



Oral contraceptives do not appear to affect cystic fibrosis disease severity

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ABSTRACT: Several studies suggest that sex may affect cystic fibrosis (CF) disease severity, with females with CF being more severely affected. In this context, it has been suggested that sex hormones may influence the CF phenotype. A large proportion of females with CF regularly use oral contraceptives (OCs), but the effect of their use on disease severity is unclear. Here, we retrospectively assessed the effects of OCs on clinical outcomes in females with CF.

Data from 681 females were available, of whom 42% had taken OCs for varying periods of time. We first performed an inter-patient analysis comparing annual change in % predicted forced expiratory volume in 1 s, body mass index and total days of intravenous antibiotic use over a 5-yr study period in 57 females exposed to and 57 females not exposed to OCs. There were no differences between the two groups. We next performed an intra-patient analysis of the same outcomes over a 3-yr period of OC exposure and a 3-yr period of no OC exposure in the same patient (exposure followed by non-exposure, n=27; non-exposure followed by exposure, n=23), but again did not detect any differences in any of the clinical outcomes.

Our data suggests that the use of OCs does not affect CF disease severity.

KEYWORDS: Cystic fibrosis transmembrane conductance regulator, hormones, lung disease, oestrogen

Pulmonary disease progression in cystic fibrosis (CF) is heterogeneous. This may in part be related to the different classes of cystic fibrosis transmembrane conductance regulator (*CFTR*) mutations having different effects on protein function [1]. However, even patients with the same mutation frequently differ with respect to pulmonary disease progression. It has been shown that socio-economic status, adherence and access to treatment as well as modifier genes contribute to the progression of CF lung disease [2]. In addition, it has been suggested that sex may have an effect on CF mortality, with females dying earlier than males [3–5], and on morbidity, since it has been suggested that females with CF acquire chronic *Pseudomonas aeruginosa* infection earlier and that this is linked to an accelerated decline of pulmonary function [6, 7]. VERMA *et al.* [8] suggest that the sex gap in childhood and adolescence closes when both sexes receive standardised aggressive treatments in a single centre.

It has been suggested that sex hormones may directly influence the CF phenotype by altering ion transport in epithelial cells. These effects have been investigated *in vivo* by SWEZEY *et al.* [9], who showed that the amiloride-insensitive component of nasal potential difference (NPD) varies during

the menstrual cycle in CF females. During the luteal (high progesterone) phase, amiloride-insensitive NPD was significantly higher than during the follicular (low progesterone) phase. This suggests that elevated progesterone during the luteal phase may alter ion transport across the respiratory epithelium, which may alter the height of the periciliary liquid and, therefore, mucociliary clearance.

In vitro studies assessing the effects of sex hormones on ion transport have produced conflicting results, with some studies reporting inhibition of chloride transport [10, 11] and others showing increased *CFTR* transcription after sex hormone exposure [12–14]. FANELLI *et al.* [15] observed an increase in the processing of misfolded *CFTR* leading to improved ion transport in a CF human bronchiolar epithelial cell line, and proposed that oestrogens rescue misfolded $\Delta F508$ *CFTR* from proteosomal degradation, thereby increasing the amount of functional *CFTR* at the cell membrane. In addition to direct effects on ion transport and *CFTR* expression and maturation, CHOTIRMALL *et al.* [16] have recently shown that oestrogens inhibit interleukin (IL)-8 secretion *via* upregulation of the secretory leukoprotease inhibitor (SLPI) in an immortalised CF cell line. This

AFFILIATIONS

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pathway may directly affect the host response during high-oestrogen phases of the menstrual cycle. Sex hormones have also been implicated in regulating immune and inflammatory responses in various pre-clinical and clinical models [17].

Combined, these results imply that sex hormones may have an effect on CF lung disease. Only one short-term study of 12 females assessed lung function in relation to sex hormone levels, showing that lung function (as measured by forced expiratory volume in 1 s (FEV₁)) changed in relation to the menstrual cycle. FEV₁ was significantly higher during the luteal phase (high levels of oestrogen and progesterone) compared with ovulation (high oestrogen and low progesterone) and menstruation (low oestrogen and progesterone) [18].

As the median age of survival increases, more females with CF reach child-bearing age and use oral contraceptives (OCs) [19, 20]. The use of OCs by females with CF provides a clinical model in which to test the hypothesis that exogenous oestrogen and/or progesterone may have a significant clinical effect on the course of the disease. Here, we performed a retrospective inter- and intra-patient comparison study to assess whether OC use affects disease severity in CF as measured by annual change in pulmonary function (% predicted FEV₁), body mass index (BMI) and the need for intravenous antibiotics.

METHODS AND MATERIALS

Database

We used information collected at annual review, during a period of clinical stability, for patients under the care of the Adult Cystic Fibrosis Unit of Royal Brompton Hospital, London, UK. Information on the use of OCs was available for the years 1981–2010 and recorded in each year as “yes” or “no”, or as “unknown” if the information was missing. Females were excluded from analysis if they had unknown OC use for all years of follow-up available in the database. We also extracted annual information on FEV₁ % pred, BMI and *i.v.* antibiotic use over the previous year (total days of treatment). Data was taken from the Royal Brompton and Harefield Trust CF database. Ethics committee (Royal Brompton, Harefield and NHLI Research Ethics Committee) approval has been granted and all patients on the database gave signed consent for anonymous data to be used for research purposes.

Inter-patient cohort comparison

We identified all females who had used OCs for a minimum of 4 out of 5 yrs during a continuous 5-yr period. Females in this “exposed” cohort were matched by age to a female who, during the same 5-yr period, had never used OCs (“not exposed”). Information on FEV₁ % pred, BMI and *i.v.* antibiotic use was extracted for each year of the relevant 5-yr period; annual changes in FEV₁ % pred and BMI were estimated using linear regression. Days of *i.v.* antibiotic use during each period were summed. To confirm that the two cohorts were similar at the start of analysis we compared FEV₁ % pred, BMI and days on *i.v.* antibiotics at the start of analysis.

Intra-patient comparison

In addition to the inter-patient comparison, we also decided *a priori* to perform an intra-patient comparison. We identified females (n=27) who used OCs for three consecutive years (“exposed” period) followed by 3 yrs of non-use (“not exposed” period); and, conversely and separately, females who did not take OCs for three consecutive years followed by 3 years of continuous use (n=23). As above, annual changes in FEV₁ % pred and BMI were estimated for each exposed and not exposed period using linear regression, and days of *i.v.* antibiotic use during each period were summed. Data obtained for the exposed and not exposed period were then compared for each female.

Statistical analysis

Statistical analysis was performed using the GraphPad PRISM 4.0 software package (GraphPad Software Inc., San Diego, CA, USA). After assessment of normal distribution, parametric data were analysed using an unpaired or paired t-test, as appropriate. Non-parametric data were analysed using the Mann-Whitney U-test and the Wilcoxon matched pairs test for unpaired data and paired data, respectively. The null hypothesis was rejected at p<0.05.

RESULTS

Patient demographics

In the period from 1981 to 2010, 42% of females on the database (n=286 females aged 16–49 yrs) reported use of OCs on at least one annual review. 43% (n=290) had never used OCs and 15% (n=104) had never responded either “yes” or “no” and were

TABLE 1 Comparison of age, forced expiratory volume in 1 s (FEV₁) body mass index (BMI) and days on *i.v.* antibiotics in females with cystic fibrosis (CF) using oral contraceptives (OCs) (“exposed” cohort) and in females with CF not using OCs (“not exposed” cohort) in the first year of analysis

Parameter	All females	Exposed cohort	Not exposed cohort	p-value [#]
Age yrs	23 (16–45)	23 (16–45)	22 (17–44)	0.995
Subjects	112	56	56	
FEV₁ % pred	52.5 (12.8–119.6)	56.2 (20.4–111.1)	48.4 (12.8–119.6)	0.73
Subjects	110	55	55	
BMI kg·m⁻²	20.1 (15.0–28.2)	20.1 (15.2–28.2)	20.1 (15.0–26.1)	0.98
Subjects	110	55	55	
Days on <i>i.v.</i> antibiotics	0 (0–126)	0 (0–70)	7 (0–126)	0.38
Subjects	108	54	54	

Data are presented as median (range) or n, unless otherwise stated. % pred: % predicted. [#]: p-values relate to comparisons between females exposed and not exposed to OCs.

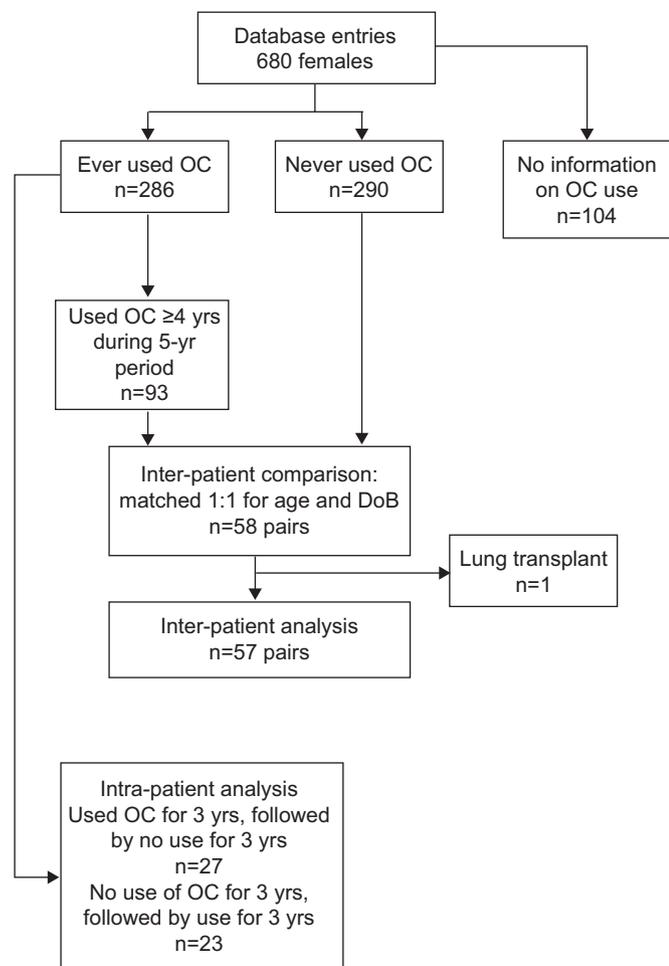


FIGURE 1. Flow chart of patient selection for “exposed” and “not exposed” cohorts for inter- and intra-patient comparison. OC: oral contraceptive; DoB: date of birth.

therefore excluded from subsequent consideration (table 1). Years of OC use ranged from 1 (n=101) to 21 yrs (n=1), but ~33% (n=93) of females had continuously used OCs for 4–5 yrs. This frequency dropped to ~10% (n=31) for females who had continuously used OCs for 6–7 yrs.

Inter-patient cohort comparison

Females were assigned to the exposed and not exposed cohorts as outlined in figure 1. 93 were identified for the exposed cohort; the database was then screened for “matching” females who had never taken OCs (not exposed cohort) and 57 pairs were identified. However, incomplete data for the study period led to the subsequent exclusion of a further two (for FEV1 % pred and BMI analysis) or three (analysis of *i.v.* antibiotic use) matched pairs.

Comparison of subjects in the exposed and non-exposed groups at the start of the analysis period (year 1) showed that they were of similar age. Females who had not taken OCs had a lower median FEV1 % pred and more days on *i.v.* antibiotics, but this did not reach significance (table 1).

There were no differences between the two cohorts in annual change in FEV1 % pred during the period of analysis (n=55 per group; exposed, -1.87% (-11.5–10.4%); not exposed, -1.03%

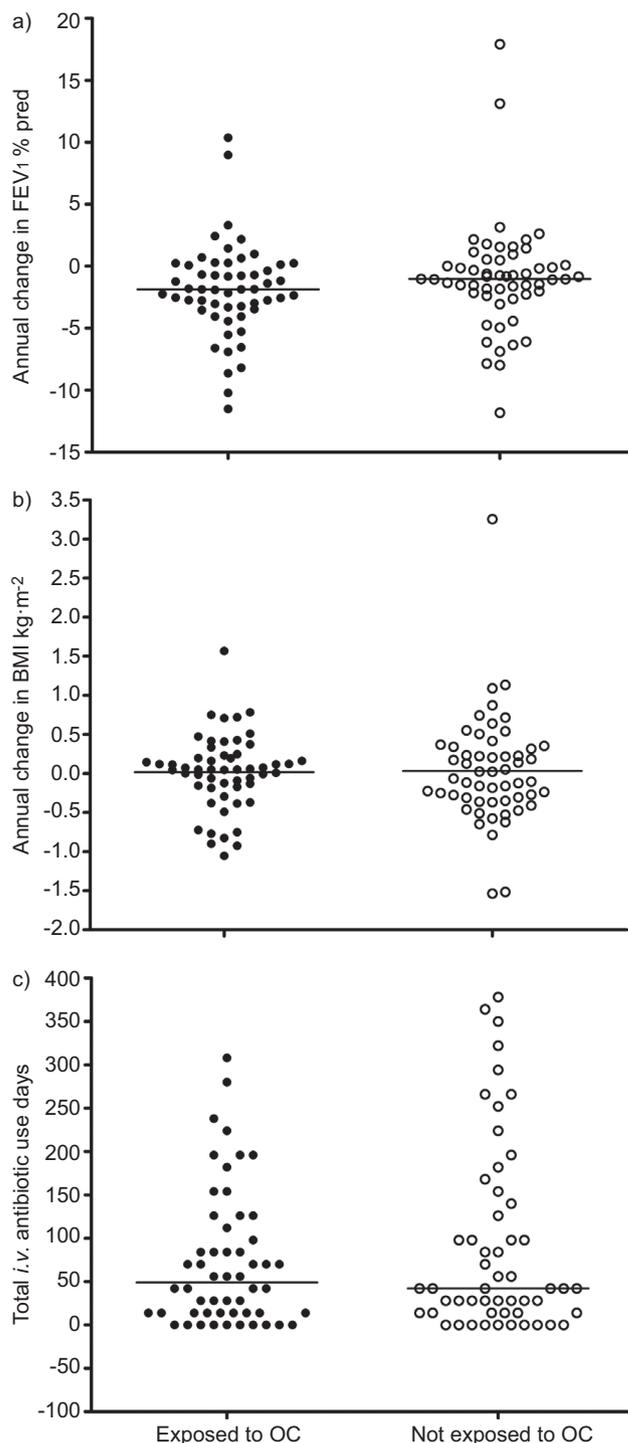


FIGURE 2. Comparison of disease severity during the 5-yr study period in females with cystic fibrosis (CF) using oral contraceptives (OCs) (“exposed” cohort) and females with CF not using OCs (“not exposed” cohort). a) Annual change in forced expiratory volume in 1 s (FEV1) % predicted (% pred); b) annual change in body mass index (BMI); c) total number of days of *i.v.* antibiotic use during the 5-yr study period. Each symbol represents one individual. Parametric and non-parametric statistical analysis was performed as appropriate. Horizontal bars indicate group mean (b) or median (a and c) as appropriate for parametric or non-parametric data.

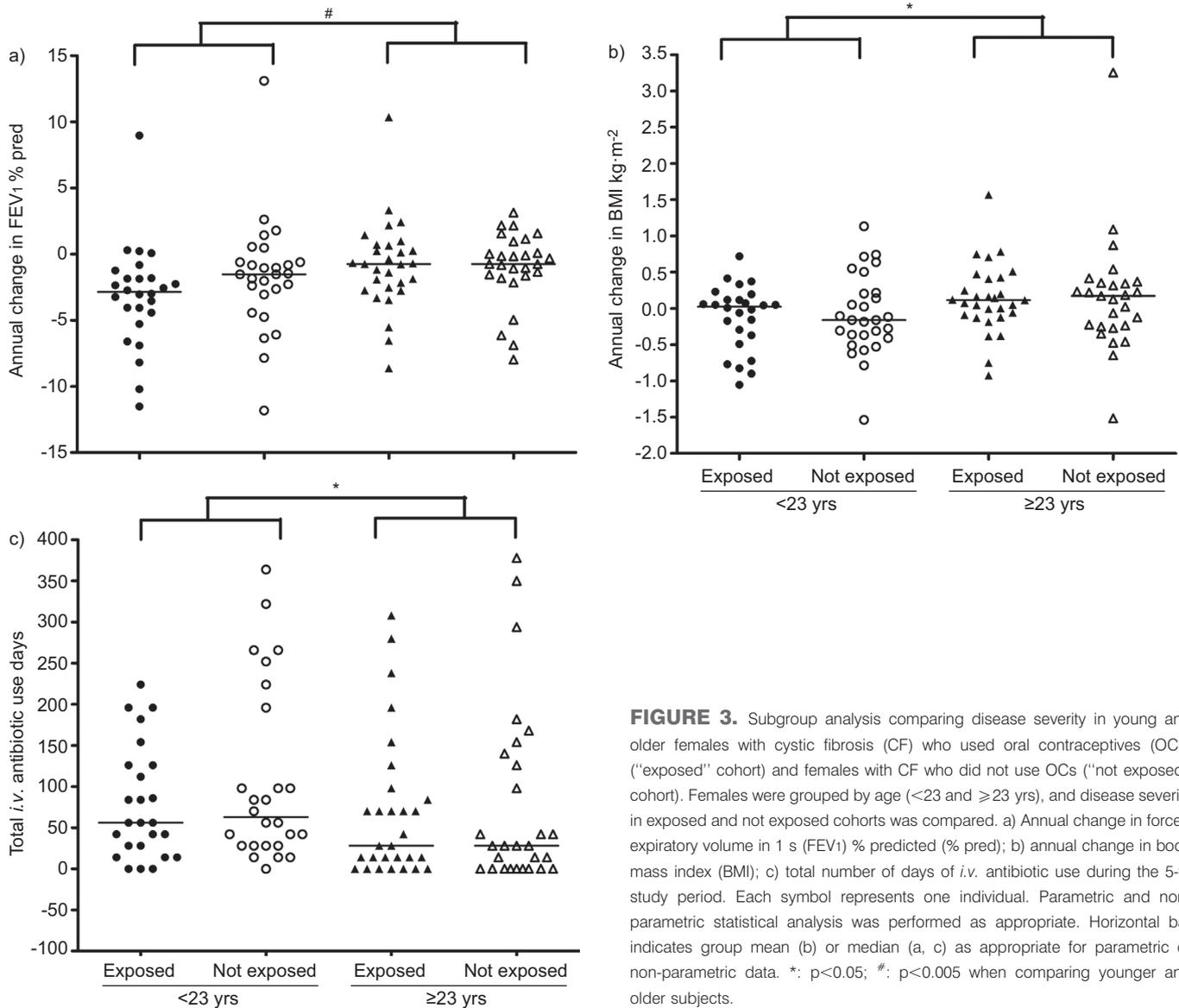


FIGURE 3. Subgroup analysis comparing disease severity in young and older females with cystic fibrosis (CF) who used oral contraceptives (OCs) (“exposed” cohort) and females with CF who did not use OCs (“not exposed” cohort). Females were grouped by age (<23 yrs and ≥23 yrs), and disease severity in exposed and not exposed cohorts was compared. a) Annual change in forced expiratory volume in 1 s (FEV₁) % predicted (% pred); b) annual change in body mass index (BMI); c) total number of days of *i.v.* antibiotic use during the 5-yr study period. Each symbol represents one individual. Parametric and non-parametric statistical analysis was performed as appropriate. Horizontal bar indicates group mean (b) or median (a, c) as appropriate for parametric or non-parametric data. *: p<0.05; #: p<0.005 when comparing younger and older subjects.

(-11.9–17.9%); p=0.115) (fig. 2a); in annual change in BMI (n=55 per group; exposed 0.05 (-1.05–1.57) kg·m⁻²; not exposed, -0.07 (-1.54–3.25) kg·m⁻²; p=0.891) (fig. 2b) or in total days of *i.v.* antibiotics (n=54 per group; exposed, 49 (0–308) days; not exposed, 42 (0–378) days; p=0.685) (fig. 2c).

Subgroup analysis using less than or greater than or equal to the median age of 23 yrs was also performed. Within each age group there was no difference in annual change in FEV₁ % pred (<23 yrs, p=0.062; ≥23 yrs, p=0.762), BMI (<23 yrs, p=0.869; ≥23 yrs, p=0.876) or total days of *i.v.* antibiotics (<23 yrs p=0.468; ≥23 yrs p=0.860) (fig. 3a–c and table 2). When all subjects <23 yrs of age at the start of analysis were compared to all subjects ≥23 yrs of age at the start of analysis there was a significant difference between the groups for all three parameters during the 5-yr period (annual change in FEV₁ % pred, p<0.005; annual change in BMI, p<0.05; total days of *i.v.* antibiotics, p<0.05) (fig. 3a–c and table 2), indicating that younger females with CF have a steeper rate of decline in FEV₁ and BMI, and a greater *i.v.* antibiotic usage than older females.

Intra-patient cohort comparison

We next assessed annual change in FEV₁ % pred and BMI and total days on *i.v.* antibiotics over a period of OC use (exposed period) and a period of no OC use (not exposed period) in the same female (fig. 1). There were no differences in any of the parameters when comparing periods of OC use to periods of no OC use regardless of order (table 3).

DISCUSSION

The literature assessing the effects of sex hormones on ion transport in airway epithelial cells is conflicting, and studies proposing potential beneficial and detrimental effects of oestrogen on ion transport in CF have been published. Here, we conducted a retrospective study aimed at assessing the effect of OC use in females with CF. Our data suggest that OC use does not affect CF disease severity.

As the median age of survival increases, more females with CF reach child-bearing age and use OCs [19, 20]. In our centre, ~43% of females with CF reported OC use at least once when

TABLE 2

Inter-patient comparison of annual change in forced expiratory volume in 1 s (FEV₁) and body mass index (BMI), and total days on *i.v.* antibiotics over a 5-yr period in females with cystic fibrosis aged <23 yrs and ≥23 yrs who were using oral contraceptives (OCs) ("exposed" cohort) and not using OCs ("not exposed" cohort)

Patients compared	Subjects	Annual change in FEV ₁ % pred	p-value*	Subjects	Annual change in BMI kg·m ⁻²	p-value*	Subjects	Total <i>i.v.</i> antibiotic use over 5 yrs days	p-value#
All									
<23 yrs	54	-2.25 (-11.81–13.1)	<0.005	54	-0.08 (-1.53–1.13)	<0.05	51	56 (0–364)	<0.05
≥23 yrs	56	-0.74 (-8.63–10.4)		56	-0.12 (-1.52–3.25)		57	28 (0–378)	
<23 yrs									
Exposed	26	-2.86 (-11.51–8.97)	0.062	26	-0.025 (-1.05–0.719)	0.869	25	56 (0–224)	0.468
Not exposed	27	-1.52 (-11.82–13.11)		28	-0.16 (-1.54–1.13)		26	63 (0–364)	
≥23 yrs									
Exposed	29	-0.74 (-8.63–10.36)	0.762	29	0.11 (-0.93–1.57)	0.876	29	28 (0–308)	0.86
Not exposed	27	-0.74 (-7.98–3.14)		27	0.17 (-1.52–3.25)		28	28 (0–378)	

Data are presented as n or median (range), unless otherwise stated. % pred: % predicted. #: exposed versus not exposed to OCs.

questioned during annual follow-up, a percentage similar to frequencies reported by CONWAY *et al.* [21]. However, the effects of OCs on disease severity have not been extensively studied. We are aware of only one comparatively small and short study, which assessed the effect of OCs on lung function. FITZPATRICK *et al.* [19] studied 12 females using OCs and reported that lung function did not decline over a 6-month period. Our study was conducted over 5 yrs of follow-up, which may provide a better opportunity to observe either detrimental or beneficial effects of OCs on CF disease. We observed a drop of ~1–2% pred in FEV₁ per year in our cohorts, which is consistent with most recently published data [22], although reported rates of decline vary (see later). Importantly, the decline in lung function in females using and not using OCs was similar. In addition, subgroup analysis in younger (<23 yrs) and older (≥23 yrs) females did not indicate an effect of OC use on disease severity.

Consistent with data collated from other registries [22], we have shown here that lung function in females with CF aged <23 yrs declines more rapidly than in older females. The reasons for the steeper decline in FEV₁ from early adolescence to early adulthood are poorly understood, but we show here that the younger population also has a lower BMI and an increased need for *i.v.* antibiotics. Importantly, we have shown that OC use does not alter the decline in lung function in this younger cohort.

The reported rate of decline in lung function (FEV₁ % pred) varies from study to study ([23] and references therein), and also appears to be birth-cohort dependent. Reported values range from 0.6% to 4% decline in FEV₁ per year. In addition, several studies have shown that anti-inflammatory drugs such as prednisone and ibuprofen improve, or slow, the decline of lung function over a 2–4-yr study period [24–26]. It is, therefore, reasonable to expect a significant positive or negative effect of OC use to become apparent over a 3–5-yr study period. Milder detrimental or protective effects of OCs would probably become more obvious the longer the period of continuous use of OCs. This, however, needs to be balanced against the inverse relationship between length of OC use and available patient numbers. For example, only ~10% (n=31) of CF females in our database had taken OCs for a continuous period of 6 or 7 yrs. The inter-patient cohort comparison had sufficient statistical power to detect a ~10% difference in the absolute value of FEV₁ % pred in females exposed and not exposed to OCs. Smaller effects of OCs on lung function may have been missed, but are unlikely to have been of clinical significance. For the inter-patient analysis, we matched females for year of birth and age to minimise birth cohort- and age-related disease severity effects. Furthermore, we assessed the effects of OC use on three key markers of CF disease severity; lung function as measured by FEV₁ % pred, BMI and *i.v.* antibiotic use. To assess if age at time of OC use is an important variable, we also performed a subgroup analysis in younger and older females, but again OCs did not appear to have an effect.

In addition to inter-patient analysis we also performed an intra-patient analysis, by comparing periods of OC use to subsequent periods of no OC use in the same patient and *vice versa*. Intra-patient analysis is less likely to be affected by confounding factors such as genetics, environment or medication. As noted above, we had to balance the need for prolonged use of OCs

TABLE 3 Intra-subject comparison of disease severity during adjacent 3-yr periods of oral contraceptive (OC) use ("exposed" period) and no OC use ("not exposed" period) in females with cystic fibrosis

Parameter	First 3-yr period			Second 3-yr period		
	Exposed period	Not exposed period	p-value [#]	Not exposed period	Exposed period	p-value [#]
Δ FEV1 % pred	-0.40 (-22.4–7.55) [†]	-2.00 (-16.70–4.85) [†]	0.265	-1.83 (-15.50–6.60) [§]	-2.35 (-9.30–6.65) [§]	0.426
Δ BMI kg·m ⁻²	-0.02 (-0.64–1.64) [†]	-0.02 (-1.4–1.54) [†]	0.316	-0.01 (-1.62–1.30) [‡]	-0.27 (-1.87–1.38) [‡]	0.618
Total <i>i.v.</i> antibiotics days	28 (0–84) ⁺	28 (0–168) ⁺	0.567	42 (0–140) [§]	56 (0–238) [§]	0.725

Data are presented as median (range), unless otherwise stated. Females with 3 yrs of OC use (exposed period) followed by 3 yrs of no OC use (not exposed period) or 3 yrs of no OC use (not exposed period) followed by 3 yrs of OC use (exposed period) were included in the analysis. Annual change (Δ) in % predicted forced expiratory volume in 1 s (FEV1 % pred), annual change in body mass index (BMI) and total number of days of intravenous antibiotics were compared for each 3-yr study period.

[#]: p-values indicate comparison between values for age of females; [†]: n=27; [‡]: n=25; [§]: n=23; ^{||}: n=24.

against available patient numbers and, therefore, restricted the intra-patient analysis to 3-yr periods of OC/no OC use.

The lack of effect in our study is in contrast to previous studies in asthma where oestrogens have been shown to affect disease severity in female asthmatics. During low-oestrogen phases of the menstrual cycle hospitalisation and exacerbation rates appear to be increased and lung function reduced [27–29]. In addition, OC use has reduced exacerbation rates and improved lung function in some asthmatic females [17].

Similar to other retrospective studies, our study has a number of limitations. 1) We have no information about the type of OC used. OCs have been available in the UK since 1960, with the original pill containing oestrogen and progesterone [30]. However, more recently, progesterone-only "mini pills" have become widely available, although combination pills are still in use. *In vitro* studies have shown that progesterone can also impair chloride ion transport [31] or increase *CFTR* expression [14]. 2) Females with CF routinely require antibiotics and it has been well described that antibiotics can impair the effectiveness of OCs by reducing circulating oestradiol levels [32]. We do not have pharmacokinetic data assessing hormone concentrations in our patient population and, therefore, cannot completely exclude the possibility that circulating oestrogen and/or progesterone levels may have been affected by simultaneous antibiotic intake and may have been too low to affect ion transport properties. However, to the best of our knowledge, the rate of unplanned pregnancies in females with CF is not higher than in the general population, which implies that circulating hormone levels are not significantly reduced by the antibiotics. 3) We have no information as to why females were using OCs. Avoidance of pregnancy is the most likely explanation, but prevention of irregular menstruation may also be a reason. Females may be using OCs because they are very well and want to avoid pregnancy or they make take OCs because their health has declined and would, therefore, not be able to cope with pregnancy. It is conceivable that these scenarios may have led to significant differences in disease severity between the two cohorts. However, we assessed stratification of the cohorts at the start of the study period and showed that there were no differences in disease severity as measured by FEV1 % pred, BMI and use of *i.v.* antibiotics. 4) We carefully matched cohort subjects for year of birth and time period and era of OC use or

non-use, but did not match for other variables that may influence disease, such as age of first *P. aeruginosa* isolate and treatments during the study period, which would have further reduced numbers. 5) Missing data or the lack of appropriate control subjects can be a problem in retrospective studies; we had to exclude 15% of females because we did not have any information related to OC use or because appropriate controls could not be identified. However, we think it improbable that any missing or erroneous information on the database would be systematically related to our study hypothesis and, thus, is unlikely to have produced any bias in our findings. 6) The study is based on data collected in a single centre and reproducibility of the results should ideally be assessed nationwide. However, current UK and US registry data do not record this. We are currently assessing whether questions related to types of OC used and reasons for OC use could be usefully added to the annual review questionnaire, to allow us to collect longitudinal data, which may help to reinforce the conclusions drawn in this study.

In summary, OC use did not affect FEV1 % pred, BMI or the need for *i.v.* antibiotics. These findings, therefore, suggest that the use of OCs does not affect CF disease severity.

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STATEMENT OF INTEREST

None declared.

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