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Effect of a combined estrogen and progesterone oral contraceptive on circulating adipocytokines adiponectin, resistin and DLK-1 in normal and obese female rhesus monkeys[☆]

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Abstract

Background—Hormonal contraception is the most common medication used by reproductive aged women but there is little understanding of the impact of hormonal contraception on obesity and metabolism. Adipokine levels (adiponectin, resistin) and markers of adipocyte development (DLK-1) are altered in obese animals and humans and are associated with increased cardiovascular risk. We sought to determine the effect of combined hormonal oral contraceptive pills (COCs) on circulating adiponectin, resistin and DLK-1 levels in obese and normal-weight rhesus macaque monkeys.

Methods—Serum adiponectin, resistin and DLK-1 levels in reproductive-age female rhesus macaques of normal (n = 5, mean = 5.76 kg) and inherently obese (n = 5, mean = 8.11 kg) weight were determined before, during and 2 months after cessation of 8 months of continuous treatment with COCs.

Results—The obese group alone showed a significant decrease (p<.01) in weight with COC use, which returned to baseline after COC cessation. Baseline adiponectin levels prior to COC treatment were lower in the obese group (p<.05). Adiponectin levels increased from baseline in both groups, but more so in the obese group (p<.05). Resistin levels were similar at baseline, with an increase in both groups following treatment. Circulating resistin remained elevated above baseline levels after COC cessation, particularly in the obese group (p<.05). While DLK-1 levels did not change significantly in either group, a trend for higher levels in obese animals was observed.

Conclusions—COC use may alter metabolic processes via direct (resistin) or indirect (adiponectin) means, while unchanging DLK1 levels suggest they do not affect adipocyte development. COCs may directly increase resistin levels, as observed in both groups. As

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adiponectin is inversely related to adipocyte mass, increased levels in the obese group are likely attributed to weight loss.

Keywords

Obesity; Hormonal contraception; Metabolism; Adipocytokines; DLK-1

1. Introduction

Obesity is a rapidly growing problem in the United States. In 1991, 12.0% of the US population was considered obese [body mass index (BMI) $>30 \text{ kg/m}^2$] [1], but by 2009, the prevalence skyrocketed to 26.7–33.8% with 68% of Americans currently overweight or obese (BMI $>25 \text{ kg/m}^2$) [2,3]. Obesity is associated with multiple medical problems. Specifically for women, there are adverse implications for reproduction, concerning both fertility and the prevention of pregnancy [4–7]. As hormonal contraception is the most common medication used by reproductive-aged women, understanding the impact of contraception on obesity and metabolism is essential to the overall health of this population.

Adipose tissue was once thought of primarily as a storage organ but is now recognized as an endocrine organ and part of the innate immune system [8]. Adipose involvement in metabolism and inflammation makes it a key factor in the development of obesity-associated metabolic abnormalities and insulin resistance [9–11]. As part of the hypoxia-induced adipose inflammation that occurs in obesity, alterations in the secretion of specific adipokines such as adiponectin and resistin occur, which in turn further affects free fatty acid uptake, ectopic fat deposition, as well as overall metabolism and lipotoxicity [11]. Adiponectin is released by adipocytes and appears to have a protective effect through antiatherogenic and anti-inflammatory mechanisms [10,11]. Adiponectin has also been shown to decrease serum glucose levels, free fatty acids and triglycerides, and improve insulin sensitivity while decreased serum levels are associated with male gender, obesity, diabetes and cardiovascular disease [10]. In rhesus macaques, serum adiponectin levels have been shown to decrease in parallel with progression to type 2 diabetes, further supporting the role of adiponectin in insulin sensitivity [12].

Resistin is a peptide released by adipocytes and macrophages [11]. With obesity, there is an increase in both adipocytes and macrophages and circulating resistin levels increase. In animal models, increased resistin levels are associated with insulin resistance [11]. Although an association with cardiovascular risk is less established than with adiponectin, increased circulating levels of resistin have been shown to be an independent risk factor for new onset heart failure in older persons [13,14].

Adipocytes arise from pre-adipocytes that, in turn, are derived from multipotent mesenchymal stem cells. DLK-1 (delta-like protein 1) is secreted by pre-adipocytes and directs the stem cells toward chondrogenic lineage (cartilage formation) and inhibits differentiation into adipocytes. Down-regulation of DLK-1 is required for adipose differentiation [14,15]. Increased DLK-1 expression may serve as a negative feedback signal to prevent further adipose differentiation. In animal models, over-expression of soluble DLK-1 has been shown to decrease adipose mass and improve insulin sensitivity, while deficiency has been shown to cause rapid weight gain with increased fat mass [15].

There is little understanding of how hormonal contraception may affect adipocytokines and adipocyte development factors in reproductive-aged women. Additionally, hormonal contraception may have a differential effect on metabolic health based on initial BMI (normal versus obese) [16]. Therefore, we sought to determine the effect of an oral

contraceptive on adiponectin, resistin and DLK-1, as well as whether there is any difference between normal and obese cohorts.

2. Materials and methods

Our serum samples were obtained from a previously published study researching the effects of oral contraceptives on body weight while keeping caloric intake stable [16]. In this study, obese ($n=5$) and normal-weight ($n=5$) rhesus macaque monkeys were used due to their similarity to humans in both their reproductive systems and ovulatory cycles. In this animal model, activity levels, metabolic changes and caloric intake can be measured and controlled in a way that is not possible in human subjects. Animals had their body weight monitored over 8 months on combined oral contraceptive pills (COCs) while maintaining a diet that resulted in stable body weight prior to initiation of COCs. In the parent study, the obese monkeys showed a significant decrease in body weight (8.6%) and body fat (12%) while taking COCs compared to normal weight monkeys who had no significant change in either [16]. Approval for the study was obtained through the Oregon National Primate Center Institutional Animal Care and Use Committee (IACUC).

2.1. Study population

Serum samples were obtained from normal ($n = 5$, mean body weight = 5.76 kg) and obese ($n = 5$, mean body weight = 8.11 kg), reproductive-age adult rhesus macaque (*Macaca mulatta*) monkeys from a previously published study [16]. The obese group was inherently obese with naturally increased food intake and decreased activity levels as compared to the normal BMI controls. Weight stability in both groups was monitored for 2 months prior to initiation of COCs. A combination COC with 30 mcg ethinyl estradiol (EE)/150 mcg levonorgestrel (LNG) (Seasonale, Pomona, NY, USA) was dosed in a continuous fashion (no placebo days) for a treatment period of 8 months (237 days) [17]. Dosing studies prior to study initiation were performed for each monkey in order to ensure a comparable human equivalent dose for this COC [EE 145 ± 45 pg/mL, LNG 5.6 ± 1.5 ng/mL (mean $\pm$ SD)] [18]. Each monkey had a set dose to achieve this plasma level, which ranged from 1/8 to 1/2 tablet daily. EE and LNG levels were stable over the course of the study, exhibiting a 7.5% and a 10.1% variation, respectively. Subjects were then studied for 2 months after the cessation of COCs to determine reversibility of effect of COCs on metabolic markers of interest [16]. Serum time-points analyzed included baseline (prior to initiation of COCs), 2 and 6 months after initiation and 2 months after cessation of COCs.

2.2. Measurements of adiponectin, resistin and DLK-1

Blood sampling occurred in the morning during a fasting state. Serum levels of adiponectin, resistin and DLK-1/Pref-1 were measured with enzyme immunoassays according to the manufacturer instructions. Each sample was measured in duplicate. Adiponectin levels were measured using rhesus macaque Adiponectin Competitive ELISA kit from Adipo-Gen, Inc (Seoul, Korea). DLK-1 levels were measured using Quantikine® ELISA kit from R&D Systems (Minneapolis, MN, USA). Resistin levels were measured using Human Resistin ELISA kits from Biovendor Research and Diagnostic Products (Czech Republic). These assays were previously validated for use with rhesus macaque serum by our lab.

The data were then analyzed with SAS 9.3 using nonparametric testing (Wilcoxon and Kruskal-Wallis Test) to compare normal and obese groups and change in serum levels as compared to baseline over time, accounting for correlation. Percent increase from baseline, mean and standard deviations at each time-point were also calculated.

3. Results

3.1. Adiponectin

One outlier in the normal group was identified at the 2-month time-point (Fig. 1A). At baseline, the mean serum levels of adiponectin were significantly lower in the obese group (mean 0.41 mcg/mL, SD 0.12) compared to the normal group (0.94 mcg/mL, SD 0.23) ($p < .05$) (Table 1). There was an increase in serum adiponectin levels over time on COCs in both groups (Figs. 1, 2). After 2 months of COCs, mean serum levels increased above baseline values in both the obese group (0.63 mcg/mL, SD 0.31) and the normal-weight group (1.79 mcg/mL, SD 0.73) (Fig. 2). Mean serum levels continued to increase after 6 months of COC treatment in the obese group (0.89 mcg/mL, SD 0.40) and remained elevated in the normal weight group (mean 1.54 mcg/mL, SD 0.63). The response to COC treatment was variable in both groups with a certain proportion of animals being responsible for the overall change in adiponectin levels. For example, in the obese group (Fig. 1B), two animals exhibited only minimal increases in circulating adiponectin levels at 6 months relative to baseline levels, whereas the remaining three animals had levels that were greater than twofold above their baseline levels. At 6 months, the percent increase from mean baseline levels was significantly higher in the obese group than in the normal weight group (117% vs. 65%, $p < .05$). Two months after cessation of COC treatment mean adiponectin levels remained elevated above baseline in obese animals (mean 0.79 mcg/mL, SD 0.54) while normal weight animals had mean levels which decreased towards baseline (mean 0.83 mcg/mL, SD 0.33), but neither reached statistical significance.

3.2. Resistin

At baseline, mean resistin levels were similar in both the obese (mean 2.50 ng/mL, SD 0.55) and normal groups (mean 3.04 ng/mL, SD 0.63) ($p = .24$) (Table 1). After 2 months of COC use, mean resistin levels were not significantly elevated in obese and normal weight groups relative to baseline levels (Fig. 3). Mean resistin levels increased significantly from baseline after 6 months of COC treatment in the normal weight group (mean 7.88 ng/mL, SD 2.69), but the change did not reach statistical significance in the obese group (mean 4.68 ng/mL, SD 2.22; $p = 0.12$). Mean resistin levels remained elevated above baseline in both groups 2 months after cessation. Mean resistin levels remained elevated compared to baseline in both groups. Mean levels in the obese group remained 140% above mean baseline levels ($p < .05$; mean 6.01 ng/mL, SD 1.39), and the mean levels in the normal group remained 100% above baseline ($p < .05$; mean 6.07 ng/mL, SD 1.33). In both the normal-weight and obese groups, resistin levels 2 months after cessation were nearly equivalent.

3.3. DLK-1

At baseline, there was no significant difference in mean serum DLK-1 levels between obese (mean 0.36 ng/mL, SD 0.32) and normal-weight (mean 0.24 ng/mL, SD 0.26) animals (Table 1); standard deviations within groups were large (Fig. 4). At the 2- and 6-month COC treatment intervals, as well as after cessation of treatment, there were no significant differences in mean circulating DLK-1 levels relative to baseline values. While mean DLK-1 levels were greater in obese animals prior to, during, and following COC treatment relative to normal weight rhesus macaques, this difference was not statistically significant.

4. Discussion

As obesity rates increase, more understanding of the relationship between adiposity and metabolism becomes both possible and necessary. Since oral contraceptive pills are often the only medication that women take consistently through their reproductive years, they are often blamed for weight gain that is likely attributable to lifestyle, environment and genetic

factors [19]. A study by Edelman et al. [16] using non-human primates was designed to investigate the effects of COCs on weight in a setting where activity and food intake could be controlled, which could not be performed in women. The results revealed COCs increased basal metabolic rate without a changing activity or food intake, leading to a decrease in body weight and percent body fat that was significant in the obese animals [16]. However, what other changes occur with COC use that may impact metabolic health? The link between adiposity and metabolism has been well established and can be measured with surrogates such as adiponectin, resistin and DLK-1.

As expected with a change in basal metabolic rate shown in the study population, adipocytokines were affected by COC use and the effect varied by body weight. Baseline mean adiponectin levels were lower in the obese group, which as expected correlates with the greater adipose mass. Mean adiponectin levels increased from baseline in both groups, but a greater increase was seen in the obese group. However, the obese group lost more body weight and fat during the parent study [16], which may account for this difference. Mean resistin levels in both groups were similar at baseline, with an increase in both groups following COC treatment. Circulating mean resistin levels remained elevated above baseline levels after COC cessation in both groups with a greater increase in the obese group. The role of resistin remains controversial in humans, but its elevation has been associated with increased cardiovascular (CV) risk [9]. In contrast, adiponectin has been well established as having a protective effect on CV risk. These results may indicate an improvement in the metabolic profile with COC use, not only in body weight, but also with adipokine levels and CV risk. More research is needed to investigate which adipokine dominates in its effect and impact on cardiovascular health. This would ideally be done in an animal model where variables can be controlled and cardiovascular markers measured.

While DLK-1 levels did not change significantly in either group, a trend for higher levels in obese animals was observed. The variability in the serum values and the small sample size make this difficult to interpret. The findings do suggest that COCs do not affect differentiation of mesenchymal stem cells to adipocytes (or adipocyte development) despite observed changes in body weight and fat composition, although more research is needed in this area.

As reported in the parent study, with COC use the obese animals lost a significant amount of both their body weight and fat, determined by dual-energy X-ray absorptiometry scans, in the absence of any changes in caloric intake or activity levels [16]. Thus, the lowered adiposity appears due to changes in the basal metabolic rate. Although, body weight and fat are known modulators of adipokines and the obese group followed the expected increase in adiponectin with decreased body fat seen with COC use, the normal-weight group also showed a trend towards increased adiponectin with COC use. The mean adiponectin levels in the obese group did not reach the levels in the normal weight group, likely due to the fact that the obese animals never fully achieved the BMI of the normal weight animals. This result suggests that the increased serum adiponectin levels may be directly related to COC use.

The advantage of animal studies is the ability to tightly control and monitor certain variables, while a disadvantage is the small sample sizes that are feasible in primate models. Animal models also pose limitations in that there is a difference between animal and human behavior as well as a difference in the type of body fat seen in humans versus animals (white versus brown adipose) [20]. This analysis requires further studies to investigate adiponectin levels, as currently the majority of direct evidence comes from animals, while human studies to date are largely associative [10]. Although findings in early animal studies appear to correlate with more recent human studies [21], more research is needed.

The impact of COCs on metabolic health and adipocytokines is sparse and focuses mostly in a polycystic ovarian syndrome model [22,23]. One study showed a decrease in mean adiponectin levels with short-term use of a progestin-only subdermal implant [24]. Another showed no change or a decrease in mean resistin levels in females taking COCs for 6 months [25]. Neither study looked at the effect of body weight.

A major limitation of this study was the small sample size, which increased variance and, as seen with adiponectin, as few as 2 animals were responsible for the trends seen. Despite the small sample size, our study revealed trends in adipocytokines that reached statistical significance. Trends for increase in adiponectin and resistin seen in both obese and normal weight animals may indicate a direct effect of the COCs on these metabolic markers. More human research is needed to evaluate the effects of COCs on obesity and metabolism as well as the clinical significance of these metabolic surrogate markers.

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Fig. 1. Serum adiponectin levels of individual normal weight (A) or obese (B) monkeys prior to (baseline), during (2 and 6 months), and following (2 months) COC treatment. Each line represents an individual animal through the course of the study interval.

Fig. 2. Mean serum adiponectin levels over time in obese and normal weight rhesus macaque monkeys on COCs. Significance ($p < .05$) is indicated for comparisons between groups at each time-point (brackets) and the change from baseline levels within each group (*). Error bars signify SD.

Fig. 3.

Mean serum resistin levels in obese and normal weight rhesus macaque on COCs.

Significance ($p < .05$) is noted for a change from baseline levels within each group (*). No significant differences were observed between groups at each time-point. Error bars signify SD.

Fig. 4. Mean serum DLK-1 levels in obese and normal weight rhesus macaque on COCs. No significant differences exist between groups at each time-point or from baseline levels within each group. Error bars signify SD.

Table 1

Mean serum levels before, during and after treatment with COCs for 8 months

	Normal weight		Obese	
	<i>n</i> =5		<i>n</i> =5	
	Mean serum level	SD	Mean serum level	SD
Adiponectin (mcg/mL)				
Baseline	0.94	0.23	0.41	0.12
2 months	1.79	0.73	0.63	0.31
6 months	1.54	0.63	0.89	0.40
2 months after	0.83	0.33	0.79	0.54
Resistin (ng/mL)				
Baseline	3.04	0.63	2.50	0.55
2 months	3.95	1.77	3.14	0.81
6 months	7.88	2.69	4.68	2.22
2 months after	6.07	1.33	6.01	1.39
DLK-1 (ng/mL)				
Baseline	0.24	0.26	0.36	0.32
2 months	0.29	0.26	0.45	0.34
6 months	0.24	0.23	0.35	0.29
2 months after	0.38	0.26	0.51	0.38