Salivary SIgA responses to acute moderate-vigorous exercise in monophasic oral contraceptive users.

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Abstract

The purpose of this study was to examine the effect of oral contraceptive (OC) use on salivary secretory immunoglobulin A (SIgA) levels at rest and in response to an acute bout of moderate-vigorous exercise during two phases of the 4 week OC cycle corresponding to different phases of the synthetic menstrual cycle.

Ten healthy active females completed a cycling at 70% $\dot{V}O_{2peak}$ for 45 min at two time points of an OC cycle: during the equivalent in time to the mid-follicular phase (day 8 ± 2) and the mid-luteal phase (day 20 ± 2). Timed unstimulated saliva samples were obtained before, immediately post exercise and 1 h post exercise and analyzed for salivary SIgA.

Salivary SIgA secretion rate was 26 % (95% Confidence limits, CI, 6-46) lower at post-exercise compared with pre-exercise during the synthetic follicular phase ($p = 0.019$) but no differences were observed during the synthetic luteal trial. Saliva flow rate was 11% (95% CI, 8-30) lower at post-exercise compared with pre-exercise (main effect for time; $p = 0.025$).

In conclusion, the pattern of salivary SIgA secretion rate response to moderate-vigorous exercise varies across the early and late phases of a monophasic OC cycle, with a transient reduction in salivary SIgA responses during the synthetic follicular phase.

These findings indicate that monophasic OC use should be considered when assessing

mucosal immune responses to acute exercise.

INTRODUCTION

Secretory Immunoglobulin A (SIgA) is known as 'the first line of defence' against

pathogens and antigens presented at the mucosa. A number of exercise studies have

assessed salivary SIgA as a non-invasive marker of mucosal immunity with several

studies reporting an association between long-term intensive physical activity, transient

depressions of salivary SIgA and increased upper respiratory symptoms (URS, e.g.,

runny or stuffy nose, sore throat, headache, cough) (Fahlman and Engels 2005, Gleeson

et al. 2012, Neville et al. 2008). Fahlman and Engels (2005) demonstrated that salivary

SIgA concentration and salivary SIgA secretion rate were decreased across a 12-month

training period in American football players with salivary SIgA secretion rate found to

be predictive of URS incidence. Furthermore, athletes who reported recurrent symptoms

of URS had a higher training load and a lower salivary SIgA secretion rate than the

symptom-free counterparts (Gleeson et al. 2012). URS cluster around competitions and

detrimentally affect performance in elite swimmers (Pyne et al. 2001).

A recent study suggests that female athletes may be more susceptible than their male

counterparts to URS (He et al. 2014). A lower level of oral-respiratory mucosal

immunity may explain this as it is reported that females present a lower salivary SIgA

concentration and secretion rate compared with males (Gleeson et al. 2011, Allgrove et al. 2009). However, menstrual cycle and oral contraceptive (OC) use was not controlled for in these studies and a positive association between IgA levels in cervical mucosal secretions has been related to levels of both natural and synthetic oestrogens in naturally cycling and oral contraceptive users (Franklin and Kutteh 1999). Furthermore, oestrogen-mediated cross talk between mucosal and reproductive organs during secretory IgA antibody induction has been demonstrated (Cha et al. 2011). On the other hand, oral mucosal immunity may be less affected by menstrual phase than markers of systemic immune function, at least in non-OC users; salivary SIgA concentration and secretion rates are reported to be unaffected by menstrual cycle at rest (Burrows et al. 2002, Gillum et al. 2014) and after acute exercise (Gillum et al. 2014).

OCs modify endogenous sex hormonal status via the provision of exogenous synthetic oestrogens and progesterone to primarily prevent ovulation and result in more consistent circulating hormone levels. OC use reduces cycle-length variability and provides a consistent 28-day cycle (Burrows and Peters 2007); in this regard OCs provide females with an attractive option for controlling their menstrual cycle. Furthermore, reductions in premenstrual symptoms and menstrual blood loss, possible lowered risk of

musculoskeletal injury and increases in bone density in those with menstrual disturbances are additional potential benefits of OC use for female athletes (Bennell et al. 1999). In the general population, OC remain the most common form of primary contraceptive used in the United Kingdom (HSCIC 2013) and the United States (Guttmacher Institute 2013) and it is estimated that use among female athletes at least matches that of the general population (Bennell et al. 1999). The findings of a large study of the genito-urinary mucosal secretions of 69 OC users and 85 non-OC users suggests that both menstrual phase and OC use are significant determinants of cervical SIgA and SIgG levels, with higher levels of SIgA and SIgG in OC users at the time in the cycle equivalent to the follicular phase (the 'synthetic' follicular phase) compared with the synthetic luteal phase (Safaeian et al. 2009). This is despite a constant amount of synthetic oestrogen being ingested during those times. However, although not as extreme as in non-OC users, these times in the synthetic menstrual cycle also correspond to significant, albeit small, differences in levels of circulating oestrogen concentrations in OC users (Timmons et al. 2005). It is not known if OC have similar effects on salivary SIgA levels. Interestingly, reported incidences of the common cold were also associated with lower cervical SIgA levels (Safaeian et al. 2009).

The aim of the present study was therefore to determine the effect of OC use on salivary concentrations of 17β-estradiol, progesterone and salivary SIgA at rest and salivary SIgA responses to an acute bout of moderate-vigorous exercise during two phases of the 4 week OC cycle corresponding to different phases of the synthetic menstrual cycle.

MATERIALS AND METHODS

Participants

Ten healthy regularly active (but not specifically endurance trained) females (mean ± SD: age 20.8 ± 1.7 years; height 169.9 ± 4.5 cm; body mass 63.0 ± 6.7 kg; $\dot{V}O_{2peak}$ 43.3 ± 6.6 mL/kg/min; peak power output 224.0 ± 29.5 watts) volunteered to participate in the study. All participants had been regularly taking 21 day monophasic combined oral contraceptives (0.030-0.035 mg ethinyloestradiol and either 0.25 mg norgestimate, 0.5 mg norethisterone or 0.15 mg levonorgestrel) for at least 6 months prior to recruitment and had regular synthetic menstrual cycles.

All participants were fully informed (written and verbally) about the study and possible benefits and risks. Participants provided written consent to participate in the study which was approved by the Loughborough University Ethical Committee. At an initial

health screening, participants did not report taking any other medication or experiencing symptoms of infection within the previous two weeks. All participants were non-smokers.

Preliminary procedures

In a preliminary session, peak oxygen uptake ($\dot{V}O_{2peak}$) was estimated using a continuous incremental exercise test performed on an electromagnetically braked cycle ergometer (Lode Excalibur, Groningen, Netherlands). Participants began cycling at a work rate of 35 watts, with increments of 35 watts every 3 min, continuing until volitional fatigue. Expired gas samples were collected into Douglas bags during the third minute of each work rate increment and the final minute of the exercise test, and heart rates were measured continuously using short-range telemetry (Polar FS1, Polar Electro Oy, Kempele, Finland). The percentages of O_2 and CO_2 in the expired gas samples were measured using a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer (series 1400, Servomex, Crowborough, East Sussex, UK), respectively. These were used alongside a dry gas meter (Harvard Aparatus, Edenbridge, Kent, UK) to determine $\dot{V}E$, $\dot{V}O_2$, and $\dot{V}CO_2$. Gas analyzers were calibrated according to the manufacturer's instructions using a zero gas (100% N_2), a calibration mix (16% O_2 and

10

4% CO_2), and atmospheric air. The work rate equivalent to 70% $\dot{V}O_{2peak}$ was interpolated from the $\dot{V}O_2$ (L/min)-work rate (Watts) relationship.

Participants returned to the laboratory for a second occasion to undertake a familiarization period that consisted of cycling for 45 min at a work rate corresponding to 70% $\dot{V}O_{2peak}$. Heart rates were monitored during cycling, and 1-min expired gas samples were taken during the 15th, 30th and 45th min to ensure that the participants were exercising at the required intensity. If this was not the case, work rate was adjusted accordingly.

Main trials

Participants completed one experimental trial during the phase of the OC cycle that is equivalent in time to the mid-follicular phase (8 ± 2 days after start of menstruation) and one during the phase of the OC cycle that is equivalent in time to the mid-luteal phase (20 ± 2 days after start of menstruation). For simplicity, these will be subsequently referred to as the follicular phase and the luteal phase. To counterbalance the order in which women completed the experimental sessions, six started during the follicular phase and four during the luteal phase. This design required some women to complete

all testing within one menstrual cycle, and some required testing to carry over into their next cycle.

Participants were instructed to maintain their regular exercise routines throughout the study, but no strenuous exercise was allowed during 24 h before each main trial. In an effort to standardize their nutritional status, participants were asked to eat the same foods and drinks during the 24 h prior to both experimental trials. Each participant was also asked to refrain from caffeine and alcohol intake during 24 h before each main trial. After at least a 4 h fast, participants arrived at the laboratory at 11:45 h to ensure that timing of exercise and saliva collection was consistent across all participants and all main trials. Participants were not allowed to eat until trial was completed, but water was provided to maintain hydration during the trial.

Participants were required to empty their bladder before body mass was recorded. Subsequently, participants sat quietly for 10 min before an initial pre-exercise (baseline) saliva sample was collected into a pre-weighted sterile plastic containers. For this, participants rinsed their mouth with water, swallowed the whole fluid in their mouth and sat still with their head slightly tilted forward with minimal orofacial movement.

Collection time for each participant was designed to ensure a total saliva sample of between 0.5 and 1.5 mL. Following this, participants began cycling on an electromagnetically braked cycle ergometer at 70% $\dot{V}O_{2peak}$ for 45 min. Participants were allowed to consume water ad libitum during both main trials, apart from 5 min before each saliva sample collection. Heart rate and RPE were measured every 10 min throughout exercise. At 15, 30 and 44 min of exercise, 1-min expired gas samples were collected to determine $\dot{V}O_2$ and $\dot{V}CO_2$, to ensure that the participants were exercising at the correct intensity. Further unstimulated saliva collections were obtained at immediately post exercise and 1 h post exercise. After the cessation of exercise, body mass and volume of water consumed was recorded. Mean laboratory conditions were 22.1 ± 1.0℃ and 26 ± 6% relative humidity.

Saliva analysis

Following the saliva collection, samples were weighed to the nearest 10 mg. Saliva volume was estimated assuming saliva density to be 1.00 $g.mL^{-1}$ (Cole and Eastoe 1988) and saliva flow rate calculated from saliva volume and collection time. Samples were then centrifuged at 12,000 rpm for 2 min at room temperature, the cell free supernatant removed and stored at -20°C for later analysis. Salivary 17β-estradiol and

progesterone concentrations were measured using high sensitivity enzyme immunoassay (EIA) kits (Salimetrics, Newmarket, UK). Salivary SIgA concentration was measured using a sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, 96 well microplates (Nunc-Immunoplate, Thermo Fisher Scientific, UK) were coated with rabbit anti-human IgA capture antibody (Dako, Ely, UK), washed and blocked with 2% bovine serum albumin in PBS. Samples were diluted 1:2000 in PBS and analysed in duplicate against a standard of purified IgA from colostrum (Sigma-Aldrich, Dorset, UK) and incubated overnight 4ºC. Plates were washed before adding HRP-conjugated polyclonal rabbit anti human-IgA (Dako, Ely, UK) to all wells. Following a second incubation at room temperature for 90 min and a final wash, a colour substrate (OPD substrate, Dako, Ely, UK) was added, and the absorbance of the individual samples was subsequently determined at 490 nm. Salivary SIgA secretion rate was calculated by multiplying SIgA concentration with saliva flow rate. All samples from the same participant were analyzed in duplicate on a single microplate. The coefficient of variation of the methods on the basis of analyses of these duplicate samples was 2.2% for 17β-estradiol, 1.7% for progesterone and 2.8% for salivary SIgA.

Statistical analysis

A student's paired t-test was used to assess the differences in basal salivary hormone levels between the follicular and luteal phases. A two-way (trial x time) analysis of variance with repeated measures was used to examine the differences in salivary SIgA levels. When a main effect was observed, *post hoc* tests using Bonferroni adjustment was used for multiple comparisons. Statistical significance was set at $p < 0.05$. Effect sizes (ES) based on eta^2 are provided for all significant data to give an indication of the proportion of the variation attributable to trial or group, using Cohen's definition of eta^2 of 0.01, 0.06 and 0.14 representing 'small', 'medium' and 'large' effects, respectively (Cohen, 1988). Statistical analyses were performed using PASW Statistics 18 software (SPSS, Chicago, IL, USA). Data are expressed as mean ± SD.

RESULTS

Baseline salivary 17β-estradiol and progesterone concentrations in each synthetic phase are presented in Table 1. The salivary 17β-estradiol level was significantly higher in the synthetic follicular phase compared with the synthetic luteal phase at baseline ($p = 0.029$, ES = 0.43) (Table 1). There were no significant differences in salivary progesterone levels (Table 1). Mean relative $\dot{V}O_{2peak}$ during exercise did not differ between the trials (follicular: 69.8 ± 4.4%, luteal: 69.7 ± 5.3%). Similarly, there were no

differences in heart rate (follicular: 159 ± 9 beats.min^{-1}, luteal: 162 ± 11 beats.min^{-1}), work rate (follicular: 138 ± 20 W, luteal: 138 ± 19 W) and RPE (follicular: 13 ± 2, luteal: 13 ± 1) between the trials. Body mass loss (corrected for fluid intake) was also similar between trials (follicular: 0.03 ± 0.30 kg, luteal: 0.06 ± 0.35 kg).

Saliva responses

Saliva flow rate was 11% (95% Confidence limits, CI, 8-30) lower at post-exercise compared with pre-exercise (main effect for time; $p = 0.025$, ES = 0.15; Fig. 1a), but there was no effect of trial or interaction effect. There was no significant trial x time interaction or main effects for trial or time evident for salivary SIgA concentration (Fig. 1b). However, there was a significant interaction between trial and time for salivary SIgA secretion rate (interaction; $p = 0.049$, ES = 0.19; Fig. 1c), with salivary SIgA secretion rate 26% (95% CI, 6-46) lower at post-exercise compared with pre-exercise values ($p = 0.019$) during the synthetic follicular phase. Values rose by just 7% (95% CI, -13-27) (not significantly different from pre-exercise; $p = 1.00$) following exercise in the luteal trial (Fig. 1c).

DISCUSSION

The principal finding of the present study was that the salivary SIgA secretion rate was significantly reduced by 45 min cycling at 70% $\dot{V}O_{2peak}$ in the synthetic follicular phase only, yet the salivary SIgA concentration was unaffected by both the phases of the OC cycle and exercise. We also report the salivary resting 17β-estradiol concentration was significantly lower in the synthetic luteal compared with the synthetic follicular phase but levels of progesterone were constant.

In the present study, there were no differences in resting saliva flow rates, salivary SIgA concentration and salivary SIgA secretion rates between the synthetic follicular and luteal phases of the OC cycle. Similar observations have been reported regarding salivary SIgA in naturally menstruating females who do not use OC (Burrows et al. 2002, Gillum et al. 2014). The findings of the present study are in contrast to those in cervical secretions (Safaeian et al. 2009), suggesting that the effect of OC on basal SIgA concentrations and secretion rates is local to the reproductive mucosa, whereas salivary SIgA production is not affected by physiological variations of ovarian hormones across a single OC cycle.

To our knowledge, the present study is the first to report differences in salivary SIgA

secretion rate between synthetic menstrual phases in active females who use

monophasic OC. In the synthetic follicular phase, salivary SIgA secretion rate fell by

26% (95% CI, 6-46) after performing 45 min moderate-vigorous intensity exercise but

there was no effect of exercise on salivary SIgA secretion rate in the synthetic luteal

phase. A limitation of the present study is that we did not assess the non-OC users as a

control group. However, in a similar protocol to the present study, Gillum et al. (2014)

found no influence of menstrual phase on salivary SIgA and other salivary

anti-microbial protein responses to a 45-min treadmill running at 75% $\dot{V}O_{2peak}$ in

non-OC users with a regular menstrual cycle. Exogenous ethinyl estradiol and

progestogen act to suppress endogenous estrogen and progesterone production in OC

users. The mean concentration of female hormones (salivary 17β-estradiol and

progesterone) from the current study was lower than that of previously reported non-OC

users (Gillum et al. 2014) and this may influence salivary SIgA responses to acute

exercise.

In the present study there was a significant (albeit small) difference in 17β-estradiol

concentration between the synthetic luteal and follicular phases, with values higher

during the synthetic follicular phase. Given that estrogens are typically reported to

increase antibody production from B cells (Oertelt-Prigione 2012) this is perhaps surprising. However, the kinetics of the acute effects of exercise on salivary SIgA responses are more likely related to the availability of the transmembrane polymeric Ig receptor (pIgR) necessary for transport of the IgA dimer across the glandular epithelial cell than altered B cell antibody production (Bishop and Gleeson 2009). Nevertheless, *in vitro* studies in humans suggest that estrogen augments IgA transcytosis via increased pIgR expression in a dose-response manner (Diebel et al. 2011). This would suggest a higher IgA secretion at times of higher estrogen concentrations, in contrast to the observations of the present study. However, the doses used in the study by Diebel et al. were in the μmol/l^{-1} range, much larger than that seen in the circulation in OC users.

Furthermore, cortisol is known to be higher in OC users (Timmons et al., 2005) and the presence of cortisol decreases expression of pIgR mRNA in rabbit mammary gland organ culture (Rosato et al. 1995). Although it is not clear if the same effect is present in humans, it could be speculated that an altered cortisol response to exercise between the two phases could have contributed to the altered salivary SIgA response to exercise observed here. It is acknowledged that further exploration of this theory is limited as cortisol levels were not determined in the present study.

The present study suggests that in order to assess the responses of SIgA to exercise in females OC use may need to be taken into account, particularly in view of the prevalence of OC use within the general population and in athlete populations (Bennell et al. 1999; Burrows and Peters 2007). In the recent findings from a cohort of 210 athletes that female athletes are more susceptible than males to URS, and 40% of the female participants were OC users (He et al. 2014). Low SIgA concentration or secretion rate has been identified as a risk factor for development of URS in physically active individuals (Fahlman and Engels 2005, Gleeson et al. 2012, Neville et al. 2008). The response of the SIgA (concentration and secretion rate) to maximal incremental swimming exercise was not influenced by menstrual cycle phase in female swimmers who were non-OC users (Morgado et al. 2014). In contrast, the difference of the SIgA secretion rate response was observed in the OC cycle in this study. Therefore, the findings of the study provide an initial indication of the potential importance for OC-using female exercisers, female sports performers and their coaches. If the acute moderate-vigorous intensity exercise is performed towards the mid follicular phase, that may cause transient reductions in mucosal immune protection after performing.

In conclusion, the pattern of salivary SIgA secretion rate response to moderate-vigorous exercise varies across the early and late phases of a monophasic OC cycle. This is associated with fluctuations in resting salivary oestrogen (17β-estradiol) concentrations. Although the reduction in salivary SIgA responses during the synthetic follicular phase was transient, it may represent a temporary impairment of mucosal immune defence associated with monophasic OC use.

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Table 1. The concentration of the resting salivary female hormones during the synthetic

follicular and luteal phases of the OC cycle.

	Synthetic Follicular Phase	Synthetic Luteal phase
17β-estradiol (pg.ml^{-1})	2.73 (0.52)	2.38 (0.43) *
Progesterone (pg.ml^{-1})	77.1 (35.6)	83.6 (59.6)

n=10. Values are mean ± SD.

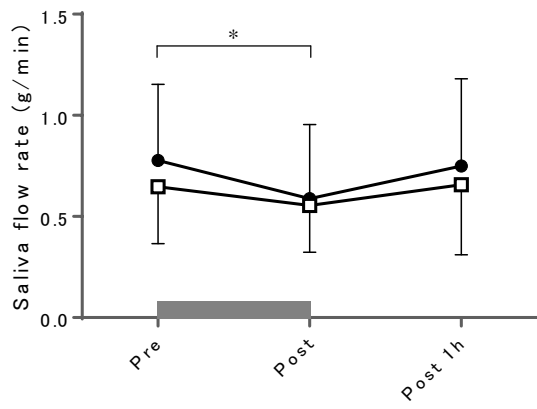* Significantly different between synthetic follicular and luteal phases at

$p = 0.029$

Figure 1. Effect of 45 min cycling at 70% $\dot{V}O_{2peak}$ on a) saliva flow, b) salivary SIgA concentration and c) salivary SIgA secretion rate during the synthetic follicular phase (●) and luteal phase (□) of the OC cycle. Values are mean ± SD (n=10).

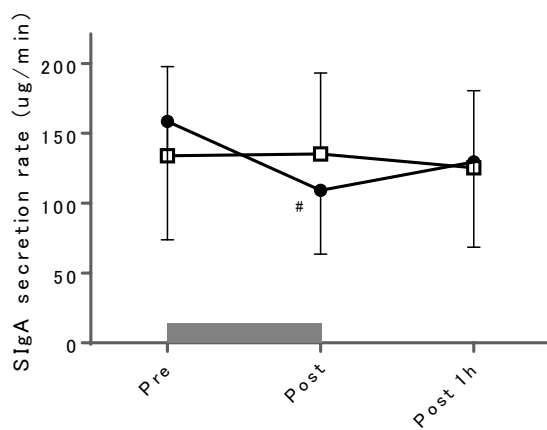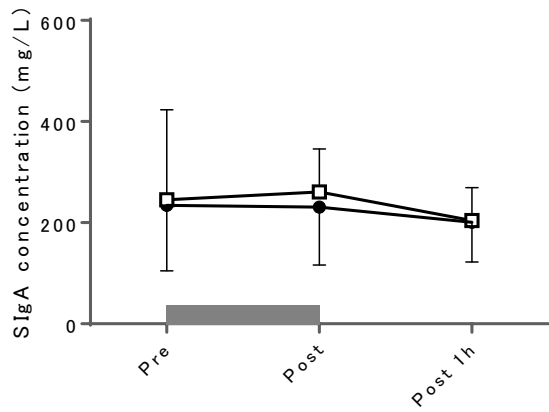* Main effect of time compared to pre-exercise, $p = 0.048$.

[#] A significant trial x time interaction, $p = 0.049$. SIgA secretion rate was decreased at post-exercise compared with pre-exercise in the synthetic follicular phase only.

a)

b)

c)