Clofazimine pharmacokinetics in patients with TB: dosing implications

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Background: Clofazimine is in widespread use as a key component of drug-resistant TB regimens, but the recommended dose is not evidence based. Pharmacokinetic data from relevant patient populations are needed to inform dose optimization.

Objectives: To determine clofazimine exposure, evaluate covariate effects on variability, and simulate exposures for different dosing strategies in South African TB patients.

Patients and methods: Clinical and pharmacokinetic data were obtained from participants with pulmonary TB enrolled in two studies with intensive and sparse sampling for up to 6 months. Plasma concentrations were measured by LC-MS/MS and interpreted with non-linear mixed-effects modelling. Body size descriptors and other potential covariates were tested on pharmacokinetic parameters. We simulated different dosing regimens to safely shorten time to average daily concentration above a putative target concentration of 0.25 mg/L.

Results: We analysed 1570 clofazimine concentrations from 139 participants; 79 (57%) had drug-resistant TB and 54 (39%) were HIV infected. Clofazimine pharmacokinetics were well characterized by a three-compartment model. Clearance was 11.5 L/h and peripheral volume 10 500 L for a typical participant. Lower plasma exposures were observed in women during the first few months of treatment, explained by higher body fat fraction. Model-based simulations estimated that a loading dose of 200 mg daily for 2 weeks would achieve average daily concentrations above a target efficacy concentration 37 days earlier in a typical TB participant.

Conclusions: Clofazimine was widely distributed with a long elimination half-life. Disposition was strongly influenced by body fat content, with potential dosing implications for women with TB.

Introduction

Drug-resistant TB remains a major obstacle to achieving ‘End TB’ targets.1 A key driver of the drug-resistant TB epidemic has been a lack of effective therapy, leading to low cure rates, amplification of resistance and ongoing transmission. New therapeutic options with improved efficacy and tolerability have recently become available, including the new anti-TB agents bedaquiline and delamanid, and the repurposed drugs linezolid and clofazimine. As a result, there has been a shift in treatment guidelines towards shorter injection-free regimens.2 Clofazimine is recommended by the WHO and the American Thoracic Society for patients with rifampicin-resistant TB3,4 and also has a potential role in treatment-shortening regimens for drug-susceptible TB.5 First discovered in 1957, clofazimine...
has been used almost exclusively in combination therapy for leprosy. There have been no studies evaluating dose–exposure relationships in patients with drug-resistant TB, and the optimal dose for this condition is unknown. Clofazimine is highly protein bound and undergoes duration-dependent accumulation in fat, tissue macrophages and reticuloendothelial organs, resulting in an extremely long terminal half-life, with implications for risk of adverse effects and emergence of resistance. Furthermore, because clofazimine may be a substrate of cytochrome P450 (CYP) and the P-glycoprotein transporter, there are potential pharmacokinetic (PK) drug–drug interactions, including with antiretrovirals and other anti-TB agents. Though such research has been undertaken, it is unknown how well murine models of clofazimine dosing predict human PK. As a consequence of this limitation, plus the lack of PK data from TB patients, pharmacodynamic (PD) targets for efficacy and toxicity for clofazimine in TB treatment have not been established.

A model-based approach that can account for the unusual PK characteristics of clofazimine and predict individual exposures is required to estimate dose–response relationships and inform dose optimization in TB treatment. Using data from two cohorts of South African patients with TB, we developed a model to describe the population PK of clofazimine, evaluate covariate effects on PK variability and simulate exposures for optimized dosing strategies.

Patients and methods
Study design and population
Clinical and drug concentration data were obtained from a prospective observational cohort study (PROBeX) of adults treated with bedaquiline for pulmonary XDR and pre-XDR TB recruited from three TB hospitals in South Africa. Most participants were treated with regimens that also included clofazimine 100 mg daily, as per local standard of care. Detailed clinical and laboratory data were collected at monthly study visits over 6 months. Sparse PK sampling was performed at roughly 1, 2 and 6 months after starting clofazimine at a single pre-dose timepoint after self-reported dosing. A subgroup of consecutive participants at a single site consented to intensive sampling (i.e. pre-dose and at 1, 2, 3, 4, 5, 6, 8 and 24 h after an observed dose and a standard meal of peanut butter on brown bread) at the Month 2 visit. Clofazimine plasma concentrations were measured at the Division of Clinical Pharmacology at the University of Cape Town using a validated LC-MS/MS assay. The lower limit of quantification (LLOQ) was 0.00781 mg/L. The inter-day accuracy ranged from 101% to 105%, and the precision (%CV) ranged from 3.3% to 4.6% during sample analysis.

Additional data were obtained from a 14-day Phase 2A early bactericidal activity (EBA) trial of clofazimine alone or in combination with bedaquiline, pretomanid and pyrazinamide. Clofazimine was administered as a loading dose of 300 mg daily on Days 1–3, followed by 100 mg daily on Days 4–14. PK sampling was performed pre-dose and 5 and 10 h after observed doses on Days 1, 2, 3 and 8; pre-dose, hourly up to 5 h, and at 10, 16 and 24 h after observed doses on Day 14, plus an additional sample at Day 28 (2 weeks after clofazimine discontinuation). Bioanalysis for drug plasma concentrations was conducted by PRA (Lenexa, KS, USA) using liquid–liquid extraction and ultra-performance LC-MS/MS. The LLOQ was 0.004 mg/L; inter-day accuracy ranged from 98.5% to 103%, and the precision (%CV) ranged from 2.3% to 3.7% during sample analysis.

PK analysis
Non-linear mixed-effects modelling was used to analyse clofazimine concentrations from both clinical cohorts simultaneously using NONMEM version 7.4.3. Pirana, Perl-speaks-NONMEM (PsN) version 4.9.0 and Xpose were used for the analysis and simulation. We tested one-, two- and three-compartment disposition models. For the absorption process we tested first-order absorption with and without lag, saturable absorption, sum of inverse Gaussians and chain of transit compartments. Values below the LLOQ were excluded from the dataset.

Allometric scaling was applied on all disposition parameters to account for the effect of body size descriptors, including total body weight (TBW), fat-free mass (FFM) and body fat. Individual values of FFM were derived from observed TBW, height and sex using a validated formula (provided in Supplementary data, available at JAC Online). Fat mass was obtained as the difference between TBW and FFM. Age, sex, race, HIV status, clofazimine dose, treatment arm, duration on treatment and use of lopinavir/ritonavir (a strong CYP3A4 and P-glycoprotein inhibitor) were evaluated as additional covariates. Sampling importance resampling (SIR) was used to assess the robustness of the final parameter estimates and to obtain 95% CIs. Details of the modelling approach are provided in the Supplementary data.

Simulations
Using the final model, we simulated time to steady-state for typical body size descriptors of female and male participants observed in our cohort. The final model was also used to simulate different dosing regimens to shorten the time to average daily exposure above a putative target concentration of 0.25 mg/L extrapolated from a murine model while avoiding excessive peak values above those attained at steady-state with standard dosing to reduce risk of QT prolongation. Various loading doses and durations were explored, including 300 mg daily for 1–2 weeks and 200 mg daily for 2–8 weeks. For the latter simulations, characteristics of a typical TB patient weighing 56 kg and different proportions of fat mass (13%, 20% and 34%) to reflect body size composition in the average TB patient and genders in the cohort, including random interindividual variability, were repeated 10 000 times. Simulations were performed using PsN, Berkeley Madonna version 9.1.19, and NONMEM version 7.4.3.

Results
Demographics and clinical profile
Seventy-nine participants on clofazimine-based regimens with complete 6 month follow-up data were included from the PROBeX observational cohort, in addition to 60 participants from the EBA trial. Overall, median age was 31.0 years (IQR 24.0–39.5), 68 (48%) were female and 54 (39%) were HIV infected. Median weight was 55.1 kg (IQR 48.1–60.9) and percentage body fat was significantly higher in women [median 33.8% (IQR 30.9–37.7) versus 13.9% (IQR 12.0–16.6) in men] (Table 1 and Figure S1).

Model development and PK parameters
Clofazimine concentrations from 1570 plasma samples (n = 139 participants) were included in the analysis: 367 observations from three sampling visits in the PROBeX cohort (including intensive sampling from 22 participants) and 1203 observations from sampling over the 14 day EBA trial. Six samples had concentrations below the LLOQ and were excluded from the analysis. Twenty-five sparse samples from PROBeX participants with poor treatment adherence or missing dosing history had concentrations significantly lower than predicted on visual inspection and were also excluded.

Clofazimine PK were best characterized by a three-compartment disposition model with first-order elimination and absorption in transit compartments (Figure 1). Size descriptors were normalized to median population values. The best size descriptors for scaling of disposition parameters were: TBW for
total (CL) and intercompartmental (Q1, Q2) clearance and volume of distribution in the ‘shallow’ peripheral compartment (Vp2); FFM for central volume (Vc); and fat mass for volume of distribution in the ‘deep’ compartment (Vp1). The latter parameter resulted in the largest change in objective function value, indicating a significant effect, when included in the model. Age, sex, race, HIV status, duration of treatment and treatment arm were not identified as influential covariates according to stratified visual predictive checks and change in objective function, performed using the final model. The model detected an effect of lopinavir/ritonavir on clofazimine exposure, leading to higher bioavailability; however, the estimate was not sufficiently robust in terms of change in objective function to be included in the final model. Residual unexplained variability was best described by a combined model utilizing both additive and proportional components, with separate additive error estimates for the sparse dataset from PROBeX (due to uncertainty in self-reported dosing times and adherence). A prediction-corrected visual predictive check suggested adequate fit for the pooled data (Figures 2 and S2).

The final model parameter estimates are summarized in Table 2. The typical value for clearance (CL/F) was 11.5 L/h, and 10 500 L for the ‘deep’ peripheral volume. Women had much larger ‘deep’ peripheral volume (13 700 L for a typical woman versus 5720 L for a typical man) explained by significantly higher body fat percentage compared with men. The model detected an increase in bioavailability over the first few days of treatment, from a baseline of 68% to 95% of the reference value by the fourth dose. Because participants received a larger daily dose (300 mg versus 100 mg) in the first 3 days of the EBA trial, dose effect was investigated as a potential explanation, but a time-dependent exponential increase model (described in the Supplementary data) fitted the data better, even after testing several alternative absorption processes.

### Table 1. Baseline participant characteristics

| Variable                        | PROBeX^a (n = 79) | Phase 2A trial (n = 60) | Total (n = 139) |
|---------------------------------|-------------------|------------------------|----------------|
| Age, years                      | 32.5 (26.5–40.0)  | 29.5 (23.0–39.0)       | 31.0 (24.0–39.5) |
| Females, n (%)                  | 45 (57)           | 23 (38)                | 68 (49)        |
| Ethnicity, n (%)                |                   |                        |                |
| black                            | 53 (67)           | 29 (48)                | 84 (60)        |
| mixed race                       | 24 (30)           | 31 (52)                | 55 (39)        |
| white                            | 2 (3)             |                        | 2 (1)          |
| Weight, kg                      | 54.0 (48.0–60.0)  | 55.9 (50.0–61.7)       | 55.3 (48.1–61.5) |
| females                         | 54.2 (50.0–58.4)  | 57.9 (50.4–62.2)       | 56.0 (50.0–60.4) |
| FFM, kg                         | 40.3 (35.0–46.2)  | 44.9 (37.1–50.1)       | 42.0 (36.3–48.3) |
| Height, cm                      | 164 (157–168)     | 167 (161–173)          | 164 (158–172)  |
| BMI, kg/m²                      | 20.0 (18.0–22.5)  | 20.0 (17.9–22.0)       | 20.0 (18–21.9) |
| CD4 count, cells/mm³            | 196 (96–437)      | 715 (515–893)          | 540 (268–831)  |
| Serum creatinine, µmol/L        | 57.0 (48.5–68.0)  | 61 (51.4–70.8)         | 58.0 (50.5–69.3) |
| Duration on clofazimine, days   | 100 (55–182)      | 7.5 (4–11)             | 94 (48–180)    |

Data are median (range) unless specified otherwise.

Inj-R, injectable resistant; FQ-R, fluoroquinolone resistant.

^aMedian was imputed for missing values in continuous variables; 3 for age, 1 for weight and height, and 2 for serum creatinine.

^bCalculated as fat mass/total body weight.
Model-predicted clofazimine exposure \(C_{\text{max}}, \text{AUC}_{0–24}\) and average daily concentration \(C_{\text{avg}}\) was higher at 2 months compared with 14 days, reflecting clofazimine accumulation over time. Estimated plasma exposures were lower in women (Table 3 and Figure 3).

Simulations

Median terminal elimination half-life was estimated at 34.2 days and was significantly longer for women (49.5 days versus 21.8 days for men), resulting from differences in body composition. Consequently, the median time to steady-state (~5 times the terminal half-life), which was 150 days overall, was much shorter for men (105 days versus 230 days for women) (Figure 4).

Simulations of a 300 mg loading dose for either 1 or 2 week durations predicted peak concentrations \(C_{\text{max}}\) that exceeded those estimated at steady-state, a potential safety concern, particularly for QT prolongation (Figure S3). A simulated schedule of 200 mg daily loading dose given for the initial 2 weeks of therapy followed by standard dosing (100 mg daily) predicted average daily plasma concentrations above the *Mycobacterium tuberculosis* WT MIC value (0.25 mg/L) 37 days earlier compared with standard


**Table 2. Final population PK model parameters**

| Parameter description | Typical value (95% CI) |
|-----------------------|-----------------------|
| Clearance, (L/h)      | 11.5 (10.5–12.5)      |
| Central volume of distribution, Vc (L) | 262 (178–375) |
| Intercompartmental clearance, Q1 (L/h) | 56.3 (49.6–62.6) |
| Peripheral volume 1, Vp1 (L) | 10 500 (9320–11 600) |
| Intercompartmental clearance, Q2 (L/h) | 86 (74.6–99.5) |
| Peripheral volume 2, Vp2 (L) | 889 (696–1070) |
| Absorption mean transit time (h) | 1.41 (1.10–1.67) |
| Number of transit compartments (NN) | 4.75 (3.01–7.65) |
| Absorption rate constant, Ka (1/h) | 0.209 (0.175–0.261) |
| Relative bioavailability baseline | 0.685 (0.615–0.771) |
| Bioavailability, F | 1 [FIXED] |
| Proportional error (%) | 11.4 (10.8–12.1) |
| Additive error (sg/L) | 0.00156 [FIXED] |
| Additive error (PROBE sparse data) (mg/L) | 0.0905 (0.077–0.107) |
| P-glycoprotein inhibition half-life (days) | 1.44 (0.683–2.85) |
| Between-subject variability (%) | 25.6 (17–33.5) |
| clearance central volume | 23.5 (8.39–35.1) |
| peripheral volume 1 | 29.6 (20.4–38.1) |
| peripheral volume 2 | 54.6 (42.3–73.9) |
| bioavailability | 30.1 (25.2–35.5) |
| Between-occasion variability (%) | 46.6 (38.1–56.9) |
| absorption mean transit time | 32.6 (27.1–38) |
| absorption rate constant bioavailability | 35.4 (31.8–39.6) |
| Terminal half-life (days) | 34.2 |
| median patient | 49.5 |
| female | 21.8 |

*D95% CI obtained with the sampling importance resampling technique using PsN software.

*Allometric scaling used for CL, Vp, Vp1, Vp2, Q1 and Q2; typical values reported for the median weight (55 kg), FFM (42 kg) and fat mass (13 kg) as reported in Table 1.

*Estimate of the additive error was not statistically significant from the lower bound (LLOQ/2) and was thus fixed to that value.

*Inhibitory effect of clofazimine on P-glycoprotein using an exponential maturation function (described in the Supplementary data).

*Between-subject variability and between-occasion variability were assumed to be log-normally distributed and reported as approximate %CV.

*Derived parameters outside the estimation software: calculated for the typical male and female median values as reported in Table 1.


dosing for a typical TB patient in our cohort. For typical male patients, with 13% body fat, the 2 week loading dose achieved a 21 day reduction in time to target concentration. However, female patients, who had an average of 34% body fat, required a longer loading period: a simulation of 4 weeks’ loading led to target attainment 56 days earlier compared with standard dosing in women (Figure 5). Predicted median Cmax with use of this loading dose was 0.277 mg/L at the end of the loading period, compared with median Cmax 0.343 mg/L at steady-state.

**Discussion**

Clofazimine PK have not been adequately studied and data from TB patients are especially scarce. Using data from a prospective observational study and an EBA trial, we developed a population PK model that describes the compartmental kinetics and accumulation of clofazimine in South African TB participants with 39% prevalence of HIV co-infection. Clofazimine disposition was strongly influenced by body fat percentage, thus resulting in initially lower plasma exposure amongst women. We simulated a loading dose that would achieve steady-state faster without increasing expected peak concentrations, hence limiting the likelihood of added toxicity. These findings have implications for clofazimine dosing in TB.

Our model-predicted PK parameters confirm a large volume of distribution (~10 000 L) and long terminal half-life of ~30 days, which is consistent with known pharmacological properties of clofazimine. Clofazimine is highly lipophilic and distributes widely into fatty tissues, and murine experiments have demonstrated that clofazimine primarily accumulates in reticuloendothelial tissues and is slow to be eliminated from the body. Our findings have implications for clofazimine disposition in people. An unpublished two-compartment disposition model using data from leprosy patients and healthy volunteers with variable dosing schedules estimated a peripheral volume of around 4000 L and a half-life of 15 days. Another study, applying a one-compartment model to quantify the effect of food on clofazimine bioavailability in healthy volunteers, estimated a mean volume of distribution 1470 L, although the authors were unable to estimate the terminal elimination half-life due to limited assay sensitivity.

The pooled data and observed variance in our cohort were well described by a three-compartment structural model that accounted for the complex multiphase disposition. Lopinavir/ritonavir use was associated with a moderate increase in clofazimine exposure. Clofazimine metabolism is poorly characterized; one study found clofazimine to be a P-glycoprotein substrate on in vitro screening and thus a potential victim of P-glycoprotein inhibition by HIV protease inhibitors. This has been an inconsistent finding, and formal drug–drug interaction studies are needed to investigate the effect of co-administration with lopinavir/ritonavir on clofazimine exposure and toxicity.

Body composition explained our key finding of major sex differences in clofazimine plasma exposure. In our combined cohorts, women had a higher proportion of body fat and consequently a larger peripheral volume of clofazimine distribution compared...
with men. Because PK sampling occurred before steady-state attainment while the drug was still accumulating in peripheral tissues, women from both cohorts had much lower observed clofazimine plasma concentrations and derived exposure parameters ($AUC_{0–24}$, $C_{\text{max}}$ and $C_{\text{avg}}$), which is in agreement with reported findings from non-compartmental analyses of the EBA trial\textsuperscript{12} and the observational PROBeX study.\textsuperscript{29} The clinical consequence of larger peripheral volumes is extended terminal half-life, prolonging the predicted number of repeated daily doses necessary to achieve clofazimine steady-state in women, demonstrated by our simulations in Figure 4.

Average clofazimine plasma concentrations measured before steady-state were much lower than the recommended critical concentration of 1 mg/L and published MIC distributions in drug-resistant \textit{M. tuberculosis} strains.\textsuperscript{30} Clofazimine is highly protein bound,\textsuperscript{6,31} resulting in free plasma drug concentrations below the MIC, indicating a potentially suboptimal antibacterial effect. However, clofazimine has been shown to partition in the cellular rim of explanted lung granulomas, achieving much higher intracellular concentrations relative to plasma.\textsuperscript{32} Tissue accumulation and the long terminal elimination half-life of clofazimine may contribute to efficacy and treatment shortening as a consequence of high site-of-disease concentrations despite failure to exceed the MIC in plasma.\textsuperscript{32,33} Clofazimine has no discernible EBA at 14 days\textsuperscript{12} and consistently demonstrates delayed and concentration-dependent activity in murine models.\textsuperscript{6,10} Simulations based on sparse data from two Korean MDR-TB patients on standard doses predicted attainment of clofazimine concentration above the efficacy target in cellular lesion compartments at steady-state.\textsuperscript{33} While steady-state tissue exposures are likely to exceed bactericidal concentrations (0.25 mg/L)\textsuperscript{34} and contribute to sterilizing activity against intracellular bacilli, early treatment efficacy could be optimized using a loading dose to achieve steady-state more rapidly.

There is no established clinical PK/PD index for clofazimine efficacy in TB, precluding empirically based dosing simulations. In a murine model, clofazimine was shown to contribute sustained

### Table 3. Model-predicted secondary PK parameters from rich sampling occasions

| Study               | Females                  | Males                  | Total                  |
|---------------------|--------------------------|------------------------|------------------------|
| PROBeX (Month 2)    |                          |                        |                        |
| $C_{\text{max}}$ (mg/L) | 0.310 (0.161–0.646)     | 0.473 (0.307–0.688)   | 0.363 (0.161–0.688)   |
| $AUC_{0–24}$ (mg·h/L) | 6.08 (3.17–13.7)        | 10.3 (6.61–14.3)      | 7.33 (3.17–14.3)      |
| $C_{\text{avg}}$ (mg/L) | 0.254 (0.132–0.572)    | 0.430 (0.275–0.596)   | 0.305 (0.132–0.596)   |
| Phase 2A (Day 14)   |                          |                        |                        |
| $C_{\text{max}}$ (mg/L) | 0.199 (0.0687–0.403)   | 0.263 (0.168–0.413)   | 0.218 (0.0687–0.4130) |
| $AUC_{0–24}$ (mg·h/L) | 3.22 (1.26–6.16)        | 4.87 (2.73–8.60)      | 4.2 (1.26–8.60)       |
| $C_{\text{avg}}$ (mg/L) | 0.134 (0.0523–0.257)   | 0.203 (0.114–0.358)   | 0.177 (0.0578–0.3580) |

Data are given as median (IQR).

$n = 22$ for PROBeX, sampled at ~2 months; $n = 57$ for the Phase 2A trial, sampled at Day 14.
antimycobacterial activity for up to 6 weeks after discontinuation, and this ‘post-antibiotic effect’ was associated with plasma concentrations above 0.25 mg/L; we therefore selected average daily concentration above this value as the target efficacy parameter for our dosing simulations. The relationship between clofazimine exposure and toxicity (QT prolongation and skin changes) is unknown. Peak serum drug concentrations generally correspond to peak effect on QT interval, and our loading dose simulations aimed to balance more rapid attainment of efficacy target concentration with anticipated effects of higher initial $C_{\text{max}}$ on QT prolongation. The principles guiding selection of dosing strategies were thus to: (i) avoid substantially higher predicted $C_{\text{max}}$ after the loading period than average steady-state peak concentrations to reduce the risk of serious QT prolongation; (ii) achieve shorter time to average daily concentrations exceeding the efficacy target; and (iii) use a simple dosing regimen with once-daily administration. A dosing schedule of 200 mg for 2 or 4 weeks depending on body fat percentage followed by 100 mg daily achieved these objectives and requires further investigation in future clinical trials to delineate the impact on important endpoints and treatment shortening.

As with all modelling exercises, our analysis has limitations. While the model presented here was able to describe complex data with relatively few parameters, mechanistic assumptions were based on very limited knowledge of clofazimine metabolism and may have limited predictive ability outside the range of observed data. There was also some uncertainty in the data, particularly around accuracy of dosing times and adherence relating to sparse PK sampling in the PROBeX cohort; we accounted for this by introducing a separate additive error estimate to the model. Furthermore, we based our model on pooled data from two separate clinical cohorts and drug assays from different laboratories, which may add unexplained variability. Our model detected a moderate increase in clofazimine bioavailability over the first few days of therapy, which we attribute to putative autoinhibition of intestinal P-glycoprotein. However, this finding could be due to the limits of model extrapolation: EBA trial participants received a larger dose for 3 days and there was a high degree of variability in bioavailability. Although time-dependent rather than dose-dependent absorption describes the data better, dose-dependent saturation of absorption remains a possibility. Finally, assumptions for our dosing simulations were made in the absence of clofazimine PK/PD data from TB patients and reflect theoretical rather than empirically based targets.

In conclusion, we successfully developed a clofazimine population PK model for South African TB participants, describing massive peripheral distribution volume and prolonged terminal elimination. Clofazimine disposition was strongly influenced by body fat content, resulting in lower plasma exposure among women. The clinical consequences are unknown but clofazimine may require dose individualization at extremes of body composition to optimize use. A 2 week loading dose may support treatment shortening by enabling more rapid attainment of efficacy targets within a safe window of peak concentrations in this population; longer loading periods may be required in patients with high fat mass. This needs to be evaluated in clinical trials. Dose optimization of clofazimine is a research priority in TB, and our model is a necessary first step to understanding concentration–response relationships of this key anti-TB drug.

**Figure 4.** Predicted clofazimine concentrations at steady-state with standard dosing (100 mg daily), stratified for typical male/female participants in the cohort. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.
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Transparency declarations
None to declare.

Supplementary data
Figures S1 to S3 are available as Supplementary data at JAC Online.

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