Full Length Research Paper

Effect of dietary n-6 to n-3 polyunsaturated fatty acid ratio on prostaglandin plasma levels and genes expression peroxisome proliferator-activated receptor (PPAR) in pregnant Sprague Dawley rats

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The peroxisome proliferator-activated receptors (PPARs) are a family of nuclear transcription factors thought to act as receptors for polyunsaturated fatty acids and to reduce production of series 2 prostaglandins (PG). The objective of this study was to investigate the effect of different ratio n-6:n-3 on the PPAR expression of rats endometrial tissue. The findings obtained from this study showed significant induction of PPARδ mRNA levels in endometrial cells treatment 1:1 group by 1.38 fold compared with the PPARδ mRNA levels in endometral cells treatment 30:1 group. This induction was due to the cellular demands for prostaglandin were high in the endometrial cells when cells were treated with high ratio n6:n3 on 30:1 group, thus, resulting in an increase in both prostaglandin PGE2 and PGF2α production by induction of PPARδ genes. On the other hand, treatment 1:1 group and control group of endometrial cells did not show any significant changes in mRNA level of PPARδ, compared with treatment ratio n6:n3 on 6:1 group and treatment high ratio n6:n3 on 30:1 group of the endometrial cells. These findings show that inhibition of uterine PGF2α synthesis by n-3 fatty acids may depend on the amount of n-6 fatty acids reaching the target tissue. In conclusion, PPARδ function in the response of rat endometrium to long chain n-6:n3 polyunsaturated fatty acids.

Key words: Polyunsaturated fatty acid, gene expression, peroxisome proliferator-activated receptor, prostaglandin, pregnancy rat.

INTRODUCTION

The PPARs are a family of nuclear receptors activated by some LCFA, eicosanoids and peroxisome proliferators. Three isoforms of PPAR, encoded by separate genes, have been identified date: PPARγ, PPARα and PPARδ which upon ligand binding, heterodimerize with the retinoid receptor and interact with specific PPAR response elements in the promoter region of target genes to affect transcription. Regulation of promoter function is complex, because it is the expression of tissue-specific PPAR and retinoid receptor subtypes, competition for the retinoid receptor binding partner and differences in binding affinity among the PPAR subtypes and among there receptor subtypes (Desvergne and Wahli, 1999).

The PPARs are well known for their roles in lipid metabolism, but they are also involved in development, maturation of the epidermis, the reproduction in several animal models and functions of the nerve, lung, kidney and cardiac tissues (Desvergne and Wahli, 1999; Berger...
and Moller, 2002).

In contrast to PPARα, PPARγ has a preference for PUFAs over MUFA or UFA (Khan and Heuvel, 2003). The PPARδ is expressed in a wide range of tissues and cells, with relatively higher levels of expression noted in brain, adipose and skin (Huín et al., 2000; Amri et al., 1995). Importantly, in the endometrium PPARδ is vital for normal fertility serving as a regulator of PG production and is required for implantation in rodent models (Lim et al., 1997, 1999). MacLaren et al. (2005) reported similar expression of PPARα and PPARδ mRNA levels in BEND cells and endometrium from cyclic and pregnant Holstein cows.

The PPARδ/α agonist cPGI had a dramatic stimulatory effect on PGHS-2 mRNA levels and synthesis of PGE2 and PGF2α, which appeared to be mediated at least in part through PPARδ (MacLaren et al., 2005). They hypothesized that PPARδ is involved in the pregnancy recognition process of cattle and that it mediates at least some of the beneficial effects of long chain omega-3 PUFAs on fertility. To date, only the PPAR that has been associated with reproductive function is PPARδ (Berger et al., 2002). Some fatty acid agonists for PPARδ are DEPA, of which the latter may be the most potent activator of this chemical class (Xiao et al., 1998). Studies with mice and rats have shown that PPARδ plays a specific role in embryonic development, especially during implantation and decidualization (Ding et al., 2003). Furthermore, a study by Lim et al. (2000) demonstrated that PGJ2 is the primary PG that is essential for implantation and decidualization and suggested that the effects of PGJ2 are mediated by activation of PPARδ. This suggests that PGs produced by PGHS-2 may exert their effects directly on the nucleus via activation of PPARs. In addition, PGHS-2 deficient mice, decidualization and implantation failures can be reversed by the administration of a PPARδ-selective agonist (Lim et al., 1997). Collectively, these observations indicate that there may be a role for one or more PPARs in rat's endometrial prostaglandin production. The objective of this study was to characterize PPAR expression activity of rats endometrial tissue in response to known PPAR ligands, as well as to key stimulators and inhibitors of series 2 prostaglandin secretion.

MATERIALS AND METHODS

Animals and diets

Twenty-eight (28) female Sprague-Dawley rats weighing (240 ± 20 g body weight) were used in this experiment. They were purchased from Veterinary Faculty, Universiti Putra Malaysia. Rats were individually housed in stainless steel cages in a room with a 12/12 h light/dark cycle at the ambient temperature of 25 to 30°C. Experiment was carried out according to the guidelines for the use of animals approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM/FPV/PS/3.2.1.551/AUP-R23).

After 2 weeks of adaptation, the rats were randomly divided into four treatment groups consisting seven rats in each group. The cod liver oil was used as the main source of n-3 PUFAs [Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and soybean oil is the main source of n-6 fatty acids and linoleic acid (LA). The treatment groups include rats fed with normal rat chow diet (control, C), rats fed chow diet supplemented with 5% (w/w) SBO and 5% (w/w) CLO (Diet 1:1); rats fed chow diet added with 8.4% (w/w) SBO and 1.6% (w/w) CLO (Diet 6:1); and rats fed chow diet added with 9.6% (w/w) SBO and 0.4% (w/w) CLO (Diet 30:1). The rats were fed 7% of body weight daily and water was provided ad libitum. The diets were prepared daily to minimize rancidity and oxidative damage, the rats were fed once daily and the left over feeds were collected before new feeding. After 6 weeks of feeding, the stage of estrous cycle of the female rats was determined by vaginal smears taken between 8 to 10 am daily (for 2 weeks). Cell types in the smear were subsequently examined microscopically to determine the estrous stage. The consecutive stages of the estrous cycle of cornified cells and the presence of nucleated cells; estrus, presence of only cornified cells; metestrus, presence of leukocytes and fewer cornified cells; and diestrus, mostly polymorphonuclear leukocytes (Turner and Bagarina, 1976). Vaginal smears of 28 female rats were performed every morning during for 2 weeks and unstained native material was observed using the microscope without the aid of the condenser lens. Using the 10× objective lens, it was easier to analyze the proportion among the three cellular types, which were present in the vaginal smear. While using the 40× objective lens, it was easier to recognize each one of these cellular types Mating was done during proestrus stage by placing one adult male and one female into individual cages for 24 h. The presence of spermatozoa in the vaginal the following morning via vaginal smear was defined as day 0 of pregnancy. Pregnant females were separated from male rats on the day of pregnancy. The rat were individually housed in polycarbonate cages (43 × 28 × 16 cm) with sawdust bedding, in controlled room temperature (23 ± 2°C) with 12 h of light and 12 h of darkness. On the day 14 prior to sacrifice, food was withdrawn at 9:00 a.m. and the animals were sacrificed at 9:00 a.m the following morning on the day 15 of pregnancy after 10 weeks (8 weeks + 15 days) of feeding rats were sacrificed by cardiac puncture for determining plasma Fatty acids profile and prostaglandin concentrations (PGE2 and PGF2α). Total RNA was isolated from fresh frozen uterus tissues kept in liquid nitrogen, by RiboPureTM RNA Isolation Kit according to the manufacturer’s instructions (Ambion, Austin, Texas, USA).

RNA extraction

Figure 1 shows the total RNA was isolated from uterus tissues using the RiboPure RNA isolation kit according to the manufacturer's instructions. The purity of the extracted RNA was determined by measuring the ratio of the optical density at 260 and 280 nm using a spectrophotometer (BioRad, USA). The total RNA concentration was determined by measuring the absorbance at 260 nm. The integrity and size distribution of the total RNA was determined by using a 1.5 agarose gel. The 18S and 28S RNA bands were visualized under UV light using gel image instrumentation.

Quantitative real time PCR

SYBR green primers specific for PPARδ and beta actin genes were designed and synthesized by (Oligo- Singapore) and supplied by Next Gene Scientific Sdn. Bhd. from t he gene sequence of rat
Table 1. SYBR green specific primers used in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR δ F</td>
<td>CAGCTGCCCTTGGTGAACCAT</td>
<td>126</td>
</tr>
<tr>
<td>PPAR δ R</td>
<td>CCTGCTCAACAGACCATCAATTCTAG</td>
<td></td>
</tr>
<tr>
<td>Beta actin F</td>
<td>CCAACTGGGACGACATGGAG</td>
<td>144</td>
</tr>
<tr>
<td>Beta-actin R</td>
<td>TCAAACATGATCTGGGTCATCTTC</td>
<td></td>
</tr>
</tbody>
</table>

F = Forward, R = reverse and bp = base pair.

Statistical analysis

Data were analyzed as a completely randomized design experiment using the general linear model of SAS 9.02 software (Statistical Analysis Systems Institute Inc., 1992). The gene expression (PPAR δ) of the rat treatment diets were analyzed across treatment groups using the one-way analysis of variance (ANOVA) method. Significantly different means were then elucidated using the Duncan's.

RESULTS

Treatment of supplementation high level soybean oil on treatment Diet 30:1 for 2 month feeding increased levels of PPAR δ of mRNA by 8.82 fold, respectively. Supplementation high ratio n6:n3 on Diet 30:1 compared with low ratio n6:n3 on Diet 1:1 (1.38) (Table 2), results in an increase in the control (Diet 4) PPAR δ mRNA
The peroxisome proliferator activated receptors (PPARs) are a family of nuclear transcription factors and are thought to act as receptors for polyunsaturated fatty acids and to reduce production of series 2 prostaglandins (PG). The evidence is rapidly accumulating that fatty acids may have additional serious major effects on the synthesis of eicosanoid in domestic animals (Baguma-Nibasheka et al., 1999; Mattos et al., 2003). Depending on the amount and type of specific fatty acids to achieve the target tissues, fatty acids can either stimulate (Burke et al., 1996; Filley et al., 1999) or inhibit (Baguma-Nibasheka et al., 1999; Mattos et al., 2003; Cheng et al., 2001) the synthesis of prostanoid. The AA that is released by phospholipid hydrolysis is acted upon by PGHS-2 to form PGH2, which then is converted to PGF2α, which is then converted to PGE2 and TXA2. The polyunsaturated fatty acids get several physiological changes in the alteration of the activity or the synthesis of nuclear PPARs (Bocher et al., 2002). In mice, PPARδ deficiency leads to placental defects and frequent mid gestational results lethality (Barak et al., 2002), suggesting that this nuclear receptor may play an important role in controlling reproductive processes in mammals. Consistent with in vivo experiment (Caldari-Torres et al., 2006), supplementation of the EPA had no detectable effects on PPARδ response to PDBu in cultured BEND cells. The results suggest that supplementation of the n-3 fatty acids may alter endometrial PGF2α production through a mechanism which does not require the induction of PPARδ gene. However, whether and how these fatty acids may control the activity of this nuclear receptor guarantees further investigation and this nuclear receptor may play an important role in the control of reproductive processes in mammalian species. In this study, the molecular mechanisms by which ratio of n6:n3 fatty acids regulated key genes that are involved in prostaglandin production on endometrial cells were investigated. Quantitative real time PCR analysis was performed to quantify the expression level of the mRNA of all the genes. For the RNA purity, the A260:A280 ratios of 1.8:2.0 were typically obtained. Thus, the purity less than 1.8 was not accepted for further experiment. In addition,

**DISCUSSION**

expression from 1.00± 0.01 to 8.82± 1.84 in Diet 30:1. In the Diet 6:1 group, the PPARδ expression level was increased significantly by 2.28 fold when compared with the Diet control group. While the PPARδ mRNA expression levels was significantly highest in Diet 30:1 group when compared with Diet 1:1 and 6:1 groups, where the level was 8.82 fold higher than the Diet control group.

For both PGF2α and PGE2, the levels were significantly higher (P < 0.05) in Diet 30:1 group when compared with other groups. There were significant difference (P > 0.05) in PGF2α concentrations between the groups fed with diet Diet 1:1, 6:1 and 30:1 in which it was higher in Diet 30:1, followed by Diet 6:1 and least in Diet 1:1 groups. By increasing the plasma arachidonic acid (AA) in Diet 30:1, the production of PGF2α and PGE2 increased significantly when compared with other groups. Amira et al; 2010 (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Average C T of PPRA δ (fold)</th>
<th>Average C T of beta actin (fold)</th>
<th>ΔC T</th>
<th>ΔΔC T</th>
<th>2^ΔΔC T (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 1:1</td>
<td>26.60± 0.04</td>
<td>18.96± 0.70</td>
<td>7.64</td>
<td>-0.46</td>
<td>1.38</td>
</tr>
<tr>
<td>Diet 6:1</td>
<td>25.26± 0.28</td>
<td>18.35± 0.56</td>
<td>6.91</td>
<td>-1.19</td>
<td>2.28</td>
</tr>
<tr>
<td>Diet 30:1</td>
<td>21.58± 0.15</td>
<td>16.62± 0.24</td>
<td>4.96</td>
<td>-3.14</td>
<td>8.82</td>
</tr>
<tr>
<td>Diet Control</td>
<td>27.47± 0.84</td>
<td>19.37± 0.75</td>
<td>8.10</td>
<td>0.01</td>
<td>1.00</td>
</tr>
</tbody>
</table>

ΔC T, C T PPRA δ - C T of beta actin, ΔΔC T = C T treated cells - C T control.

**Table 2.** The effect of n6:n3 on the expression level of PPRA δ gene in the endomatrim tissue (n = 7).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGF2α (ng/ml)</th>
<th>PGE2 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 1:1</td>
<td>469.33 ± 48.08</td>
<td>700.94 ± 25.34</td>
</tr>
<tr>
<td>Diet 6:1</td>
<td>764.49 ± 41.91</td>
<td>1124.82 ± 67.08</td>
</tr>
<tr>
<td>Diet 30:1</td>
<td>1110.56 ± 106.23</td>
<td>1971.76 ± 96.73</td>
</tr>
<tr>
<td>Diet control</td>
<td>576.51 ± 22.37</td>
<td>611.85 ± 16.98</td>
</tr>
</tbody>
</table>

Values with different superscripts within column differ significantly at P < 0.05.

**Table 3.** The plasma PGF2α and PGE2 concentrations (ng/ml) at 15 days of pregnancy (n = 7).
the two bands of RNA (28S and 18S) are clearly visible as shown in Figure 1. The 28S band showed intensity double that of the 18S band which indicate good quality of extracted RNA. It was reported that the successes in the expression analysis depends on the quality of RNA (Duggan et al., 1999).

The findings obtained in this study showed significant induction of PPARδ mRNA levels in the endometrial cells of treatment Diet 1:1 by 1.38 fold as compared to the PPARδ mRNA levels in the endometrial cells of treatment Diet 30:1 by 8.82 groups. This induction due to the cellular demands for prostaglandin were high in the endometrial cells when cells were treated with high ratio of n6:n3 in Diet 30:1 group, thus, resulting in an increase in both prostaglandin PGE2 and PGF2α production by induction of PPARδ genes (Achard et al., 1997). On the other hand, treatment Diet 1:1 and control Diet control of endometrial cells did not show any significant changes in mRNA level of PPARδ genes (Achard et al., 2001). These findings which are consistent with previous studies showed that inhibition of uterine PGF2α synthesis by n-3 fatty acids may depend on the amount of n-6 fatty acids reaching the target tissue. Increasing concentrations of LA in the cell culture system may increase the availability of AA in membrane phospholipids and therefore, decrease the competition by n-3 fatty acids for the PGHS-2 enzyme (Trujillo and Broughton, 1995; Achard et al., 1997).

In this study, we showed that PPARδ is a likely mediator of some of the effects of long chain n-6 PUFA action in the pregnant rat endometrium. The relative level of PPARδ mRNA expression in rat endometrial cells is under the influence of different ratio of n6:n3 to initiate pulsatile release of PGF2α and consequently, luteolysis (MacLaren et al., 2003; MacLaren et al., 2006). Ding et al. (2003) showed that PPARδ mRNA regulated in uterine epithelium in early pregnancy of rodent and is induced in uterine subluminal stroma by the presence of the blastocyst.

**Conclusion**

Fatty acid stimulated PGF2α production and up-regulated PPARδ gene expression in pregnant rat. These findings indicate that the net inhibition of uterine endometrial PGF2α synthesis by n-3 fatty acids may depend on the availability of n-6 fatty acids within the target tissue.

**REFERENCES**


ester-induced secretion of prostaglandin F\textsubscript{2} alpha and expression of prostaglandin endoperoxide synthase-2 and phospholipase-A\textsubscript{2} in bovine endometrial cells. Biol. Reprod. 69(3): 780-78.