

Associations between cytokines, endocrine stress response, and gastrointestinal symptoms in autism spectrum disorder



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INTRODUCTION

- Autism spectrum disorder (ASD) is characterized by impairments in social communication and abnormal repetitive behavior patterns.
- Recent studies have shown a strong association between ASD and gastrointestinal (GI) symptomatology.
- Some individuals with ASD show altered reactivity to stress, as well as altered immune markers, particularly stress-responsive cytokines including TNF- α and IL-6.
- To assess potential relationships between GI symptoms and stress response, we examined whether GI symptoms are associated with increases in stress-associated endocrine markers and cytokines in ASD.
- We also conducted exploratory analyses to examine the relationship between IL-6, TNF- α , cortisol, and intelligence, as well as the effects of the presence or absence of co-occurring medical conditions on the relationship between IL-6, TNF- α , cortisol, and GI symptoms.
- Given the aforementioned findings, we expected to find positive relationships between GI symptoms and biomarkers of stress, including cortisol levels, IL-6, and TNF- α .

METHODS

Participants
120 participants diagnosed with ASD (6-18 years of age) were recruited from the Autism Speaks Autism Treatment Network at the University of Missouri Thompson Center for Autism & Neurodevelopmental Disorders and Vanderbilt University. Of the 120 participants, 107 blood samples were suitable for IL-6 analysis and 105 blood samples were suitable for TNF- α analysis. A total of 81 pre-stress and 79 post-stress salivary cortisol samples were suitable for analysis.

Assessment of Gastrointestinal Symptomatology
The parent-report version of the Pediatric Gastrointestinal Symptoms-Rome III (QPGS-RIII) was used to assess gastrointestinal symptomatology. Continuous scores were computed using a scoring rubric to create upper and lower GI tract scores.

Salivary Cortisol
Salivary cortisol samples were obtained before and after participants were subjected to the stressors. Samples were stored at -80°C until analysis. Samples were run in duplicate and absorbance was measured using a Spectramax M3 plate reader (Molecular Devices, Sunnyvale, CA).

IL-6 and TNF- α
Fasting blood samples were collected at the beginning of the study. Blood serum was extracted via centrifugation and the samples were stored at -80°C until analysis. The samples were analyzed using Enzyme-linked Immunosorbent Assay kits (ELISA) in duplicate. Absorbance was measured using a Spectramax M3 plate reader.

Stress Reactivity Protocol
Participants sat quietly for three minutes before applying environmental stimuli in order to obtain baseline data. Cold pressor and vibrotactile stimuli were applied independently to the right and left hands of the participants for 30 seconds each with 3 minute breaks between every stimulation.

Assessment of Intelligence and Co-occurring Medical Conditions
The full scale intelligence quotient (FSIQ) for each participant from the AS-ATN database (Wechsler Intelligence Scale for Children, Wechsler Abbreviated Scale of Intelligence, or Stanford-Binet) was used as a measure of general intelligence. Co-occurring medical and psychological disorders were assessed using a yes or no format questionnaire to query history of any of the following: anxiety disorder, depression, loss of skills/regression (i.e. losing previously acquired skills such as speech or social skills), or seizures.

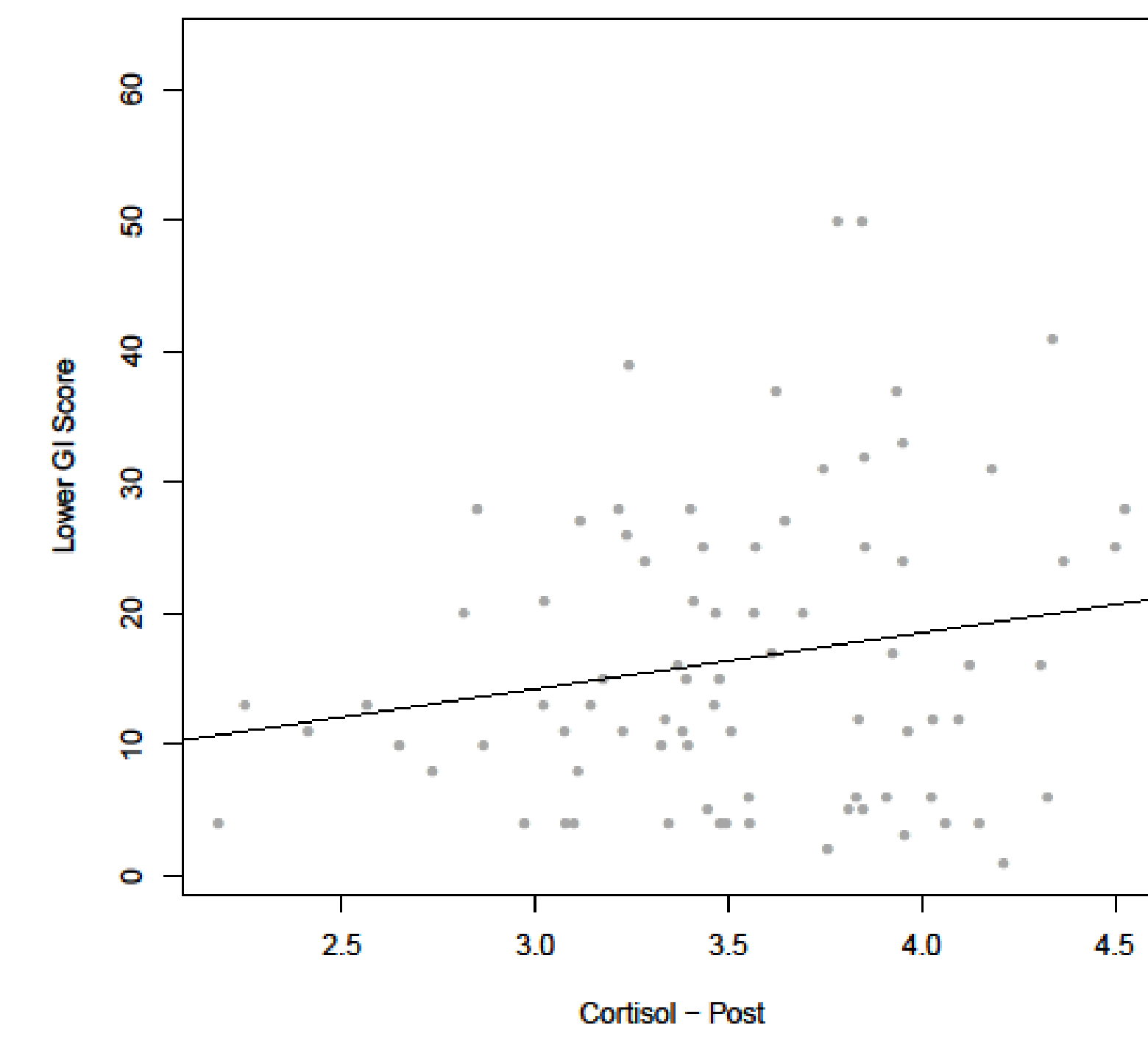


Figure 1. Linear regression for post-stress cortisol concentration and lower GI tract score from the QPGS Rome III. $p = 0.011$.

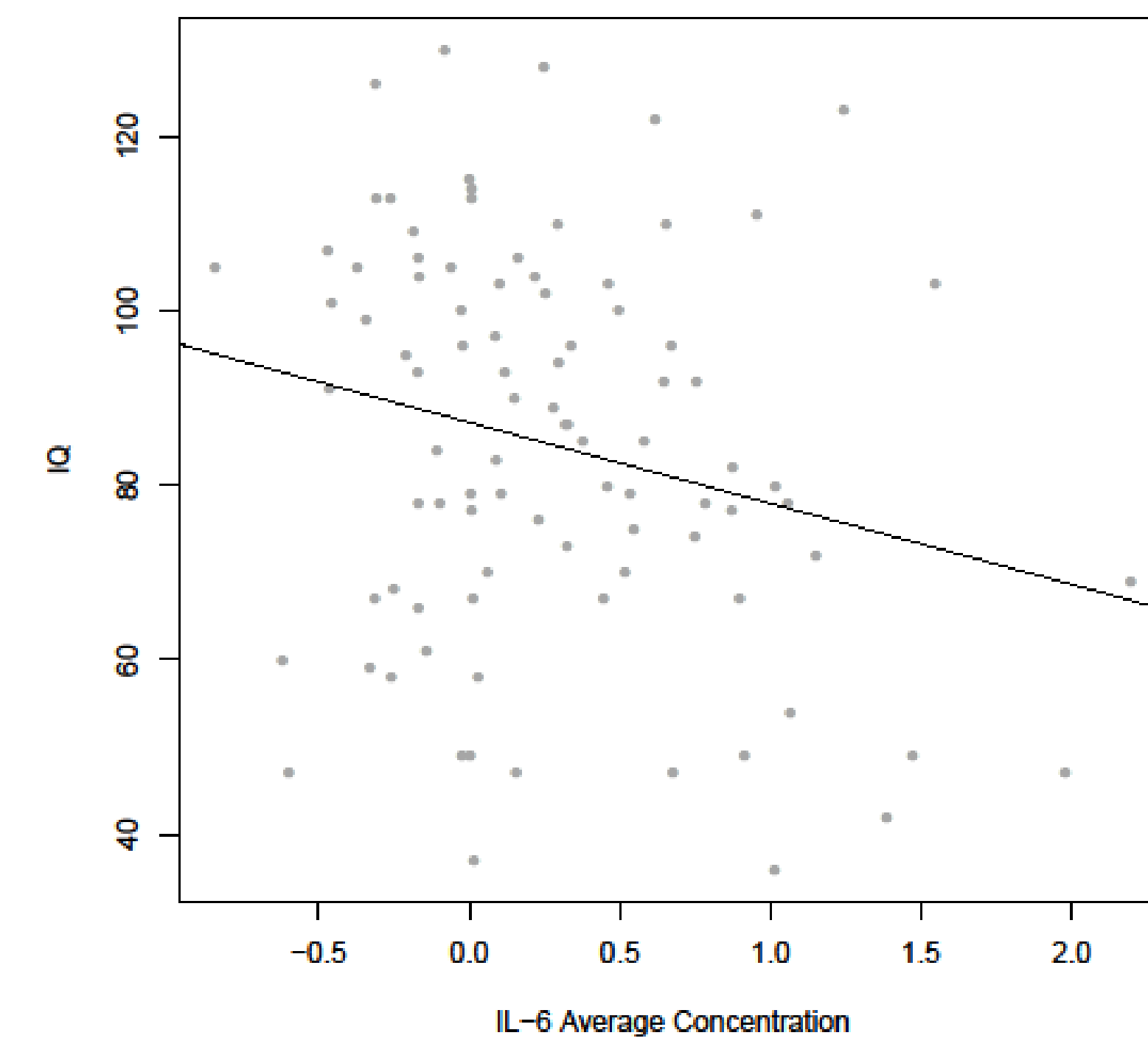


Figure 2. Linear regression for IL-6 concentration and full scale intelligence quotient. $p < 0.01$.

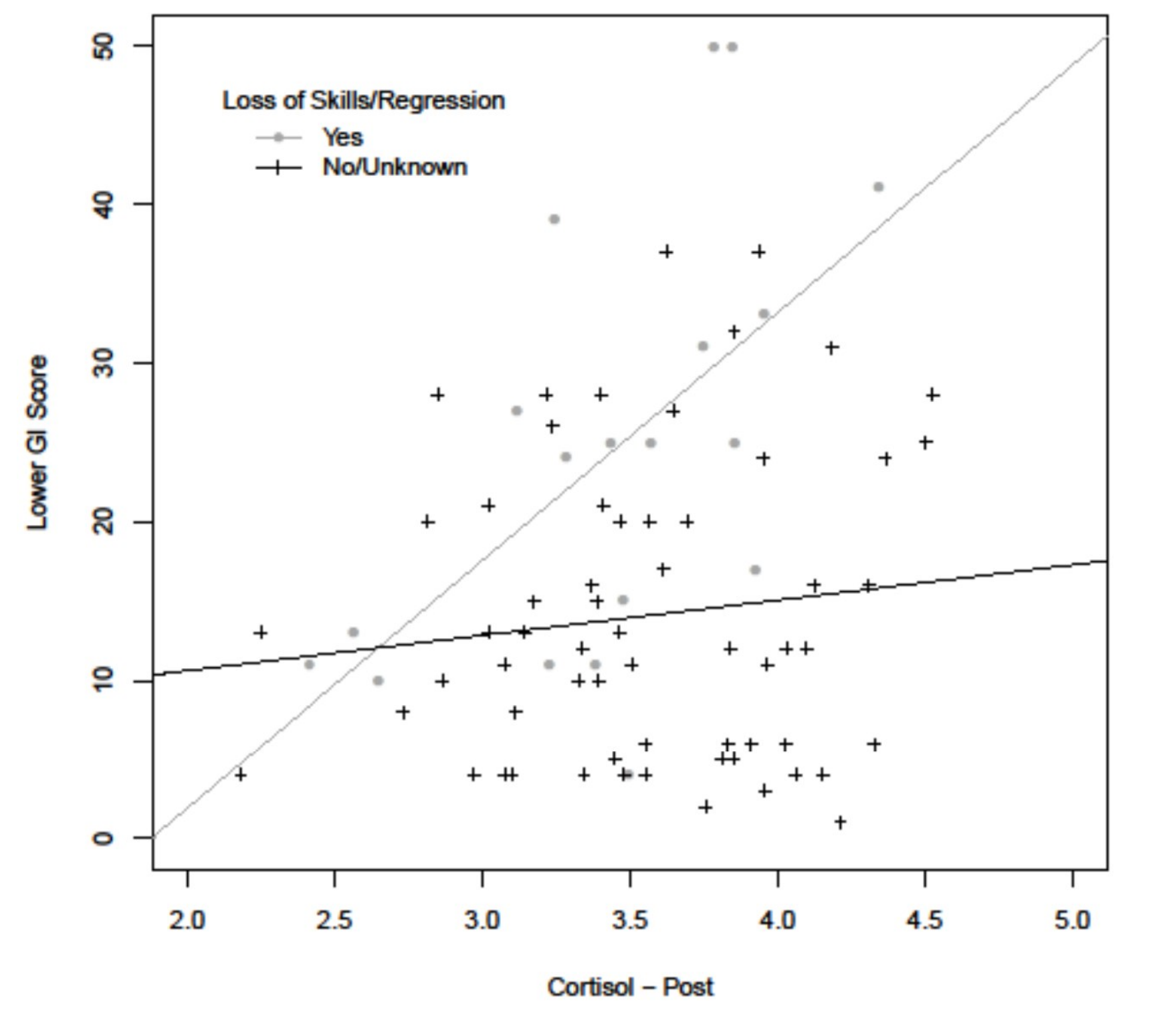


Figure 3. Effects of the presence or absence of loss of skills/regressive autism on the relationship between post-stress cortisol concentration and lower GI tract score from the QPGS Rome III. $p = 0.011$.

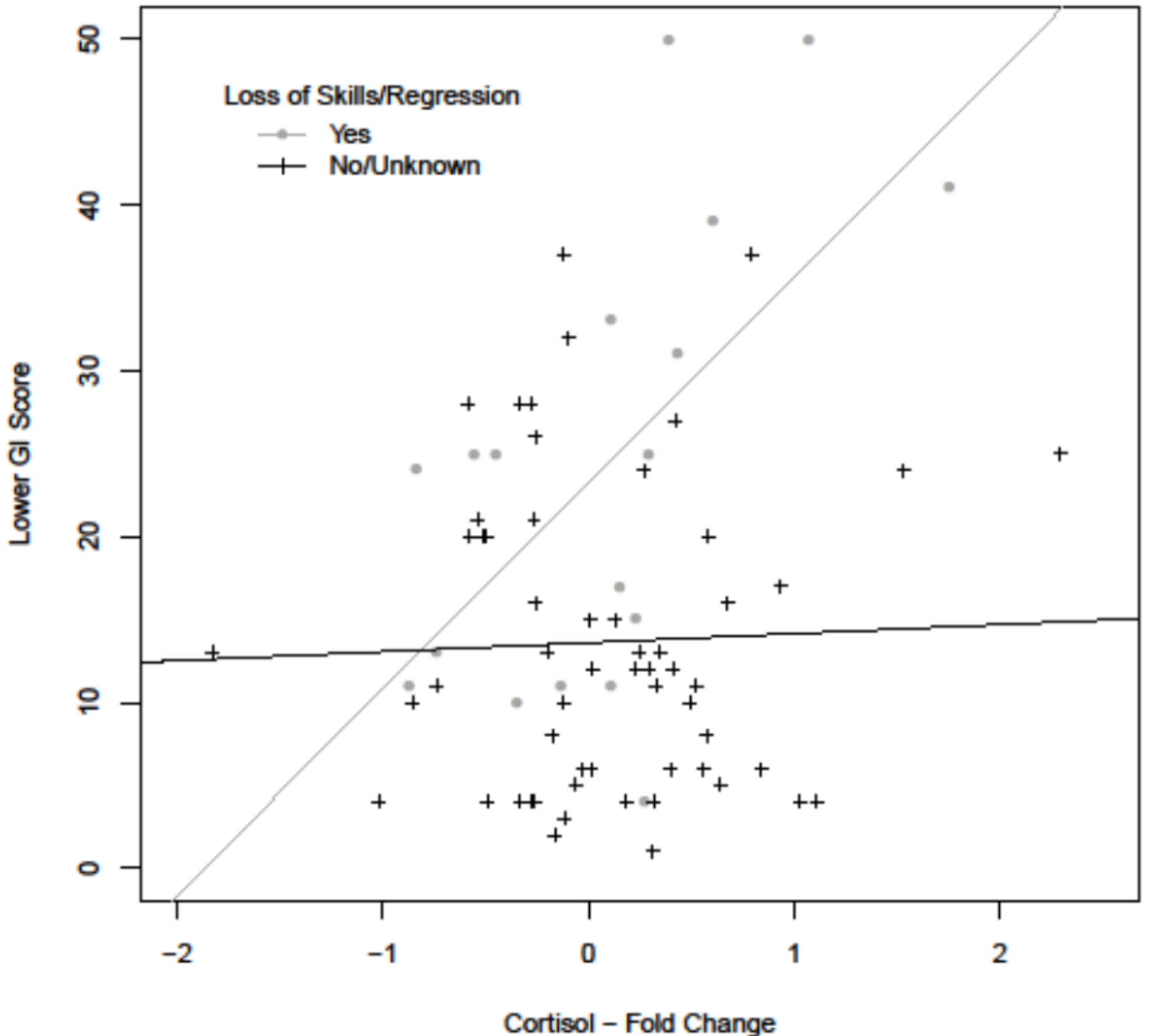


Figure 4. Effects of the presence or absence of loss of skills/regressive autism on the relationship between the fold change in cortisol concentration and lower GI tract score from the QPGS Rome III. $p = 0.004$.

RESULTS

Cortisol

- A significant positive correlation was found between post stress cortisol and lower GI tract symptom score: $p = 0.011$, $r = 0.28$, 95% CI = [0.06 – 0.48]. See Figure 1.
- A trend towards positive correlation was found between fold change cortisol concentration and lower GI score: $p = 0.086$, $r = 0.20$, 95% CI = 0.03 – 0.41.

IL-6 and TNF- α

- While controlling for age and gender, a significant positive correlation was found between TNF- α and upper GI tract symptom score: $p = 0.039$, $r = 0.20$, 95% CI [0.01 – 0.38].

Exploratory Analysis

- A robust negative relationship between IL-6 and FSIQ was observed: $p < 0.01$, $r = -0.28$, 95% CI = [-0.46 – -0.08]. See Figure 2.
- A significant negative relationship between IL-6 and Vineland Socialization domain scaled score was found: $p = 0.02$, $r = -0.27$, 95% CI = [-0.47 – -0.05]

Potential Effect Modifiers

- Loss of skills or regression was found to be a significant effect modifier for the relationship between lower GI tract symptom score and post-stress cortisol ($p = 0.011$, slope in absence of regression=2.22, slope in presence= 15.64), as well as for the fold change in cortisol concentration ($p = 0.004$, slope in absence of regression = 0.54, slope in the presence of regression = 12.44). See Figures 3 and 4, respectively.

SUMMARY

- Individuals with ASD and significant lower GI tract symptoms displayed a greater stress response, as indicated by higher levels of salivary cortisol, than those with few or no lower GI tract symptoms. However, higher levels of cortisol did not translate to an effect on the pro-inflammatory cytokines, TNF- α and IL-6.
- Higher concentrations of TNF- α were observed in participants with upper GI symptomatology as noted in previous research, but this was not related to increased post stress cortisol concentration.
- The presence of regressive autism significantly modified the relationship between post-stress cortisol and lower GI tract symptoms, suggesting increased risk for lower GI symptoms in those with ASD who lose previously acquired skills.
- Individuals with ASD and relatively high concentrations of IL-6 also had the lowest levels of intelligence, warranting further investigation.
- In light of the relationship between cortisol and lower GI tract symptoms, future studies are warranted to explore whether amelioration of the stress response might be an important aspect of treatment of some GI disorders in ASD.

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