding that serotonin turnover in the rat PFC is *reduced* by chronic FLX treatment (present results; Unceta et al., 2007) and *increased* by chronic *n*-3 fatty acid deficiency (McNamara et al., 2009c) suggests that FLX and *n*-3 fatty acids both converge on serotonin neurotransmission. This is also supported by evidence that dietary *n*-3 fatty acid fortification and FLX similarly decrease behavioral indices of depression in the forced swim test (Carlezon et al., 2005).

The significant and selective elevation in docosapentaenoic acid (DPA, 22:5*n*-6) composition in the PFC of female FLX-treated rats was not anticipated. Reciprocal elevations in rat PFC DPA composition are observed in response to chronic *n*-3 fatty acid insufficiency resulting in PFC DHA deficits (Xiao et al., 2005). It is of interest, therefore, that FLX-treated female rats, but not male rats, exhibited a trend towards lower PFC DHA composition relative to controls

($p=0.09$). These data suggest that peroxisomal-mediated DPA biosynthesis is uniquely augmented by chronic FLX treatment in the PFC of female rats. Elevated $n-6$ fatty acid biosynthesis is also supported by the finding that the 22:4/20:4 ratio was increased in the PFC of FLX-treated female rats. This effect may be mediated in part by the greater FLX and NFLX concentrations observed in female rats. It is also notable that greater DPA (22:5 $n-6$) levels were not observed in erythrocytes of female FLX-treated rats, suggesting this response is unique to the central compartment. Future studies will be required to elucidate mechanisms mediating this response in female rats.

In summary, this study demonstrates that chronic treatment with FLX, resulting in clinically-relevant plasma FLX concentrations and significant reductions in serotonin turnover, does not increase $n-3$ fatty acid biosynthesis or peripheral and central membrane $n-3$ fatty acid composition. Although additional studies will be required to evaluate the generalizability of these findings to other antidepressant medications, this evidence does not support our hypothesis that chronic FLX treatment would augment $n-3$ fatty acid biosynthesis and membrane composition. These preclinical data additionally suggest that chronic exposure to FLX at clinically-relevant concentrations likely does not contribute to the DHA (22:6 $n-3$) deficit observed in erythrocytes (Edwards et al., 1998; McNamara et al., 2010; Peet et al., 1998) and postmortem PFC (McNamara et al., 2007) of MDD patients.

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Abbreviations

PUFA	polyunsaturated fatty acids
$n-3$	omega-3
AA	arachidonic acid
DHA	docosahexaenoic acid
FLX	fluoxetine
NFLX	norfluoxetine
5-HT	serotonin
5-HIAA	5-hydroxyindoleacetic acid
cPLA ₂	cytosolic phospholipase A ₂
SREBP	sterol regulatory element-binding protein
PFC	prefrontal cortex
MDD	major depressive disorder

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Table 1

Male erythrocyte fatty acid composition

Fatty acid ¹	CON (n=20)	FLX (n=20)	p-value ²
<i>Saturated fatty acids</i>			
Palmitic acid (16:0)	23.4 ± 0.2	23.3 ± 0.2	0.55
Stearic acid (18:0)	17.6 ± 0.2	17.7 ± 0.3	0.87
<i>Monounsaturated fatty acids</i>			
Oleic acid (18:1n-9)	7.0 ± 0.3	7.2 ± 0.3	0.61
Vaccenic acid (18:1n-7)	3.1 ± 0.1	3.1 ± 0.1	0.51
<i>Polysaturated fatty acids</i>			
Linoleic acid (18:2n-6)	9.3 ± 0.3	8.4 ± 0.4	0.08
Homo-gamma-linoleic (20:3n-6)	0.5 ± 0.0	0.5 ± 0.0	0.81
Arachidonic acid (AA, 20:4n-6)	27.5 ± 0.3	27.3 ± 0.4	0.82
Docosatetraenoic acid (22:4n-6)	1.9 ± 0.1	2.0 ± 0.1	0.92
Docosapentaenoic acid (22:5n-6)	0.1 ± 0.0	0.1 ± 0.0	0.85
Eicosapentaenoic acid (EPA, 20:5n-3)	0.7 ± 0.1	0.9 ± 0.1	0.13
Docosapentaenoic acid (22:5n-3)	1.4 ± 0.1	1.5 ± 0.1	0.47
Docosahexaenoic acid (DHA, 22:6n-3)	5.1 ± 0.2	5.5 ± 0.3	0.23
<i>Ratios</i>			
AA/EPA	27.1 ± 3.0	22.6 ± 2.1	0.23
AA/DHA	5.6 ± 0.3	5.1 ± 0.2	0.22
n-6/n-3	4.3 ± 0.2	3.8 ± 0.1	0.05

¹Weight percent total fatty acids (mg/100 mg) expressed as mean ± S.E.M.

²Unpaired *t*-test (2-tail)

Table 2

Female erythrocyte fatty acid composition

Fatty acid ¹	CON (n=20)	FLX (n=20)	p-value ²
<i>Saturated fatty acids</i>			
Palmitic acid (16:0)	20.8 ± 0.2	20.9 ± 0.2	0.62
Stearic acid (18:0)	20.7 ± 0.2	20.4 ± 0.3	0.50
<i>Monounsaturated fatty acids</i>			
Oleic acid (18:1n-9)	6.4 ± 0.2	7.2 ± 0.3	0.04
Vaccenic acid (18:1n-7)	2.4 ± 0.0	2.4 ± 0.0	0.52
<i>Polysaturated fatty acids</i>			
Linoleic acid (18:2n-6)	8.1 ± 0.1	8.1 ± 0.2	0.92
Homo-gamma-linoleic (20:3n-6)	0.4 ± 0.0	0.4 ± 0.0	0.91
Arachidonic acid (AA, 20:4n-6)	28.2 ± 0.2	27.8 ± 0.4	0.38
Docosatetraenoic acid (22:4n-6)	1.8 ± 0.1	1.7 ± 0.1	0.28
Docosapentaenoic acid (22:5n-6)	0.1 ± 0.0	0.1 ± 0.0	0.85
Eicosapentaenoic acid (EPA, 20:5n-3)	0.8 ± 0.1	0.9 ± 0.1	0.49
Docosapentaenoic acid (22:5n-3)	1.7 ± 0.0	1.7 ± 0.0	0.74
Docosahexaenoic acid (DHA, 22:6n-3)	6.1 ± 0.2	6.2 ± 0.3	0.79
<i>Ratios</i>			
AA/EPA	26.2 ± 3.1	24.9 ± 2.1	0.36
AA/DHA	4.7 ± 0.3	4.5 ± 0.2	0.40
n-6/n-3	5.1 ± 0.2	5.0 ± 0.3	0.65

¹Weight percent total fatty acids (mg/100 mg) expressed as mean ± S.E.M.

²Unpaired *t*-test (2-tail)

Table 3

Male PFC fatty acid composition

Fatty acid ¹	CON (n=20)	FLX (n=20)	p-value ²
<i>Saturated fatty acids</i>			
Palmitic acid (16:0)	22.9 ± 0.6	23.7 ± 0.3	0.30
Stearic acid (18:0)	21.2 ± 0.5	21.4 ± 0.2	0.71
<i>Monounsaturated fatty acids</i>			
Oleic acid (18:1n -9)	14.9 ± 0.4	14.7 ± 0.2	0.55
Vaccenic acid (18:1n -7)	3.4 ± 0.1	3.4 ± 0.1	0.79
<i>Polysaturated fatty acids</i>			
Arachidonic acid (AA, 20:4n-6)	11.9 ± 0.3	12.4 ± 0.4	0.41
Docosatetraenoic acid (22:4n-6)	3.0 ± 0.1	3.4 ± 0.1	0.02
Docosapentaenoic acid (22:5n-6)	0.5 ± 0.0	0.5 ± 0.0	0.62
Docosahexaenoic acid (DHA, 22:6n-3)	18.8 ± 0.5	18.7 ± 0.3	0.91
<i>Ratios</i>			
AA/DHA	0.63 ± 0.0	0.66 ± 0.0	0.24
22:4/20:4	0.24 ± 0.0	0.28 ± 0.0	0.05
18:1/18:0	0.71 ± 0.0	0.69 ± 0.0	0.30

¹Weight percent total fatty acids (mg/100 mg) expressed as mean ± S.E.M.

²Unpaired *t*-test (2-tail)

Table 4

Female PFC fatty acid composition

Fatty acid ¹	CON (n=20)	FLX (n=20)	p-value ²
<i>Saturated fatty acids</i>			
Palmitic acid (16:0)	23.0 ± 0.1	22.9 ± 0.1	0.71
Stearic acid (18:0)	20.9 ± 0.1	20.7 ± 0.1	0.24
<i>Monounsaturated fatty acids</i>			
Oleic acid (18:1n -9)	15.1 ± 0.1	15.0 ± 0.1	0.72
Vaccenic acid (18:1n -7)	3.2 ± 0.1	3.2 ± 0.1	0.98
<i>Polysaturated fatty acids</i>			
Arachidonic acid (AA, 20:4n -6)	12.8 ± 0.1	12.3 ± 0.1	0.27
Docosatetraenoic acid (22:4n -6)	3.2 ± 0.1	3.4 ± 0.1	0.03
Docosapentaenoic acid (22:5n -6)	0.5 ± 0.0	0.6 ± 0.0	0.001
Docosahexaenoic acid (DHA, 22:6n -3)	18.3 ± 0.1	17.9 ± 0.2	0.09
<i>Ratios</i>			
AA/DHA	0.67 ± 0.0	0.69 ± 0.0	0.06
22:4/20:4	0.27 ± 0.0	0.28 ± 0.0	0.02
18:1/18:0	0.72 ± 0.0	0.73 ± 0.0	0.77

¹Weight percent total fatty acids (mg/100 mg) expressed as mean ± S.E.M.

²Unpaired *t*-test (2-tail)