Effects of exercise intensity on acute circulating molecular responses post-stroke

Supplemental Methods and Results

Test-retest reliability of serum collection and analyses

To assess the test-retest reliability of the serum collection and analyses across days, we compared the baseline (T0) results across the 4 testing sessions (graded exercise test and 3 exercise sessions). Separate general linear models were used for each variable with a fixed effect for protocol and an unconstrained covariance matrix to account for the within-participant repeated measures. To assess within-session test-retest reliability, we compared the 3 repeated baselines from the graded exercise test session (T-25, T-20 and T0) using a model with a fixed effect for time and a compound symmetry covariance matrix. Intraclass correlation coefficients were also calculated.

Test-retest ICCs ranged from 0.50 to 0.98 for the serum concentrations and there were no significant omnibus test-retest differences. However, T0 VEGF was significantly greater for MCT-treadmill vs. HIT-stepper and VEGF showed a significant decrease from T-25 to the T-20 repeated baseline prior to the graded exercise test (Supplemental Table).

Supplemental Table. Test-retest reliability of serum collection and analyses (N=16)			
	ICC (2,1) [95% CI]	Omnibus p for test-retest differences	Significant pairwise test-retest differences
Vascular endothelial growth factor (VEGF)			
T-25, T-20 & T0 from GXT session	0.97 [0.94, 0.99]	0.0768	T-25 > T-20
T0s from GXT & 3 exercise sessions	0.98 [0.96, 0.99]	0.2061	MCT-treadmill > HIT-stepper
Insulin-like growth factor 1 (IGF1)			
T-25, T-20 & T0 from GXT session	0.96 [0.92, 0.99]	0.3189	None
T0s from GXT & 3 exercise sessions	0.82 [0.67, 0.93]	0.4575	None
Cortisol			
T-25, T-20 & T0 from GXT session	0.79 [0.59, 0.91]	0.3928	None
T0s from GXT & 3 exercise sessions	0.50 [0.25, 0.75]	0.4718	None

ICC, intra-class correlation coefficient; GXT graded exercise test; MCT, moderate intensity continuous training; HIT, high-intensity interval training

Mediating effects of training speed on differences in serum molecular responses between gait speed subgroups

If a serum molecular response differs between participants with different baseline gait speeds, one plausible explanation could be that faster training speeds cause the molecular response differences. To evaluate this potential mechanism, we tested the mediating effects of peak treadmill speed on any differences (or lack thereof) in molecular responses between participants with baseline comfortable gait speed ≥0.4 m/s vs <0.4 m/s.

In these mediation analyses, none of the gait speed subgroup differences in molecular responses were attributable to *faster* peak treadmill speeds for the higher gait speed subgroup (Supplemental Figure). Instead, other between-subgroup differences (aside from speed-mediated effects) significantly decreased BDNF, VEGF and cortisol responses for the higher gait speed subgroup, and these non-mediated effects drove the total effects.
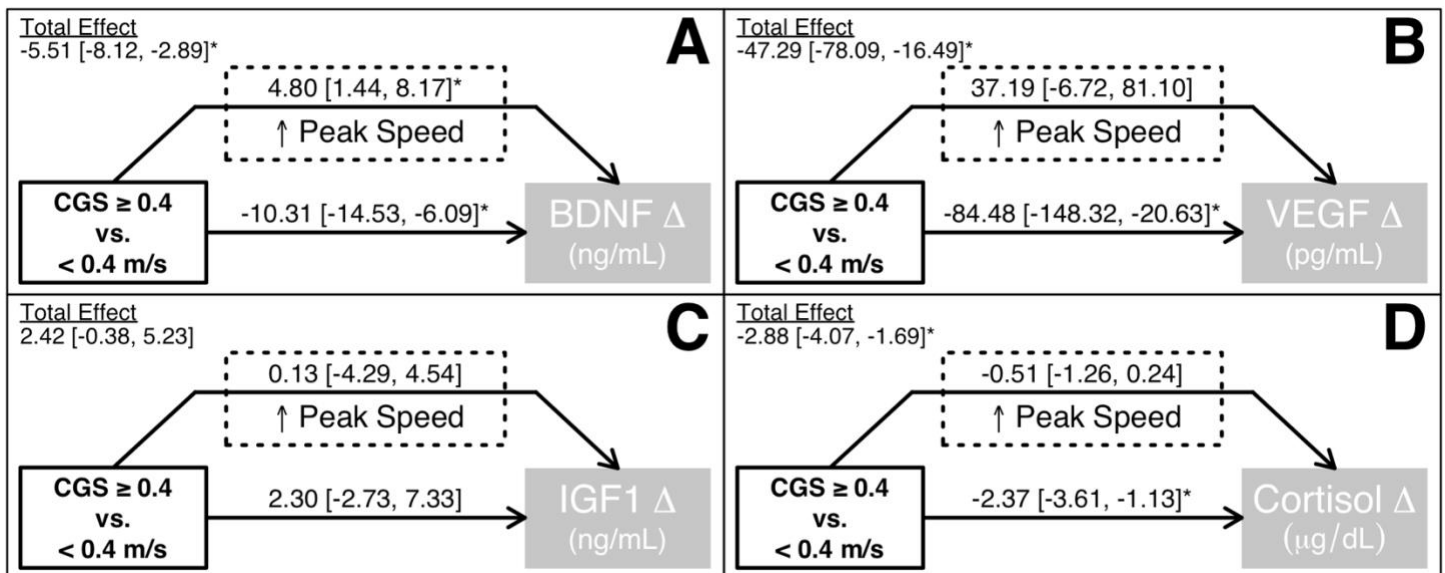
Supplemental Figure. Comfortable gait speed (CGS) subgroup differences in serum molecular responses and mediating effects of peak treadmill speed. Values are model estimates [95% CI] in the units of the molecular response. A mediated effect (arrow going through a dashed box) is the estimated amount of molecular response that is attributable to between-subgroup differences in peak treadmill speed. A non-mediated effect (arrow not going through a dashed box) is the estimated amount of molecular response that is attributable to between-subgroup differences other than peak treadmill speed. A total effect (shown in upper left corner of each panel) is the estimated between-subgroup difference in molecular response from the model and is the sum of the mediated and non-mediated effects. For example, panel A shows that the faster peak treadmill speeds for the higher vs lower gait speed subgroup increased the BDNF response by an estimated +4.80 ng/mL, while other between-subgroup differences decreased the BDNF response by an estimated -10.31 ng/mL, making the total estimated BDNF response -5.51 ng/mL less for the higher vs. lower gait speed subgroup. Biomarker response was the mean of T5, T10 and T20 minus T0. Covariates included gait speed subgroup and baseline molecular concentration. Data were each averaged across both treadmill protocols to obtain mean values for each participant. *Statistically significant ($p<0.05$) effect. BDNF, brain-derived neurotrophic factor; VEGF, vascular-endothelial growth factor; IGF1, insulin-like growth factor 1.