CHEST Online Supplement

ADRB2 Polymorphisms and Budesonide/Formoterol Responses in COPD

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e-Appendix 1.

Abbreviations

ADRB2 = β_2-adrenergic receptor gene
ANCOVA = analysis of covariance
BCSS = Breathless Cough and Sputum Scale
BUD = budesonide
CI = confidence interval
COPD = chronic obstructive pulmonary disease
DPI = dry powder inhaler
FEV_1 = forced expiratory volume in 1 second
FM = formoterol
PBO = placebo
PCR = polymerase chain reaction
PEF = peak expiratory flow
pMDI = pressurized metered-dose inhaler
SABA = short-acting β_2-adrenergic agonist.
SGRQ = St. George's Respiratory Questionnaire
SNP = single-nucleotide polymorphism

METHODS

Coprimary End Points

The coprimary clinical end points were predose forced expiratory volume in 1 second (FEV_1) and 1-hour postdose FEV_1.[1,2] Spirometry was performed according to American Thoracic Society recommendations[3] at approximately the same time (±1 hour) in the morning at each clinic visit, approximately 12 hours after the previous dose of study medication. The patient was to refrain from the use of rescue medication for at least 6 hours and ipratropium for at least 8 hours before the clinic visit.[1,2]

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Secondary End Points

A chronic obstructive pulmonary disease (COPD) exacerbation was defined as a worsening of COPD that resulted in treatment with an oral corticosteroid or hospitalization.[1,2] Dyspnea was assessed daily using the validated Breathlessness Diary, which is a single-item measure from the Breathless Cough and Sputum Scale (BCSS) score. The BCSS is a patient-reported outcome for which patients record severity of breathlessness, cough, and sputum in a daily diary before the evening dose of study medication. Each symptom is rated using a 5-point Likert-type scale from 0 to 4, with higher score indicating greater symptom severity. The total score is the sum of the three symptom scores.[4]

Health status was assessed at Months 1, 2, 6, and 12 (Trial I only) during clinic visits using the self-administered St. George's Respiratory Questionnaire (SGRQ).[1,2,5] The SGRQ is a 76-item tool that assesses health status in three areas; symptoms, activities (causing or limited by breathlessness), and impact on daily life. Each section is scored separately on a scale of 0 to 100, with 0 indicating no impairment in quality of life. Total score is based on responses to all items and is calculated using weights attached to each item in the questionnaire.[5] Morning and evening peak expiratory flow (PEF), nighttime awakenings, and rescue medication use were recorded daily in patients' diaries. Nighttime awakenings were assessed on a scale from 0 to 4, with higher scores indicating greater sleep disturbance.

Sequencing for genotyping. Taqman genotyping of 11 β_2-adrenergic receptor gene (*ADRB2*) single-nucleotide polymorphism (SNP) in Studies I and II was verified by direct sequencing in one of eight 384-well sample plates across 3 *ADRB2* coding SNPs: Cys-19Arg, Gly16Arg, and Gln27Glu. The *ADRB2* genomic sequence was exported from online databases. Primers (e-Table 1) were designed to cover the 3-SNP Cys-19Arg_Gly16Arg_Gln27Glu sequence. Primers were tested in CEPH DNA, in 384 well plates using the reagents in e-Table 2.

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PCR was carried out under the following conditions: 95ºC 10 min, followed by 40 cycles at 94ºC 30 sec, 55ºC 30 sec, 72ºC 1 min, with a final extension of 72ºC 10 min. The primers amplified well under these conditions and were carried forward to the 337 study samples. Sequencing was carried out by Sanger methodology[6] on an ABI3770. 100% concordance with TaqMan methodology was shown, giving confidence in the full TaqMan dataset to be used in further analysis.

Haplotype construction. Haplotype construction was performed using the combined de-identified genetic datasets from studies I and II. The imputation was performed for white patients only because low patient numbers in other racial groups precluded accurate prediction of haplotypes in these populations.

The E-M algorithm, as implemented in SNPHAP, was used to estimate frequencies for the 11-SNP haplotype, which were used to impute an 11-SNP haplotype pair for each patient. The 11-SNP haplotype pairs were collapsed to form the 3-SNP haplotype pairs across Cys-19Arg, Gly16Arg, and Gln27Glu. White patients with more than four missing genotypes and those predicted to carry rare haplotypes (< 5% population frequency) were not allocated a haplotype pair and were excluded from downstream haplotype pair analyses.

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Statistical Analyses

All pharmacogenetic analyses were conducted for the Gly16Arg (rs1042713) SNP. Gly16Arg was analyzed as a categorical variable with three levels, one for each possible genotype. The 11 SNPs (rs11958940, rs17778257, rs2895795, rs2053044, rs12654778, rs11959427, rs1042711, rs1042713, rs1042714, rs1800888, rs1042718) were used to infer 3-SNP haplotype pairs Cys-19Arg_Gly16Arg_Gln27Glu (rs1042711_rs1042713_rs1042714), which also were analyzed as categorical variables. Haplotype prediction was carried out independently from, and before, data analysis.

Changes in predose and postdose FEV_1, exacerbations, diary variables, and SGRQ total score were compared from baseline to the average during the treatment period (with no imputation for missing data) using analysis of covariance models, including the effects of treatment, baseline value, country, genotype/haplotype, and genotype/haplotype-by-treatment interaction. For FEV_1 and SGRQ, baseline was defined as the last available value measured before the first dose of randomized treatment. For diary-related end points, including PEF, dyspnea, BCSS total score, nighttime awakenings, and rescue medication use, baseline was defined as the mean of the last 10 days of the run-in period, excluding study day 1.

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e-Tables for Methods section

e-Table 1—ADRB2 *Primers*

	Primer Sequence (M13 tag in capitals)
Forward	ACTGTAAAACGACGGCCAGTgacaagctgagtgtgcagga
Reverse	ACCAGGAAACAGCTATGACCccagaagttgccaaaagtcc

e-Table 2—*PCR Reagents*

Reagent	Stock Concentration	Final Concentration	X1 Volume (μl)
Reddymix (2mM)[a]			11
Primer Mix	2.5 μM each	200 μM each	1.0
DNA	25 ng/μl	1 ng/μl	1.0

[a]Supplied by Thermo Fisher Scientific, Inc.

PCR = polymerase chain reaction.

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RESULTS

e-Table 3—ADRB2 *Gly16Arg Genotype-by-Treatment Interactions for Primary and Secondary End Points*

	P Value (ANCOVA)	
Variable	Study I	Study II
Predose FEV_1, L	0.674	0.197
Postdose FEV_1, L	0.414	0.125
Morning PEF	0.752	0.312
Evening PEF	0.900	0.201
Dyspnea	0.652	0.133
Rescue medication use, puffs/d	0.775	0.428
BCSS	0.378	0.279
SGRQ	0.909	0.648

ANCOVA = analysis of covariance; BCSS = Breathlessness, Cough, and Sputum Scale; FEV_1 = forced expiratory volume in 1 second = PEF, peak expiratory flow; SGRQ, St. George's Respiratory Questionnaire.

e-Table 4—*ADRB2* Cys-19Arg_Gly16Arg_Gln27Glu Haplotype Pair-by-Treatment Interactions for Primary and Secondary End Points

	P Value (ANCOVA)	
Variable	Study I	Study II
Predose FEV_1, L	0.212	0.307
Postdose FEV_1, L	0.368	0.170
Morning PEF	0.776	0.081
Evening PEF	0.513	0.072
Dyspnea	0.979	0.098
Rescue medication use, puffs/d	0.379	0.929
BCSS	0.869	0.304
SGRQ	0.562	0.386

ANCOVA = analysis of covariance; BCSS = Breathlessness, Cough, and Sputum Scale; FEV_1 = forced expiratory volume in 1 second = PEF, peak expiratory flow; SGRQ, St. George's Respiratory Questionnaire.

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FIGURE LEGENDS

e-FIGURE 1. Number of exacerbations requiring hospitalizations per patient–treatment year by *ADRB2* Gly16Arg genotype in Study I (A) and Study II (B).
BUD = budesonide; DPI = dry powder inhaler; FM = formoterol; PBO = placebo; pMDI = pressurized metered-dose inhaler.

e-FIGURE 2. Adjusted mean change from baseline (95% CI) in SGRQ score by *ADRB2* Gly16Arg genotype in Study I (A) and Study II (B).
BUD = budesonide; CI = confidence interval; DPI = dry powder inhaler; FM = formoterol; PBO = placebo; pMDI = pressurized metered-dose inhaler; SGRQ = St. George's Respiratory Questionnaire.

e-FIGURE 3. Adjusted mean change from baseline (95% CI) in predose FEV_1 by *ADRB2* Gly16Arg genotype for patients reversible and nonreversible to albuterol at baseline in Study I (A) and Study II (B).
BUD = budesonide; CI = confidence interval; DPI = dry powder inhaler; FEV_1 = forced expiratory volume in 1 second; FM = formoterol; PBO = placebo; pMDI = pressurized metered-dose inhaler.

e-FIGURE 4. Adjusted mean change from baseline (95% CI) in postdose FEV_1 by *ADRB2* Gly16Arg genotype for patients reversible and nonreversible to SABA at baseline in Study I (A) and Study II (B).
BUD = budesonide; CI = confidence interval; DPI = dry powder inhaler; FEV_1 = forced expiratory volume in 1 second; FM = formoterol; PBO = placebo; pMDI = pressurized metered-dose inhaler; SABA = short-acting β_2-adrenergic agonist.

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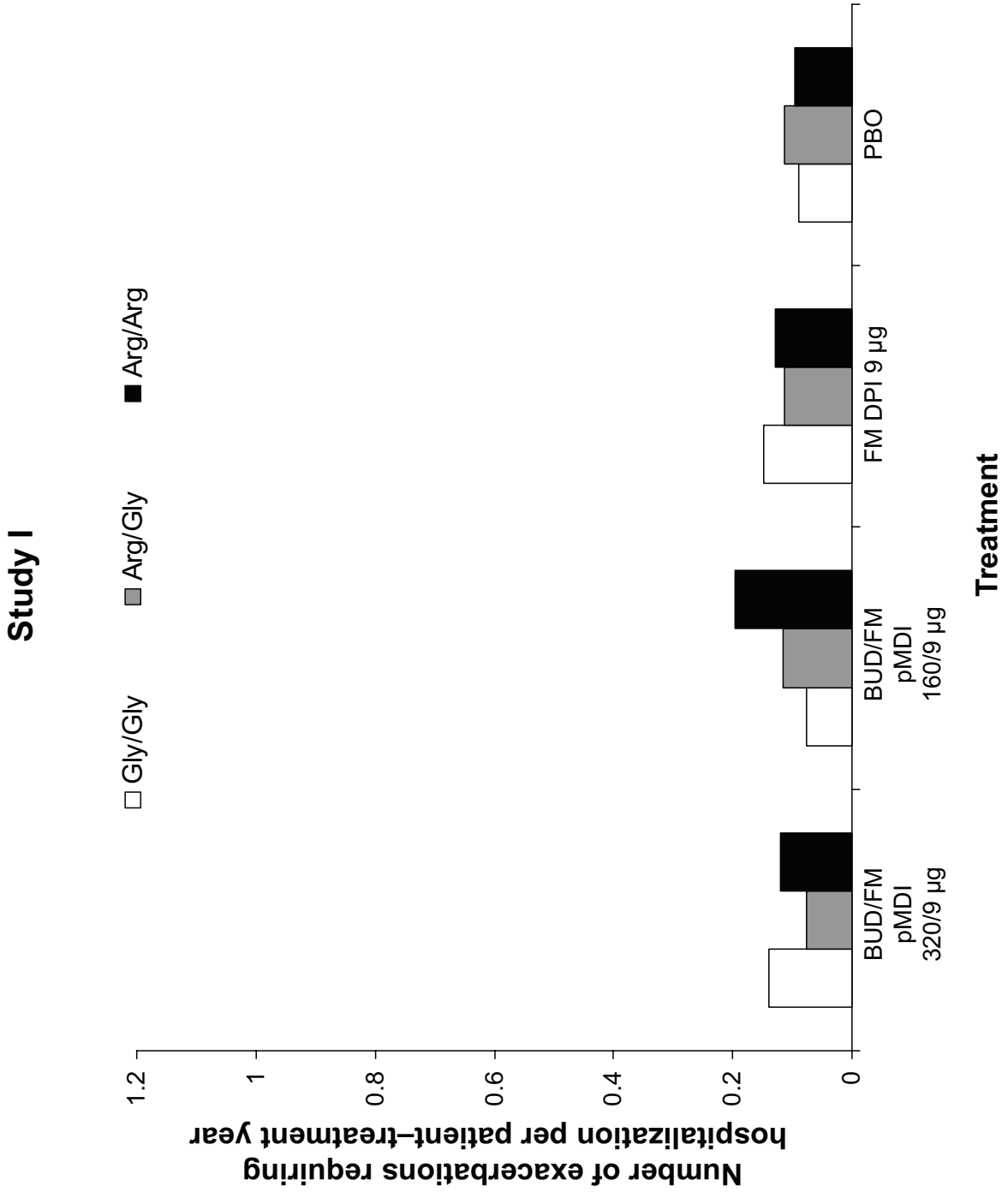
e-Table 1A.



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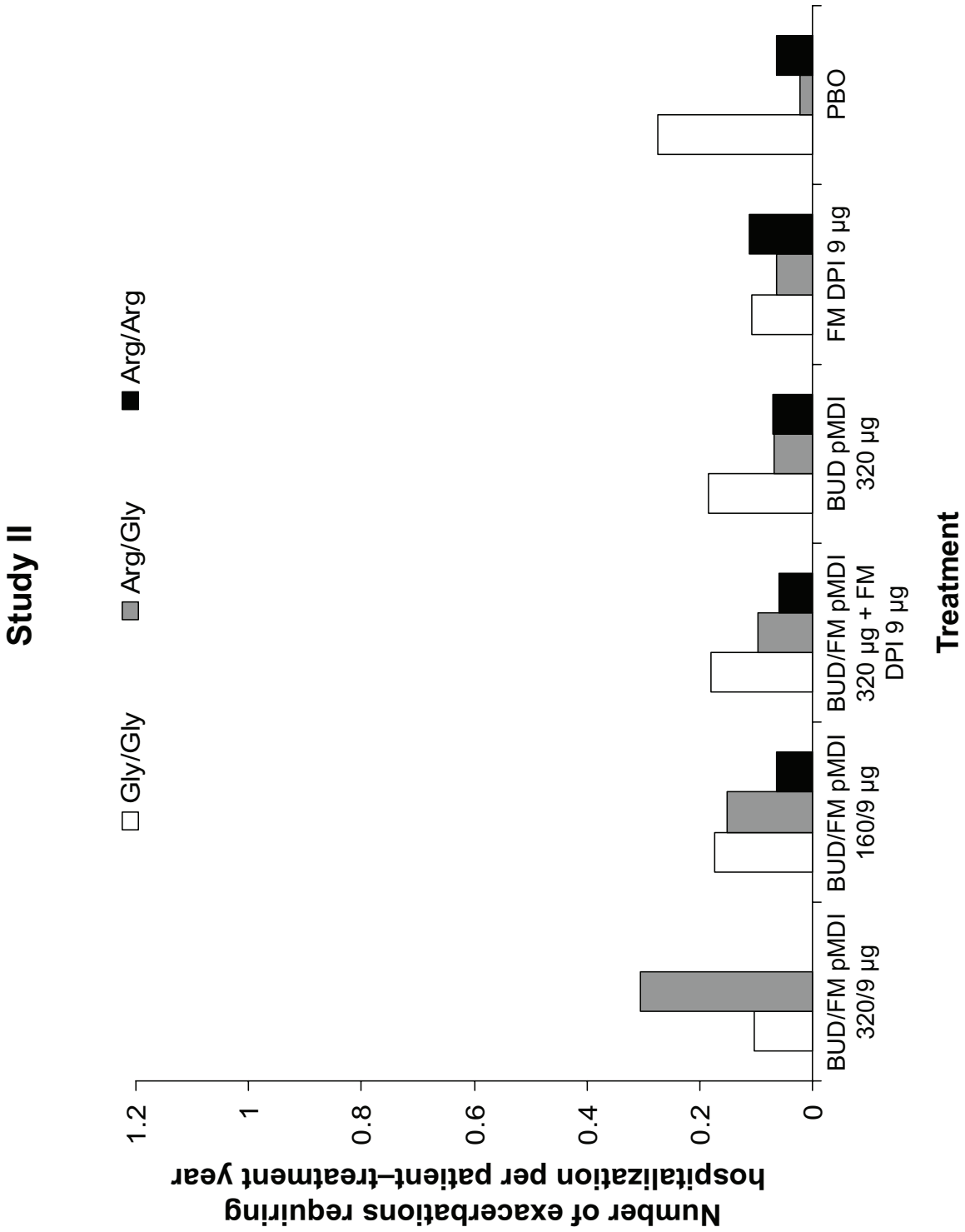

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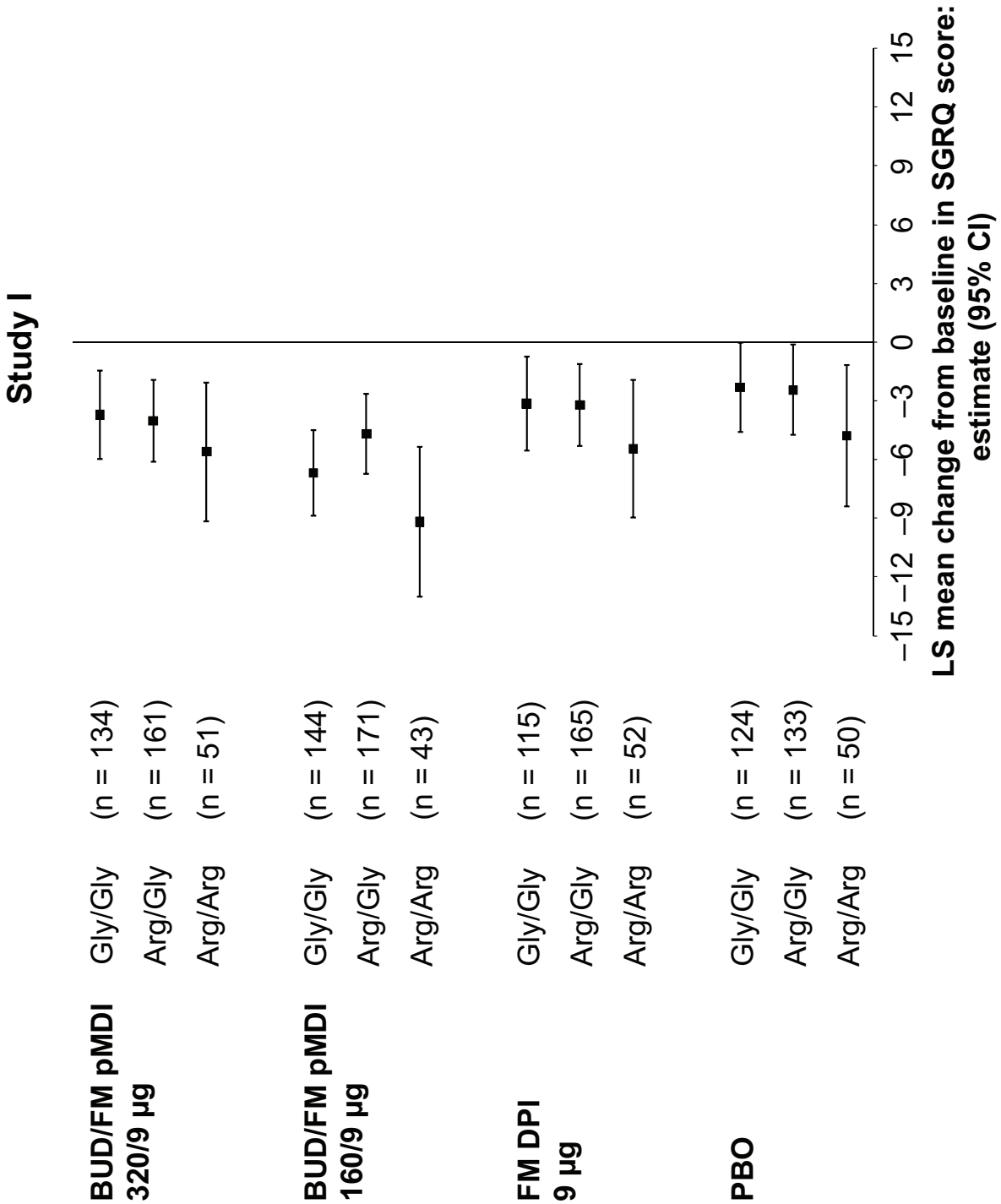

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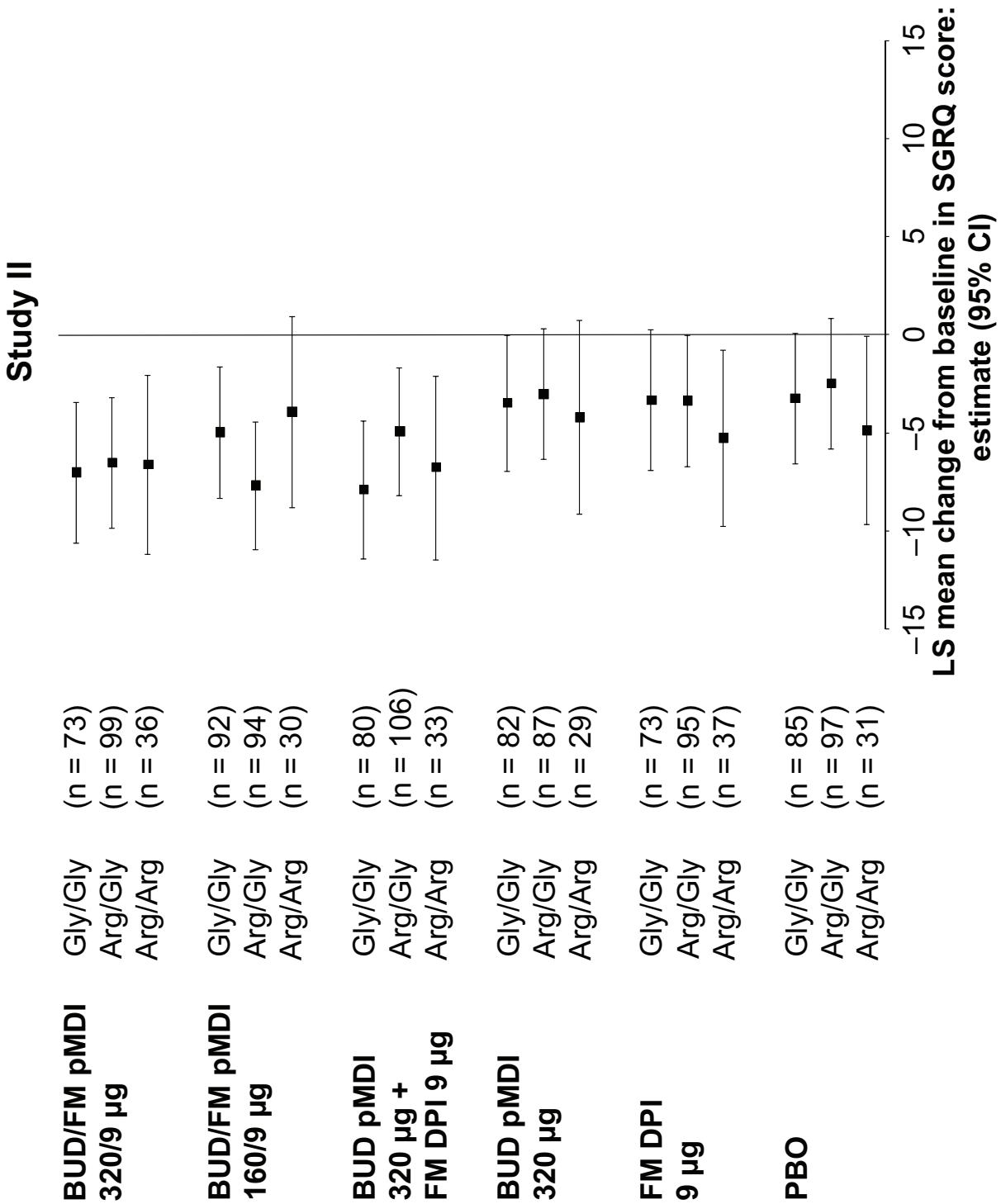

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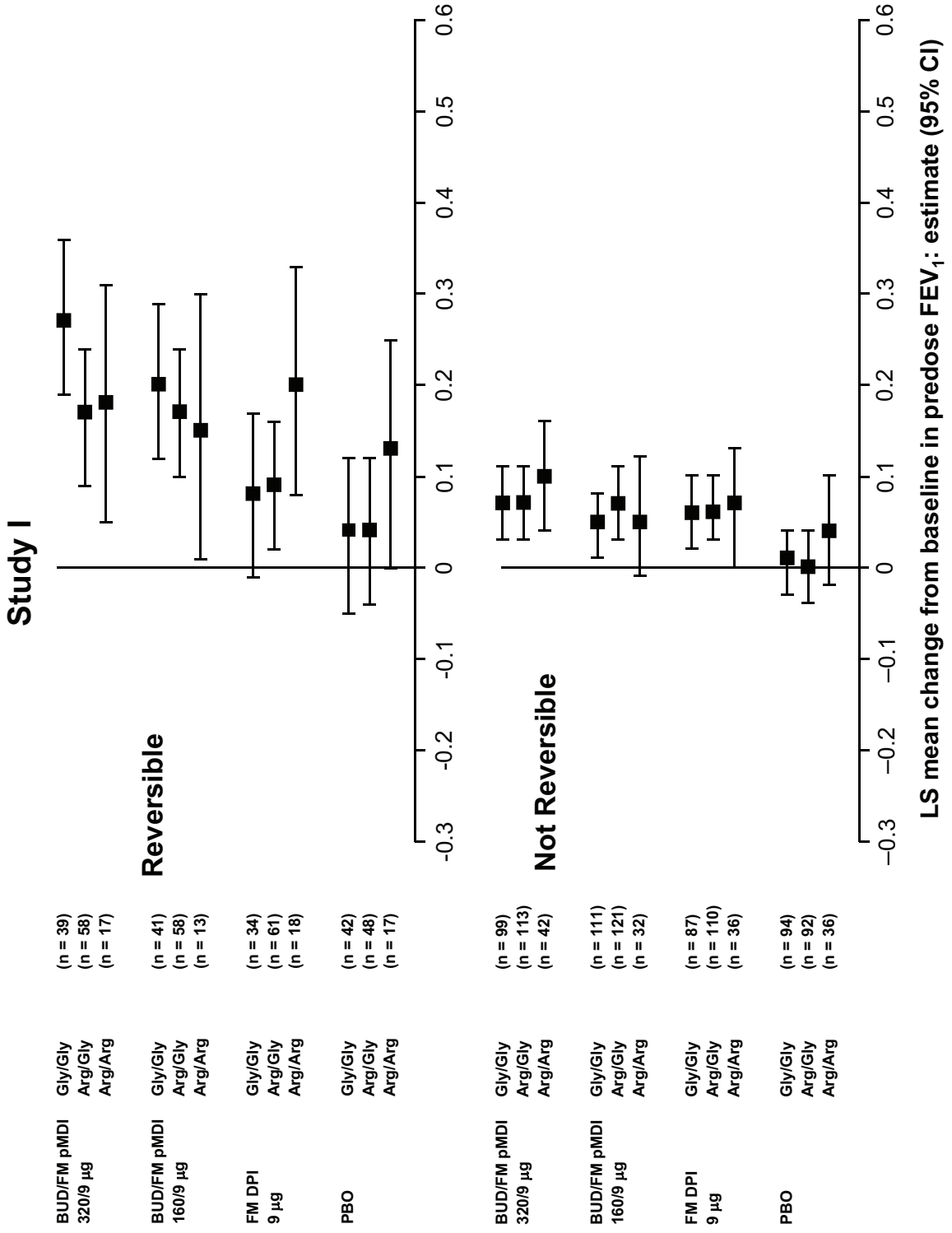
e-Table 3A.



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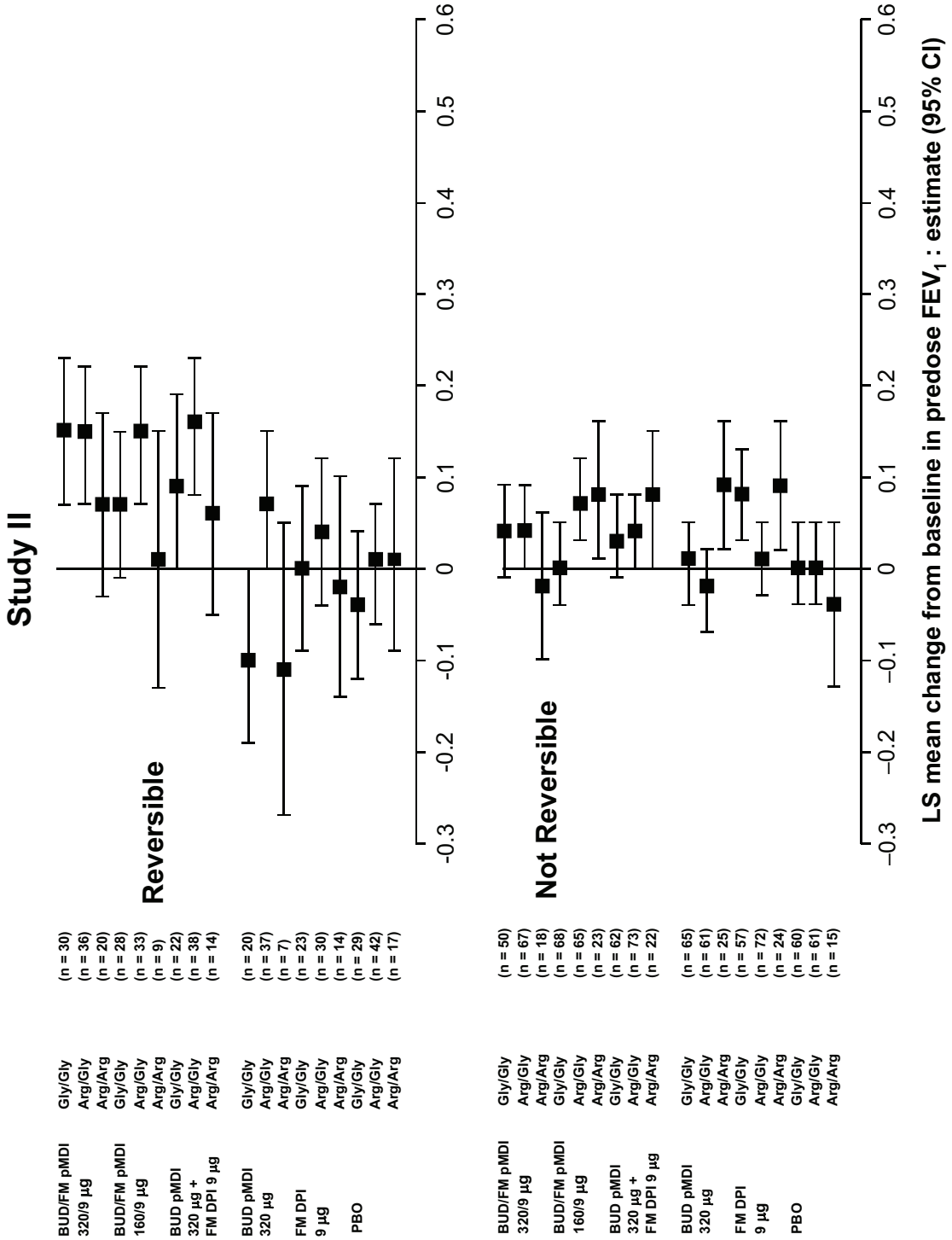
e-Table 3B.



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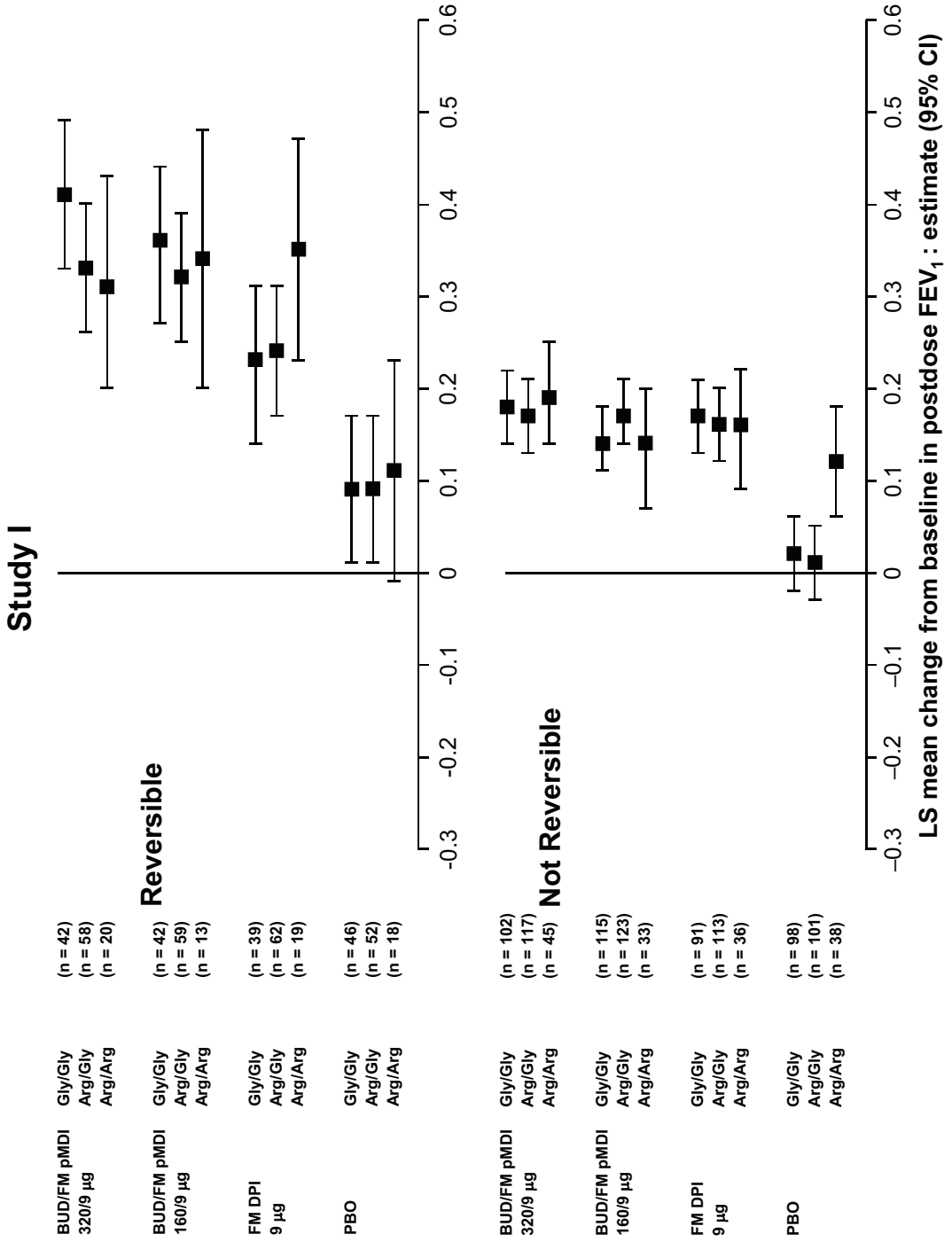
e-Table 4A.



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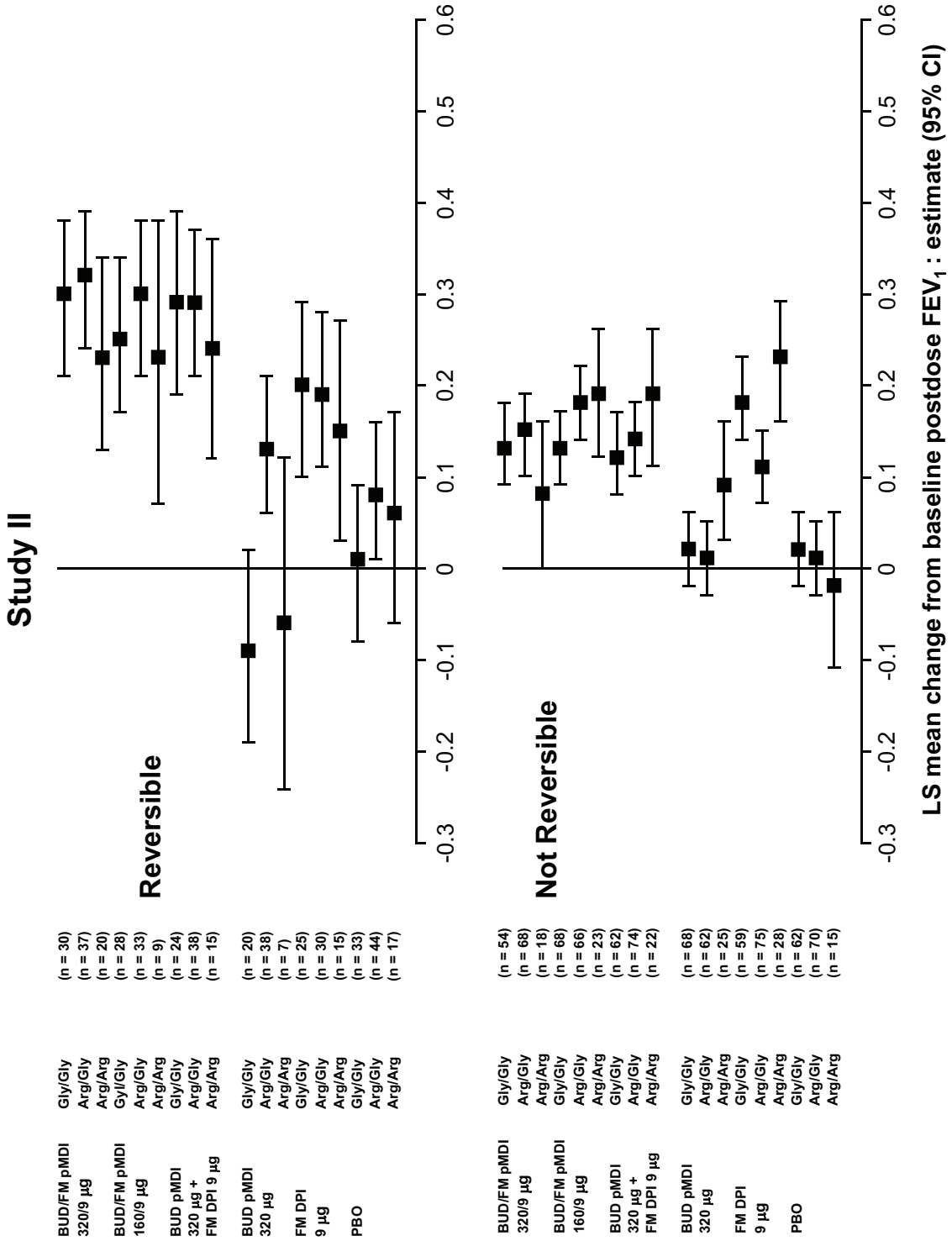
e-Table 4B.



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