The use of cyclic oral contraceptives (OC) is associated with an overall increased risk of ER-positive breast cancer, particularly in current users, and women who start OC early in life. This effect of exposure (e.g., age at initiation of use) is not seen in OC users who start later in life. Whether this effect is due to the hormone withdrawal phase associated with both cyclical OC and non-cyclic (effervescent, Cyclic OC and non-cyclic OC) is not established. We investigated the hypothesis that (i) hormone withdrawal from OC exposure increases cellularity of adipose tissue, and (ii) increased cellularity results in a higher risk of breast cancer.

Materials and Methods

Animals

BALB/c mice were purchased from Jackson Laboratory at 35 days of age. Mice were fed for 2 days on standard laboratory chow and then transferred to the cyclic (control) regimen or cyclical OC. They were maintained at a body weight of 20 g. Cyclic oral contraceptives induce a higher cellularity of adipose tissues in comparison to non-cyclic OC. Experimental use of animals was approved by the Animal Care Committee of The University of Buffalo.

Dietary model of OC exposure

Mice were grouped in 2 age categories, 1-3 and 15-19 month-old mice, and the 7-day (100% OC) of dietary regimen was carried out. Dietary models were supplemented with 0.1% estradiol (E2) or 0.1% progesterone (P) to simulate cyclic ovarian hormone cycles. Cyclic OC increased Ki-67 staining in small ducts (epithelium plus alveoli). Continuous OC increased the number of cells infiltrating the mammary adipose stroma. A 20% meal group was injected into the mammary fat pad with 10% E2-negative xenografts (TM2H mammary epithelial tumor cells, and treated for tumor latency, growth, and tumor burden. The growth of transplanted TM2H tumor cells showed a 25% increased latency, and a 33% decreased tumor burden in the mice receiving continuous OC, suggesting that the mammary environment of mice who had previously received continuous OC was favorable to the growth of transplanted tumor cells than control. Once established, growth rate was unchanged in OC. Cyclic OC showed intermediate effects in decrease tumor growth.

Results

Figure 1: Effect of OC on epithelial proliferation

Top: whole mounts of mammary glands (dorsal view for control; continuous, control or cyclic OC on 20 days post-injection of effervescently administered) were stained with H&E and were analyzed for epithelial cell proliferation (Ki-67) using ImageJ software. Bars represent mean ± SEM (n = 10) and (control and continuous or cyclic OC) (n = 11) (cyclical OC).

Figure 2: Effect of OC on mammary gland architecture

Top: representative images showing terminal structures in mammary glands (mice) stained, and immunostained for h-catenin (3). Both continuous and cyclical OC groups decreases the frequency of TEBs, and increases the frequency of adenoid buds (Ki-63). Terminal ducts were unchanged in frequency. Bottom: continuous OC is associated with an trend (P>0.05) to increased lobular complexity in paraffin sections; both OC regimens show a trend (P>0.05) with increased ducts with lobulation. Bars represent mean ± SEM (n = 10) and (control and continuous or cyclic OC) (n = 11) (cyclical OC).

Figure 3: Cyclic OC increases proliferation in the tumor epicenter

Top: representative images showing proliferation of Ki-67 stain in small ducts of mice fed continuous OC. Bottom: Cyclic OC has no significant effect on proliferation of Ki-67 stain (P>0.05). Bars represent mean ± SEM (n = 10) and (control and continuous or cyclic OC) (n = 11) (cyclical OC).

Bar graph represents significant differences from control (P<0.05).

Table 1: Effects of OC on TM2H mammary latency, survival, and multiplicity

<table>
<thead>
<tr>
<th>Latency (h)</th>
<th>Control</th>
<th>Continuous</th>
<th>Cyclic OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>87</td>
<td>68 ± 6</td>
<td>76 ± 5</td>
<td>84 ± 4</td>
</tr>
<tr>
<td>Survival (h)</td>
<td>58 ± 5</td>
<td>64 ± 4</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>Tumor latency (h)</td>
<td>15 ± 2</td>
<td>16 ± 2</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

Bar graph represents significant differences from control (P<0.05).