Oral contraceptives do not appear to affect cystic fibrosis disease severity

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ABSTRACT

Several studies suggest that gender may affect cystic fibrosis (CF) disease severity, with women 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 women with CF regularly use oral contraceptives (OC), but the effect of their use on disease severity is unclear. Here, we retrospectively assessed the effects of OC on clinical outcomes in women with CF.

Data from 681 women were available of whom 42% had taken OC for varying periods of time. We first performed an _inter-patient_ analysis comparing annual change in %predicted FEV1, body mass index (BMI) and total days of intravenous (IV) antibiotic use over a five year study period in 57 women exposed to and 57 women not exposed to OC. There were no differences between the two groups. We next performed an _intra-patient_ analysis of the same outcomes over a three year period of OC exposure and a three year period of no OC exposure in the same patient (n=23-27), but again did not detect any differences in any of the clinical outcomes.

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

INTRODUCTION

Pulmonary disease progression in cystic fibrosis (CF) is heterogeneous. This may in part be related to the different classes of *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 progression of CF lung disease.[2] In addition it has been suggested that gender may have an effect on CF mortality with women dying earlier than males;[3-5] and on morbidity since it has been suggested that women 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* suggest that the gender-gap in childhood and adolescence closes when both sexes receive standardised aggressive treatments in a single centre.[8]

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 Sweezey *et al* who showed that the amiloride-insensitive component of nasal potential difference (NPD) varies during the menstrual cycle in CF females.[9] During the luteal phase (high progesterone) amiloride-insensitive NPD was significantly higher than during the follicular phase (low progesterone). 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* observed an increase in processing of misfolded CFTR leading to improved ion transport

in a CF human bronchiolar epithelial cell line and proposed that oestrogens rescue misfolded ΔF508 CFTR from proteosomal degradation, thereby increasing the amount of functional CFTR at the cell membrane.[15] In addition to direct effects on ion transport and CFTR expression and maturation, Chotirmall *et al* have recently shown that oestrogens inhibit IL8 secretion via up-regulation of the secretory leucoprotease inhibitor (SLPI) in an immortalised CF cell line.[16] This 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 women assessed lung function in relation to sex hormone levels showing that lung function (as measured by forced expiratory volume in 1 second (FEV_1)) changed in relation to the menstrual cycle. FEV_1 was significantly higher during the luteal phase (high levels of oestrogen and progesterone) compared to ovulation (high oestrogen and low progesterone) and menstruation (low oestrogen and progesterone) .[18]

As the median age of survival increases, more women with CF reach child bearing age and use oral contraceptives (OC). [19,20] The use of OC by women 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 if OC use affects disease severity in CF as measured by annual change in pulmonary function (%FEV_1), Body Mass Index 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. Information on the use of OC was available for the years 1981-2010 and recorded in each year as "Yes", or "No" or as "Unknown" if the information was missing. Women 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 percent predicted FEV_1 (%FEV_1), Body Mass Index (BMI) and intravenous (IV) antibiotic use over the previous year (total days of treatment). Data was taken from the Royal Brompton and Harefield Trust CF database . Ethics Committee approval has been granted and all patients on the database have signed consent for anonymous data to be used for research purposes.

Inter-patient cohort comparison

We identified all women who had used OC for a minimum of four out of five years during a continuous five year period. Women in this 'exposed' cohort were matched by age to a woman who, during the same five year period, had never used OC ('not exposed'). Information on %FEV_1, BMI and IV antibiotic use was extracted for each year of the relevant five year period; annual changes in %FEV_1 and BMI were estimated using linear regression. Days of IV antibiotic use during each period were summed. To confirm that the 2 cohorts were similar at the start of analysis we compared %FEV_1, BMI and days on IV 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 women (n=27) who used OC for three consecutive years ("exposed" period) followed by three years of non-use ("not exposed" period); and, conversely and separately, women who did not take OC for three consecutive years followed by three years of continuous use (n=23). As above, annual changes in %FEV_1 and BMI were estimated for each "exposed" and "not exposed" period using linear regression and days of IV antibiotic use during each period were summed. Data obtained for the "exposed" and "not exposed" period were then compared for each woman.

Statistical analysis

Statistical analysis was performed using the GraphPad PRISM 4.0 software package (San Diego, CA). After assessment of normal distribution, parametric data were analysed using an unpaired or paired Student 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 women on the database (n=286 women between 16-49 years old) reported use of OC on at least one annual review. Forty three percent (n=290) had never used OC and 15% (n=104) had never responded either 'Yes' or 'No' and were therefore excluded from subsequent consideration (see **Table 1** for basic patient demographics). Years of OC use ranged from 1 (n=101) to 21 years (n=1), but approximately 33% (n=93) of women had continuously used OC for 4 to 5 years. This frequency dropped to ~10% (n=31) for women who had continuously used OC for 6 to 7 years.

Table 1: Comparison of age, %FEV$_1$, BMI and days on intravenous antibiotics in women with CF using oral contraceptives ("exposed" cohort) and in women with CF not using OC ("not exposed" cohort) in the first year of analysis

Parameter	All females	'Exposed' cohort	'Not exposed' cohort	p-value
Age in years	23 (16-45)	23 (16-45)	22 (17-44)	0.995
n	112	56	56	
% FEV$_1$	52.5 (12.8-119.6)	56.2 (20.4-111.1)	48.4 (12.8-119.6)	0.73
n	110	55	55	
BMI	20.1 (15.0-28.2)	20.1 (15.2-28.2)	20.1 (15.0-26.1)	0.98
n	110	55	55	
Days on IV Antibiotics	0 (0-126)	0 (0-70)	7 (0-126)	0.38
n	108	54	54	

%FEV$_1$ = percentage predicted forced expiratory volume in one second, BMI=body mass index. IV=intravenous. Median and range are shown. p values relate to comparisons between women exposed and not exposed to OC. There were no significant differences.

Inter-patient cohort comparison

Women were assigned to the "exposed" and "not exposed" cohorts as outlined in **Figure 1.** Ninety three were identified for the "exposed" cohort; the database was then screened for "matching" women who had never taken OC ("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 %FEV$_1$ and BMI analysis) or three (analysis of IV 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. Women who had not taken OC had a lower median %FEV$_1$ and more days on IV antibiotics, but this did not reach significance (**Table 1**).

There were no differences between the two cohorts in annual change in %FEV$_1$ during the period of analysis ('exposed' = -1.87 Δ%FEV$_1$ (range: -11.5 to 10.4); 'not exposed' = -1.03 Δ%FEV$_1$ (range: -11.9 to 17.9), p=0.115, n=55/group) (**Figure 2A**), in annual change in BMI ('exposed' = 0.05 ΔBMI (range: -1.05 to 1.57); 'not exposed'= -0.07 ΔBMI (range: -1.54 to 3.25), p=0.891, n=55/group) (**Figure 2B**) or in total days of IV antibiotics ('exposed' = 49 days (range: 0 – 308); 'not exposed'= 42 days (range: 0 – 378), p=0.685, n=54/group) (**Figure 2C**).

Subgroup analysis using < or ≥ the median age of 23 years was also performed. Within each age group there was no difference in annual change in %FEV$_1$ (<23: p=0.062, ≥23: n=27, p=0.762), BMI (<23: p=0.869, ≥23: p=0.876), or total days of IV antibiotics (<23: p=0.468, ≥23: p=0.860) (**Figure 3A-C and Table 2**). When all subject younger than 23 at the start of analysis were compared to all subjects equal to or older than 23 at the start of analysis there

was a significant difference between the groups for all three parameters during the five year period (annual change in %FEV$_{1:}$ p <0.005, annual change in BMI: p <0.05, total days of IV antibiotics: p <0.05) (**Figure 3A-C and Table 2**) indicating that younger women with CF have a steeper rate of decline in FEV$_1$ and BMI, and a greater IV antibiotic usage than older women.

Table 2: Inter-patient comparison of annual change in %FEV$_1$ and BMI, and total days on intravenous antibiotics over a 5 year period in women with CF younger than 23 years and 23 years or older using oral contraceptives ("exposed" cohort) and not using OC ("not exposed" cohort)

Age in years	Group	n	Annual median (range) change in %FEV$_1$	n	Annual median (range) change in BMI: units	n	Median (range) intravenous antibiotic use over 5 years (days)
<23	all	54	-2.25 (-11.81-13.1)	54	-0.08 (-1.53-1.13)	51	56 (0-364)
≥23	all	56	-0.74 (-8.63-10.4)	56	-0.12 (-1.52-3.25)	57	28 (0-378)
	p		<0.005		<0.05		<0.05
<23	'exposed'	26	-2.86 (-11.51-8.97)	26	-0.025 (-1.05-0.719)	25	56 (0-224)
<23	'unexposed'	27	-1.52 (-11.82-13.11)	28	-0.16 (-1.54-1.13)	26	63 (0-364)
	p		0.062		0.869		0.468
≥23	'exposed'	29	-0.74 (-8.63-10.36)	29	0.11 (-0.93-1.57)	29	28 (0-308)
≥23	'unexposed'	27	-0.74 (-7.98-3.14)	27	0.17 (-1.52-3.25)	28	28 (0-378)
	p		0.762		0.876		0.86

Δ%FEV$_1$ = annual change in percentage predicted forced expiratory volume in one second, ΔBMI=annual change in body mass index. IV=intravenous. Median and range are shown. p values relate to comparisons between women exposed and not exposed to OC

Intra-patient cohort comparison

We next assessed annual change in $\%FEV_1$ and BMI and total days on IV antibiotics over a period of OC use ("exposed" period) and a period of no OC use ("not exposed" period) in the same woman (see **Figure 1** for selection criteria). 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**).

Table 3: Intra-subject comparison of disease severity during adjacent 3 year periods of oral contraceptive (OC) use ("exposed" period) and no OC use ("not exposed" period). Women with 3 years of OC use ("exposed" period) followed by 3 years of no OC ("not exposed" period) or 3 years of no OC use ("not exposed" period) followed by 3 years of OC use ("exposed" period) were included in the analysis. Annual change (Δ) in percentage predicted forced expiratory volume in 1 second ($\%FEV_1$), annual change in body mass index (BMI) and total number of days of IV antibiotic were compared for each 3 year study period. Median and range are shown. p values indicate comparison between values for age of women. There were no significant differences between the groups.

Parameter	Exposed Period	Not Exposed Period	p-value
$\Delta\% FEV_1$	-0.40 (-22.4 to 7.55)	-2.00 (-16.70 to 4.85)	0.265
n	27	27	
ΔBMI	-0.02 (-0.64 to 1.64)	-0.02 (-1.4 to 1.54)	0.316
n	27	27	
Days on IV Antibiotics	28 (0 to 84)	28 (0 to 168)	0.567
n	25	25	

Parameter	Not Exposed Period	Exposed Period	p-value
$\Delta\% FEV_1$	-1.83 (-15.50 to 6.60)	-2.35 (-9.30 to 6.65)	0.426
n	23	23	
ΔBMI	-0.01 (-1.62 to 1.30)	-0.27 (-1.87 to 1.38)	0.618
n	24	24	
Days on IV Antibiotics	42 (0 to 140)	56 (0 to 238)	0.725

n	23	23	

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 oral contraceptive (OC) use in woman with CF. Our data suggest that OC use does not affect CF disease severity.

As the median age of survival increases more women with CF reach child bearing age and use oral contraceptives. [19,20] In our centre approximately 43% of woman with CF reported OC use at least once when questioned during annual follow-up, a percentage similar to frequencies reported by Conway *et al*.[21] However, the effects of OC on disease severity have not been extensively studied. We are aware of only one comparatively small and short study which assessed the effect of OC on lung function. Fitzpatrick *et al* studied 12 women using OC and reported that lung function did not decline over a six month period.[19] Our study over 5 years of follow up which may provide a better opportunity to observe either detrimental or beneficial effects of OC on CF disease. We observed an approximately 1-2% drop in FEV_1% predicted per year in our cohorts which is consistent with most recent published data,[22] although reported rates of decline vary (see below). Importantly the decline in lung function in women using and not using OC was similar. In addition, subgroup analysis in younger (less than 23 years) and older (23 years and older) women did not indicate an effect of OC on disease severity.

Consistent with data collated from other registries [22] we have shown here that lung function in women with CF below the age of 23 declines more rapidly than in older women.

The reasons for the steeper decline in FEV_1 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 IV 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 (% FEV_1) varies from study to study ([23] and references within) and also appears to be birth cohort dependent.[23] Reported values range from 0.6 to 4% decline in FEV_1 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 to 4 years 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 to 5 year study period. Milder detrimental or protective effects of OC would probably become more obvious the longer the period of continuous use of OC. 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 women in our database had taken OC for a continuous period of 6 or 7 years. The inter-patient cohort comparison had sufficient statistical power to detect an approximately 10% difference in the absolute value of %FEV_1 in women "exposed" and "not exposed" to OC. Smaller effects of OC on lung function may have been missed, but are unlikely to have been of clinical significance. For the inter-patient analysis we matched woman for year of birth and age to minimise birth cohort and age-related disease severity effects. Further we assessed the effects of OC use on three key markers of CF disease severity; lung function as measured by %FEV_1, body mass index (BMI) and intravenous antibiotic use. To assess if age at time of OC use is an important variable, we also performed a subgroup analysis in younger (less than 23 years) and older (23 years or older), but again OC 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 OC against available patient numbers and, therefore, restricted the intra-patient analysis to three year 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 hospitalization 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 women.[17]

Similar to other retrospective studies, our study has a number of limitations. (1) We have no information about the type of OC used. OC have been available in Britain since 1960 with the original pill containing oestrogen and progesterone.[30] However, more recently only progesterone containing "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) Women with CF routinely require antibiotics and it has been well described that antibiotics can impair the effectiveness of OC by reducing circulating estradiol levels.[32] We do not have pharmacokinetic data assessing hormone concentrations in our patient population and, therefore, cannot completely exclude that circulating estrogen 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 women 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 women were

using OC. Avoidance of pregnancy is the most likely explanation, but prevention of irregular menstruation may also be a reason. Women may be using OC because they are very well and want to avoid pregnancy or they make take OC 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 $\%FEV_1$, BMI and use of IV 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 which may influence disease such as age of first *Pseudomonas 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 women 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 will 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 if 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 $\%FEV_1$, BMI or the need for intravenous antibiotics. These findings, therefore, suggest that the use of OC does not affect CF disease severity.

ACKNOWLEDGMENTS

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FIGURE LEGENDS:

Figure 1: Flow chart of patient selection for "exposed" and "not exposed" cohorts for inter-patient and intra-patient comparison. OC = oral contraceptive, DoB = date of birth
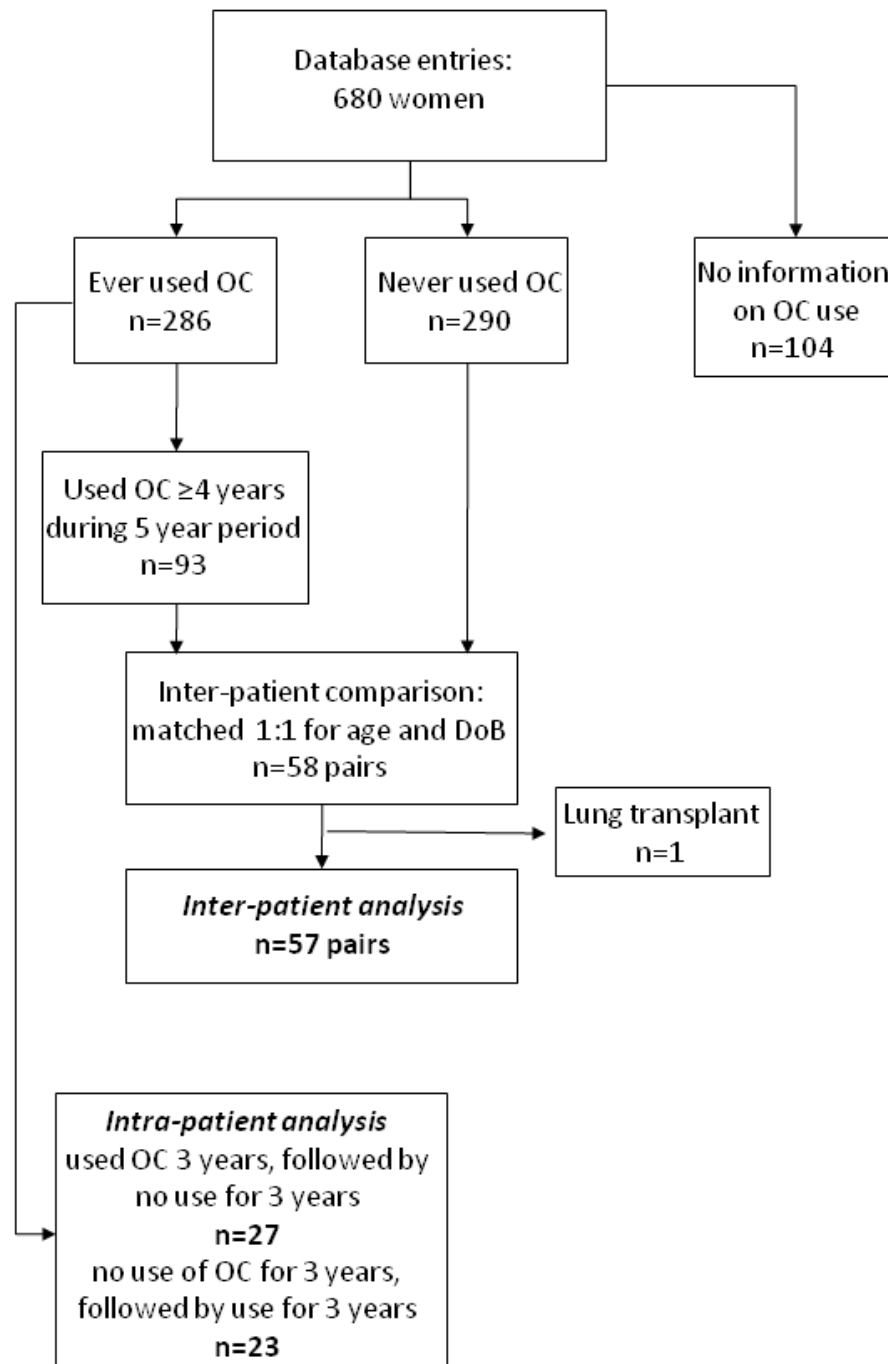
Figure 2: Comparison of disease severity during the five year study period in women with CF using oral contraceptives (OC) ("exposed" cohort) and women with CF not using OC ("not exposed" cohort).
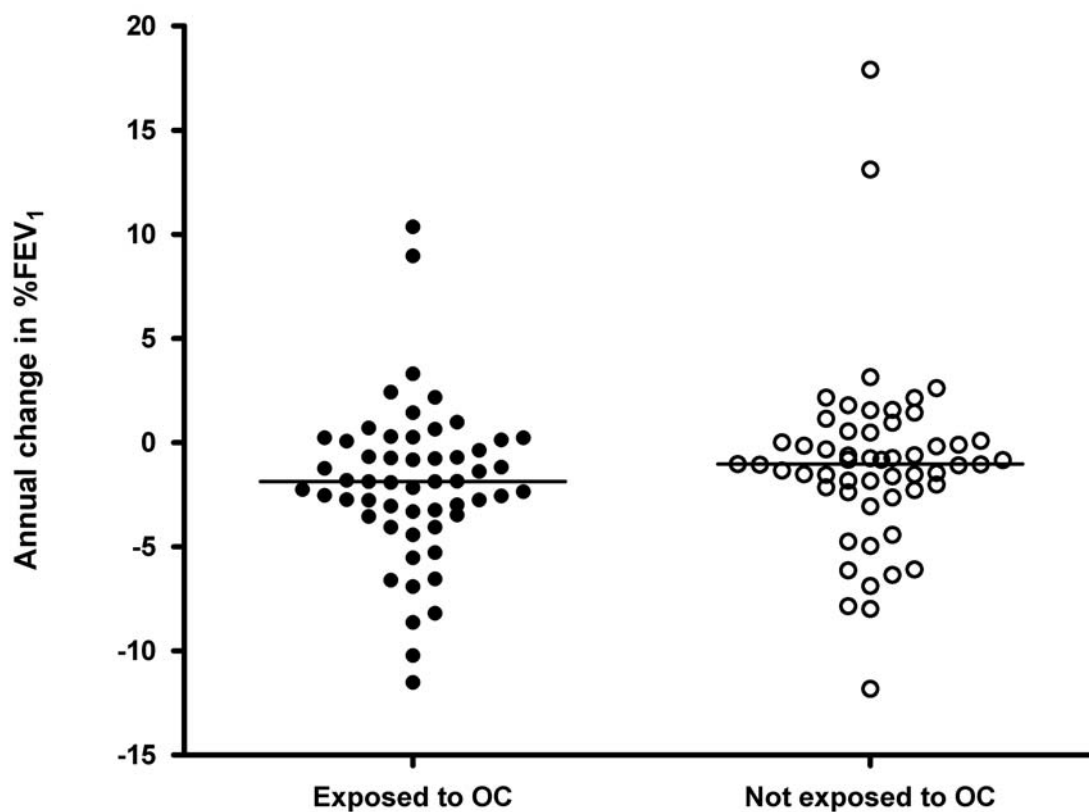

Figure 2A

(A) Annual change in percentage predicted forced expiratory volume in 1 second (%FEV$_1$),
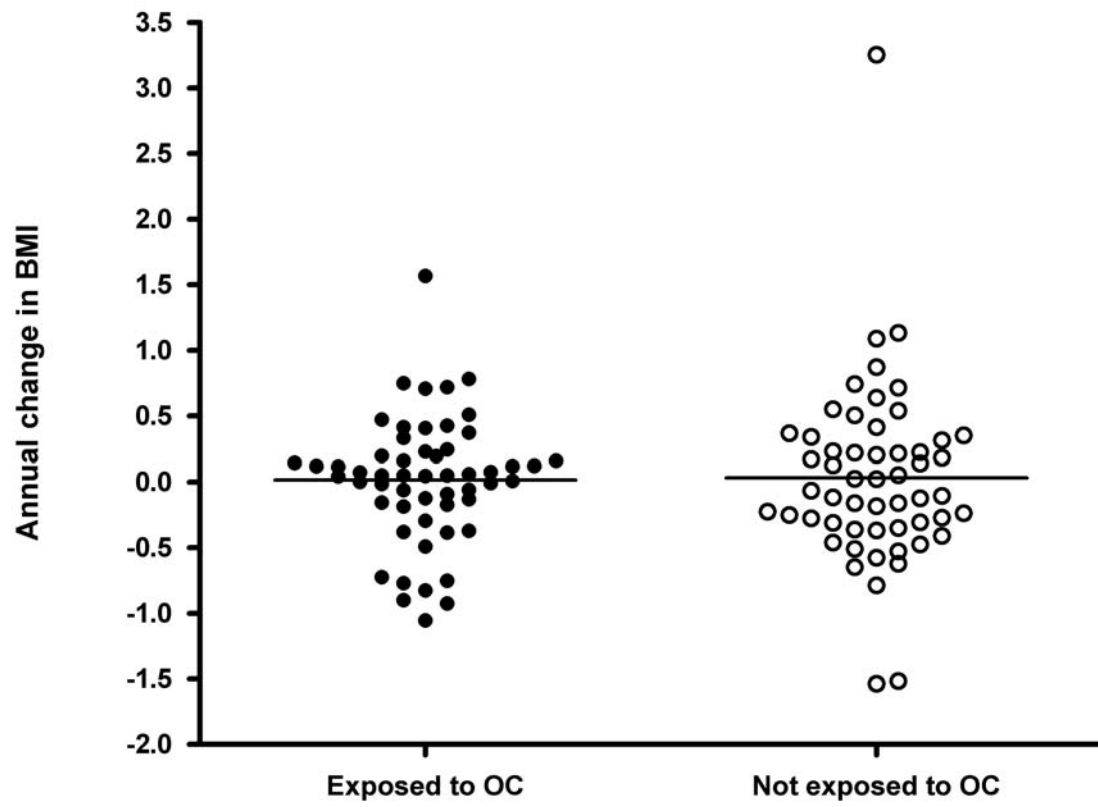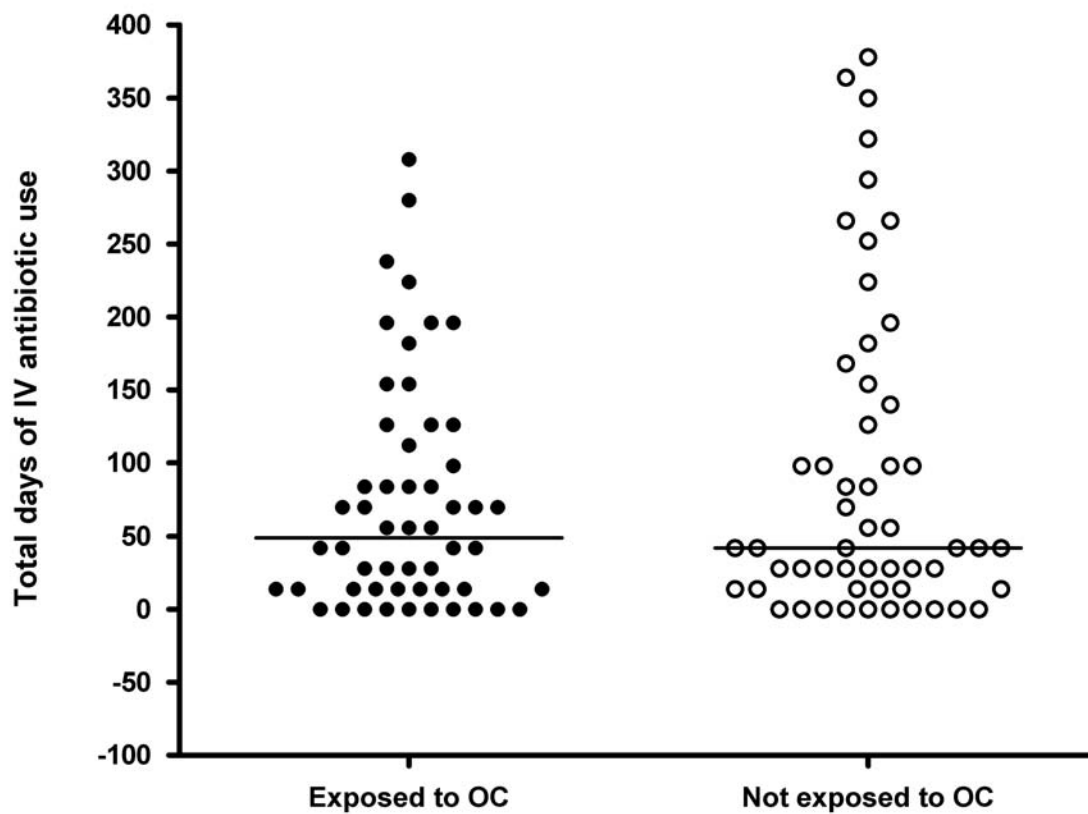
Figure 2B

(B) annual change in body mass index (BMI)

Figure 2C

(C) total number of days of IV antibiotic use during the 5 year 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+C) as appropriate for parametric or non-parametric data.

Figure 3: Sub-group analysis comparing disease severity in young and older women with CF using oral contraceptives (OC) ("exposed" cohort) and women with CF not using OC ("not exposed" cohort). Women were grouped by age (young = < 23 years, older = ≥ 23 years) and disease severity in "exposed" and "not-exposed" cohorts was compared.
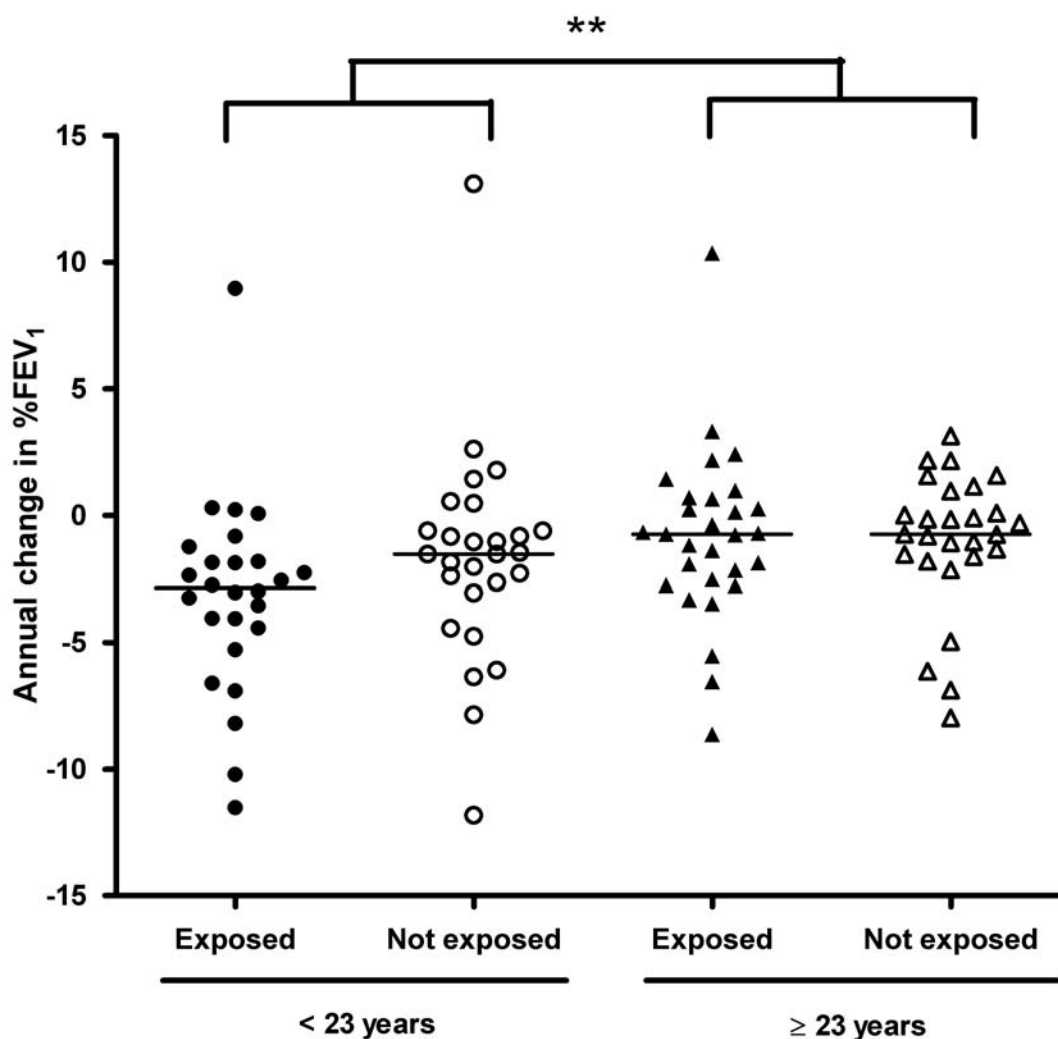

Figure 3A

(A) Annual change in percentage predicted forced expiratory volume in 1 second (%FEV$_1$),
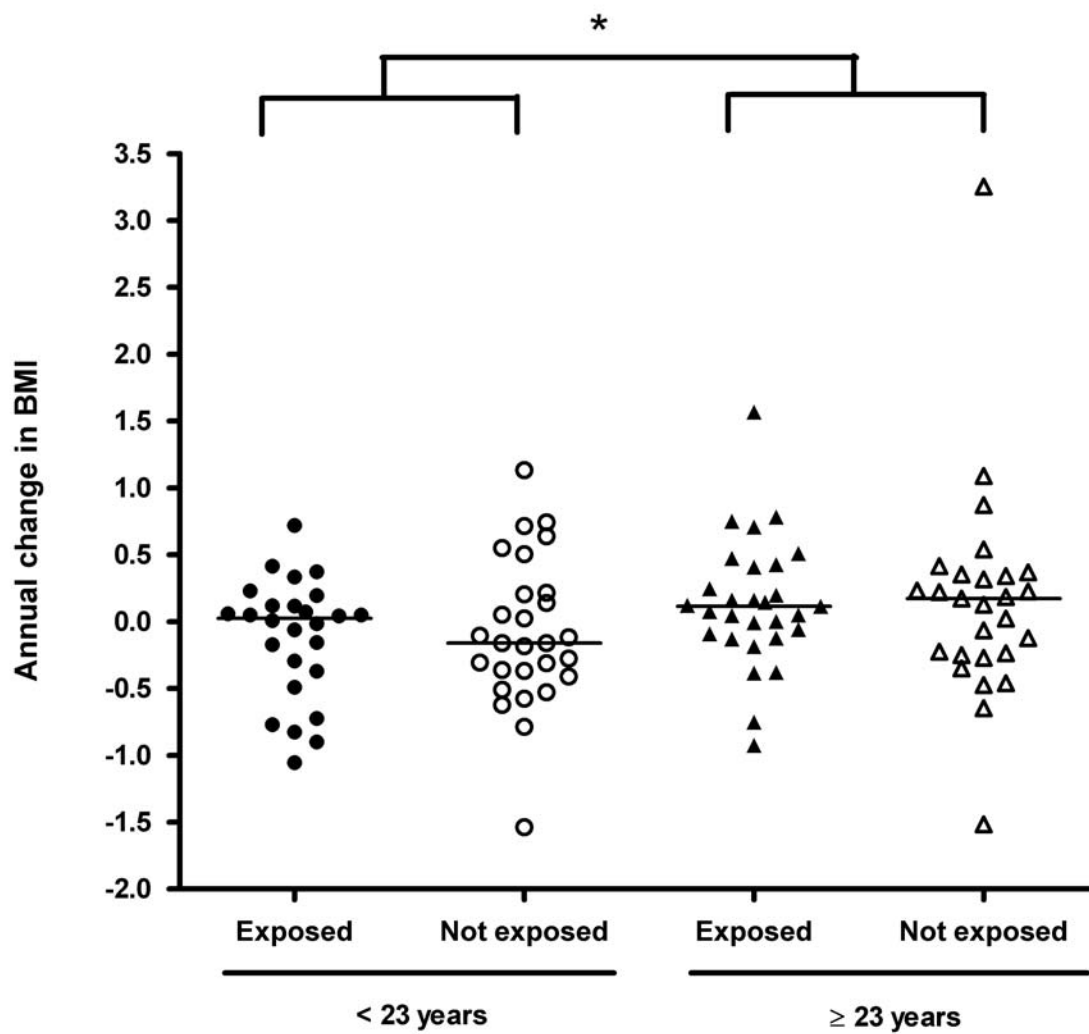
Figure 3B

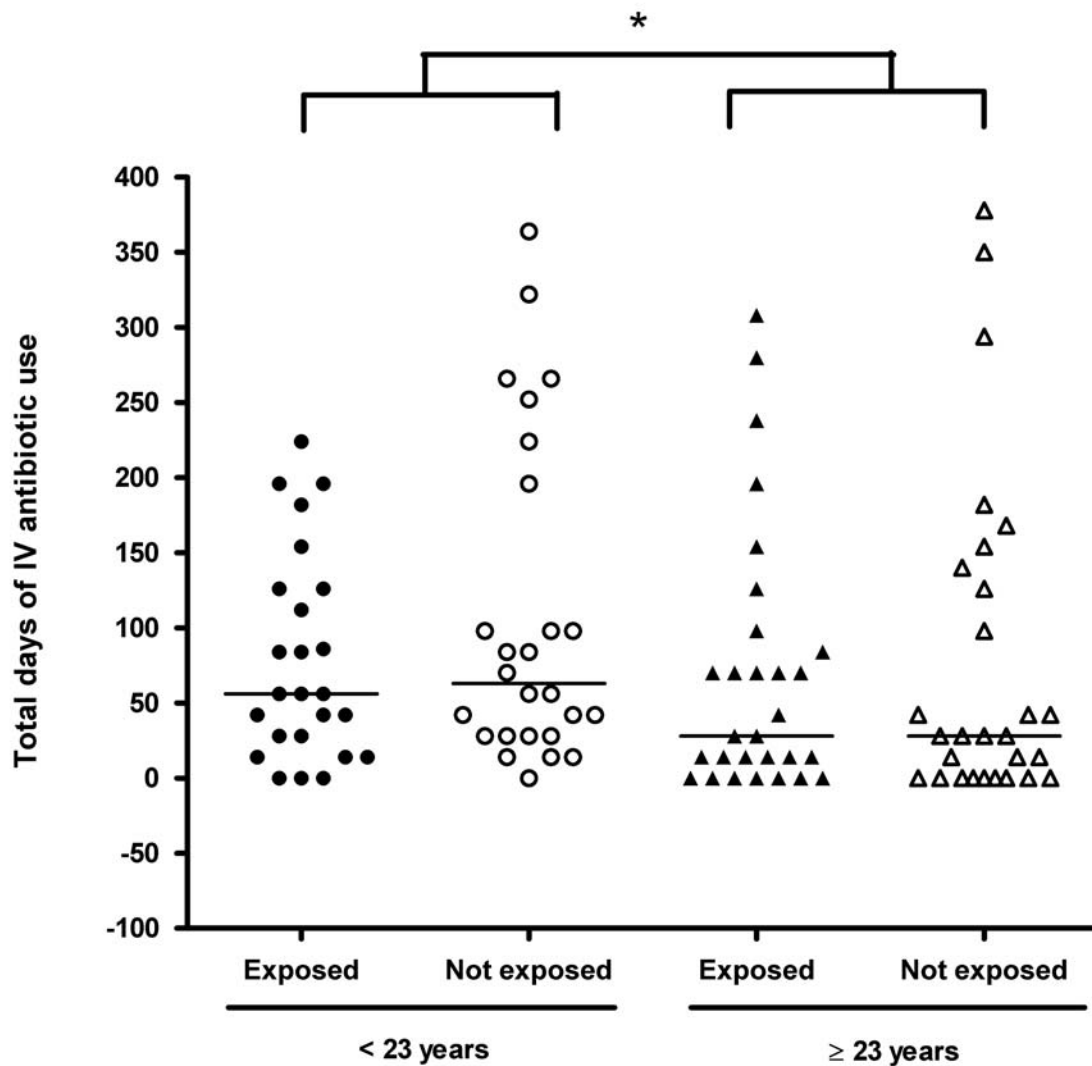**(B)** annual change in body mass index (BMI)

Figure 3C

(C) total number of days of IV antibiotic use during the 5 year 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. ** = $p<0.005$ and * = $p<0.05$ when comparing younger and older subjects.