Multiple flow FeNO in population research: R code methods demo

The following demo file provides example R code to implement six methods for cross-sectional studies with multiple flow exhaled nitric oxide (FeNO) measurements, where the goal is to relate estimated NO parameters to factors of interest (i.e., covariate(s) X) in the study population.

0. Preliminaries: set up your R session

IMPORTANT! Install JAGS, if not already installed, follow instructions at: https://mcmc-jags.sourceforge.io/

Load the following required packages, installing first if not already available. To use the R2jags package, JAGS must be pre-installed.

```
# set working directory
setwd("K:/paper/newpackage/PopFeNO")
# load required packages
require(MASS)
require(lme4)
require(nlme)
require(reshape2)
require(R2jags)
require(knitr)
```

1. Data simulation

First, set a random seed for your work.

```
set.seed(2020)
```

Next, we simulate data from a cross-sectional study with multiple flow FeNO on each partcipant and a single covariate of interest, X, which varies at the participant-level (i.e., it varies across participants, but is constant within participant across FeNO maneuvers). We have written a function DataGeneratorCS() in DataG.R, which outputs a simulated dataset containing data for each participant on mean flow and FeNO for all FeNO maneuvers as well as that participant's value of X.

In this example, we generate data for 500 participants with X following a standard normal distribution (though in practice X can have any distribution) and multiple flow FeNO (8 maneuvers per participant, two each at the target flow rates of 30, 50, 100, and 300 ml/s). Participant-level NO parameters (CANO, CawNO and DawNO) are each generated as a linear function of X, except that rather than CawNO or DawNO, we generate logCawNO and logDawNO to better replicate the approximately log normal distribution observed in practice and to avoid negative estimates in later modeling. For example, participant-level CANO_i = 1.5 + 0.1*X_i + epsilon_i where the standard deviation of the random normal error is 0.45. While we could generate each of the three NO parameters seperately for each participant, instead we generate the three NO parameters from a multivariate normal distribution to introduce correlation between NO parameters. Given the NO parameters, FeNO at each maenuvers can then be generated according to the standard two compartment model.

$$FeNO = C_{aw} + (C_A - C_{aw}) \times e^{-\frac{D_{aw}}{flow}}$$

```r
# Simulate a multiple flow FeNO dataset in a study population
source("DataG.R")        # code to generate multiple flow FeNO datasets
flow=c(rep(30,2),rep(50,2),rep(100,2),rep(300,2))
truebeta=c(0.1,0.1,0.1)
out <- DataGeneratorCS(Ndat=1000,            # number of study participants
                      alpha=c(1.5,3.5,2.5),# vector of NO parameter population means,
                                           # when X=0, in this order: CA, logCaw, logDaw
                      # function to generate participant-level covariate X
                      # as function of number of study participants
                      Xfn=function(n){rnorm(n,0,1)},
                      # vector of regression coefficients relating X to NO parameters,
                      # in this order: CA, logCaw, logDaw
                      beta=truebeta,
                      # vector of flow rates in multiple flow FeNO protocol
                      Flow=c(rep(30,2),rep(50,2),rep(100,2),rep(300,2)),

                      SD=0.1,              #SD of the error
                      sdalphaCa            = 0.45, # population SD of CA
                      sdalphalogCaw        = 0.65, # population SD of logCaw
                      sdalphalogDaw        = 0.55, # population SD of logDaw
                      coralphalogCawCa     = 0.66, # correlation of logCaw, CA
                      coralphalogCawlogDaw = -0.35,# correlation of logCaw, logDaw
                      coralphalogDawCa     = -0.38 # correlation of logDaw, CA
                     )
# output is two versions of the same dataset, plus dataset only including X and id
# one dataset for use in Bayesian methods via JAGS (datJAGS)
# one dataset for all other methods (dat)
dat     <- out$dat
datJAGS <- out$datJAGS
datX    <- out$datX
```

- The resultant dataset is in the usual ‘long’ data format:

id	eno	logeno	flow	flowTarget
1	6.928492	1.9356421	30	30
1	6.450914	1.8642218	30	30
1	5.166481	1.6421918	50	50
1	5.887342	1.7728046	50	50
1	3.782733	1.3304469	100	100
1	3.262054	1.1823571	100	100
1	1.749397	0.5592711	300	300
1	1.946140	0.6658478	300	300
2	6.715659	1.9044420	30	30
2	7.187736	1.9723762	30	30

- The intercepts and slopes of the linear regression moedls: NOparam ~ X of the simulated data, used in the plotting section:

	Ca	logCaw	logDaw
(Intercept)	1.5347190	3.5414636	2.4698980
datJAGS$X	0.0930702	0.0986973	0.0874073

2. Estimate NO parameter associations with X

2.1 TS_NLS: Two-stage nonlinear least squares

1: NLS_StageI: Estimated NO paramteres ordered by id, exist NAs

2: NLS_StageIX: Combine NO paramters and covariate X by id

3: Fit individual grouped linear regression models for each NO parameters

```r
source("TS_NLS.R")

# Stage I
NLS_StageI <- TS_NLS_StageI(dat)

# create dataset including both Stage I estimates and X
NLS_StageIX <- merge(NLS_StageI, datX, by = "id")

# Stage II - edit to include any additional Stage II
# covariates (e.g., confounder adjustments)
TS_NLS_Ca <- lme(Ca ~ X, random = ~1 | id, data = NLS_StageIX,
    na.action = na.omit)
TS_NLS_logCaw <- lme(logCaw ~ X, random = ~1 | id, data = NLS_StageIX,
    na.action = na.omit)
TS_NLS_logDaw <- lme(logDaw ~ X, random = ~1 | id, data = NLS_StageIX,
    na.action = na.omit)
```

Interpretation for CANO:

The population mean CANO is 1.38 (95% CI: 1.34,1).

For 1 unit increase in covariate X, CANO increases 0.08, (95% CI: 0.03, 0.13).

Interpretation for CawNO and DawNO from their log version:

The population mean CawNO is 3.42 (95% CI: 3.35,3).

For 1 unit increase in covariate X, CawNO increases 13.64%, (95% CI: 6.86%, 20.85%).

The population mean DawNO is 2.78 (95% CI: 2.72,3).

For 1 unit increase in covariate X, DawNO increases 4.8%, (95% CI: -1.33%, 11.31%).

- These interpretations are similar for following results.

2.2 TS_HMA: Two-stage Högman & Merilänen Algorithm

```r
source("TS_HMA.R")

# Stage I, specify target flow rates for HMA (low, medium,
# high)
HMA_StageI <- TS_HMA_StageI(dat, flowLMH = c(30, 100, 300))

# create dataset including both Stage I estimates and X
HMA_StageIX <- merge(HMA_StageI, datX, by = "id")

# Stage II - edit to include any additional Stage II
# covariates (e.g., confounder adjustments)
TS_HMA_Ca <- lme(Ca ~ X, random = ~1 | id, data = HMA_StageIX,
    na.action = na.omit)
TS_HMA_logCaw <- lme(logCaw ~ X, random = ~1 | id, data = HMA_StageIX,
    na.action = na.omit)
TS_HMA_logDaw <- lme(logDaw ~ X, random = ~1 | id, data = HMA_StageIX,
    na.action = na.omit)
```

2.3 TS_NLME: Two-stage nonlinear mixed effects model

```
if (!file.exists("TSNLME_cc.Rdata")) {
    source("TS_NLME.R")
    # Stage I
    TSNLME_StageIout <- TS_NLME_StageI(dat, tol1 = 0.1, tol2 = 0.1,
        outputFit = TRUE)  # include X for later unified version
    save(TSNLME_StageIout, file = "TSNLME_cc.Rdata")
} else {
    load("TSNLME_cc.Rdata")
}
TSNLME_StageI <- TSNLME_StageIout$ests
TSNLME_StageIfit <- TSNLME_StageIout$fit  # save fit to speed up U_NLME

# create dataset including both Stage I estimates and X
TSNLME_StageIX <- merge(TSNLME_StageI, datX, by = "id")

# Stage II - edit to include any additional Stage II
# covariates (e.g., confounder adjustments)
TS_NLME_Ca <- lme(Ca ~ X, random = ~1 | id, data = TSNLME_StageIX,
    na.action = na.omit)
TS_NLME_logCaw <- lme(logCaw ~ X, random = ~1 | id, data = TSNLME_StageIX,
    na.action = na.omit)
TS_NLME_logDaw <- lme(logDaw ~ X, random = ~1 | id, data = TSNLME_StageIX,
    na.action = na.omit)
```

2.4 UNLME: Unified nonlinear mixed effects model

- Notation: There are two ways to fit the unified NLME model. One through direct simulation, the other updates the two-stage version.

```
if (!file.exists("UNLME_cc.Rdata")) {
    set.seed(2020)
    # The function is for single X. If you want to fit with
    # multiple X, just modify the 'fixed' and start
    # statement of the function
    source("U_NLME.R")
    # direct approach
    U_NLMEout <- U_NLME_direct(dat, datX, tol = 0.1)
    # update approach
    # U_NLMEout_u<-U_NLME_update(TSNLME_StageIout,dat,datX,tol=0.1)
    # anova(U_NLMEout,U_NLMEout_u) # compare two approaches
    save(U_NLMEout, file = "UNLME_cc.Rdata")
} else {
    load("UNLME_cc.Rdata")
}
```

2.5 TS__HB: Two-stage Hierarchical Bayesian method

- Load existing results if exists.

```
source("TS_HB.R")

# Stage I
if (!file.exists("TSHB_cc.Rdata")) {
    set.seed(2020)
    TSHB_S1 <- TSHB_iter(beta0_prior = c(2, 4, 3), rhat = 1.1,
        addon.iter = 6000, Max_update = 10, n.final = 6000, N.iterT = 55000,
        N.burnin = 50000, N.thinM = 10, N.chain = 3, flow = flow,
        dat = datJAGS, tracing = c("beta0_Ca", "beta0_logCaw",
            "beta0_logDaw", "sdCa", "sdlogCaw", "sdlogDaw", "corlogCawCa",
            "corlogCawlogDaw", "corlogDawCa", "sigma_c"))
    save(TSHB_S1, file = "TSHB_cc.Rdata")
} else {
    load("TSHB_cc.Rdata")
}

TSHB_S1_dat <- data.frame(Ca = TSHB_S1$summary[grepl("^Ca", rownames(TSHB_S1$summary)),
    1], logCaw = TSHB_S1$summary[grepl("^logCaw", rownames(TSHB_S1$summary)),
    1], logDaw = TSHB_S1$summary[grepl("^logDaw", rownames(TSHB_S1$summary)),
    1])
TSHB_S1_dat$id <- as.numeric(unlist(lapply(rownames(TSHB_S1_dat),
    function(x) strsplit(strsplit(x, "\\[")[[1]][2], "\\]")[[1]])))
TSHB_S1_dat <- TSHB_S1_dat[order(TSHB_S1_dat$id), ]
TSHB_StageIX <- cbind(TSHB_S1_dat, datX)

# Stage II - edit to include any additional Stage II
# covariates (e.g., confounder adjustments)
TS_HB_Ca <- lme(Ca ~ X, random = ~1 | id, data = TSHB_StageIX,
    na.action = na.omit)
TS_HB_logCaw <- lme(logCaw ~ X, random = ~1 | id, data = TSHB_StageIX,
    na.action = na.omit)
TS_HB_logDaw <- lme(logDaw ~ X, random = ~1 | id, data = TSHB_StageIX,
    na.action = na.omit)
```

2.5.ex Convergence diagnostic for TS__HB via Rhat (See Gelman and Rubin (1992), Brooks and Gelman (1998)])

- Print out the estimation (95% CL) and Rhat (converge if <1.1)

	2.5%	mean	97.5%	Rhat
beta0_Ca	1.5090358	1.5499208	1.5910045	1.006103
beta0_logCaw	3.4134646	3.4946240	3.5793552	1.042173
beta0_logDaw	2.4181594	2.5164054	2.6083653	1.035312
sdCa	0.4225800	0.4554148	0.4888889	1.009386
sdlogCaw	0.5753498	0.6428229	0.7181116	1.094953
sdlogDaw	0.3826897	0.4936405	0.6024987	1.092135
corlogCawCa	0.5399083	0.6394098	0.7304849	1.023285
corlogCawlogDaw	-0.4425728	-0.2489738	-0.0269078	1.120939
corlogDawCa	-0.4174139	-0.2354739	-0.0193047	1.032991
sigma_c	0.0989317	0.1007837	0.1026710	1.008575

2.6 U_HB: Unified Hierarchical Bayesian method

- Load existing results if exists.

```r
source("U_HB.R")

if (!file.exists("UHB_cc.Rdata")) {
    set.seed(2020)
    UHB_sim <- UHB_iter(beta0_prior = c(2, 4, 3), betaC_prior = c(0.1,
        0.1, 0.1), rhat = 1.1, addon.iter = 12000, Max_update = 10,
        n.final = 6000, N.iterT = 1e+05, N.burnin = 90000, N.thinM = 20,
        N.chain = 3, flow = flow, dat = datJAGS, X = datJAGS$X,
        tracing = c("beta0_Ca", "beta0_logCaw", "beta0_logDaw",
            "beta1_Ca", "beta1_logCaw", "beta1_logDaw", "sdCa",
            "sdlogCaw", "sdlogDaw", "corlogCawCa", "corlogCawlogDaw",
            "corlogDawCa", "sigma_c"))
    save(UHB_sim, file = "UHB_cc.Rdata")
} else {
    load("UHB_cc.Rdata")
}
```

2.6.ex Convergence diagnostic for U_HB via Rhat

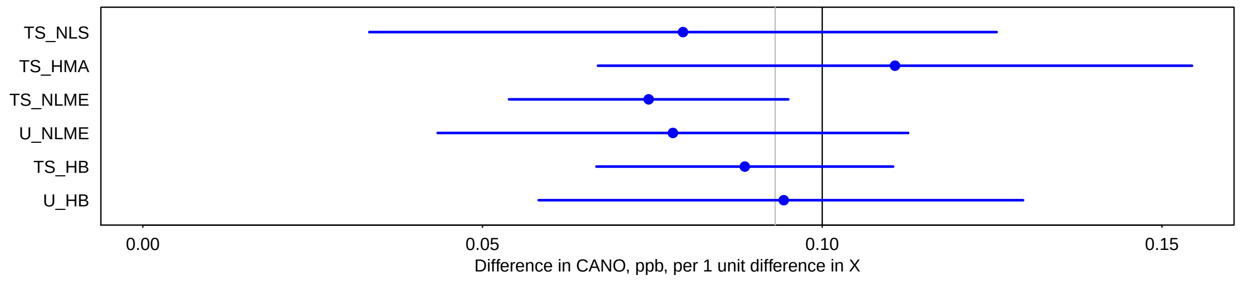
- Print out the estimation (95% CL) and Rhat (converge if <1.1)

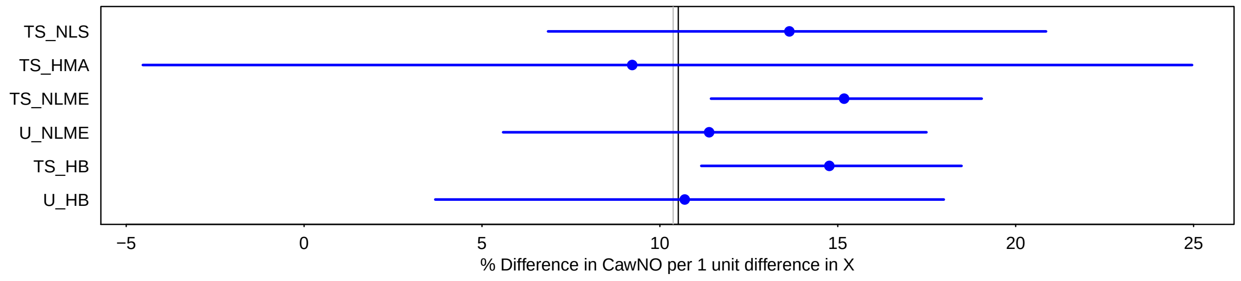
	2.5%	mean	97.5%	Rhat
beta0_Ca	1.5111985	1.5509132	1.5915931	1.005527
beta0_logCaw	3.4085700	3.4942062	3.5963433	1.033232
beta0_logDaw	2.4031259	2.5224750	2.6206695	1.032590
beta1_Ca	0.0582630	0.0943350	0.1295614	1.001966
beta1_logCaw	0.0362116	0.1016380	0.1653405	1.002494
beta1_logDaw	0.0150388	0.0835517	0.1558483	1.004417
sdCa	0.4154042	0.4468857	0.4805696	1.005201
sdlogCaw	0.5790614	0.6414866	0.7056561	1.023145
sdlogDaw	0.3833122	0.4899050	0.6050677	1.065012

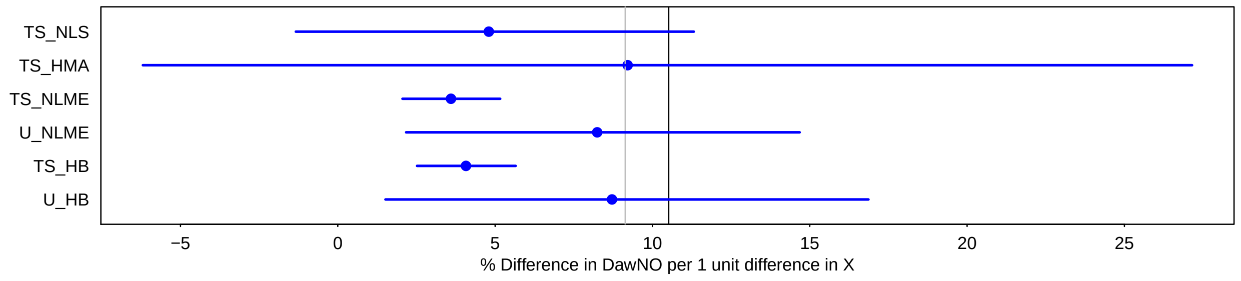
	2.5%	mean	97.5%	Rhat
corlogCawCa	0.5382412	0.6325284	0.7250663	1.010522
corlogCawlogDaw	-0.4646919	-0.3068066	-0.1044257	1.049161
corlogDawCa	-0.4791551	-0.3068774	-0.1284974	1.014665
sigma_c	0.0988964	0.1007370	0.1026550	1.007205

3. Create plot comparing estimated NO parameter associations across 6 methods

- Y axis: 6 methods
- X axis: coefficient effect size: The values used in simulation were all equaled to 0.1 for CANO, logCawNO and logDawNO. Which means for 1 unit increase in the covariate X, the corresponding NO paramters CANO, logCawNO and logDawNO increase 0.1 unit. The geometric interpretation for CawNO was that it was (exp(0.1)-1) times higher for 1 unit increase in the covariate, so was for DawNO
- Refernce lines: Black lines indicate the values used in data simulation. Gray lines indicate the values obtained by regression on the simulated NO parameters and Xs.


TS_NLS
TS_HMA
TS_NLME
U_NLME
TS_HB
U_HB
Difference in CANO, ppb, per 1 unit difference in X


TS_NLS
TS_HMA
TS_NLME
U_NLME
TS_HB
U_HB
% Difference in CawNO per 1 unit difference in X


TS_NLS
TS_HMA
TS_NLME
U_NLME
TS_HB
U_HB
% Difference in DawNO per 1 unit difference in X