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Association Between Fatty Acid Supplementation and Prenatal Stress in African Americans: A Randomized Controlled Trial

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Abstract

Objective—To test the association between docosahexaenoic acid (DHA)supplementation and perceived stress and cortisol response to a stressor during pregnancy in a sample of African American women living in low-income environments.

Methods—Sixty-four African American women were enrolled at 16–21 weeks of gestation. Power calculations were computed using published standard deviations for the Perceived Stress Scale and the Trier Social Stress Test. Participants were randomized to either 450 mg per day of DHA(n=43) or placebo (n=21).At baseline, 24, and 30 weeks of gestation, perceived stress was assessed by self-report. Cortisol response to a controlled stressor, the Trier Social Stress Test (TSST) was measured from saliva samples collected upon arrival to the laboratory and after the completion of the TSST.

Results—Women in the DHA supplementation group reported lower levels of perceived stress at 30 weeks of gestation, controlling for depression and negative life events (mean = 27.4 versus 29.5, (F [3, 47] = 5.06, p = .029, *cohen's d* = .65). Women in the DHA supplementation had lower cortisol output in response to arriving to the laboratory and a more modulated response to the stressor (F [1.78, 83.85] = 6.24, p = .004, *cohen's d* = .76).

Conclusions—Pregnant women living in urban low-income environments who received DHA reported reduced perceived stress and lower levels of stress hormones in the third trimester. DHA supplementation may be a method for attenuating the effects of maternal stress during late pregnancy and improving the uterine environment with regard to fetal exposure to glucocorticoids.

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Introduction

Consistent with the prenatal programming hypothesis,[1] there is now evidence from multiple studies using a variety of methodologies and across different species that the mother's level of psychosocial stress during pregnancy is significantly associated with suboptimal developmental outcomes in their offspring including disturbances in attention,[2–3] impaired learning and disruption in neurogenesis,[4–5] and increased anxiety-like behaviors.[2] The strength of the causal claim is based on rigorous controlled experiments in which the prenatal effect is distinguished from postnatal effects by using methods such as cross-fostering or nursery rearing. The pattern of findings in humans closely mirrors those from controlled animal studies.[6–9] The strongest candidate for the mechanism by which prenatal stress confers risk to the offspring is the maternal hypothalamic-pituitary-adrenal (HPA) axis,. Prenatal stress causes long-term alterations in the functioning of the offspring's HPA axis,[10–11] and each of the phenotypic outcomes identified above can be linked with disruptions in the HPA axis.

A few investigators have examined the effects of DHA supplementation during pregnancy on later developmental functioning. Neuroprotective effects of DHA supplementation during pregnancy on the offspring has been reported in controlled animal studies[12–14] In humans, fatty acid supplementation is also associated with reductions in stress reactivity in controlled studies.[15–18] These data converge to support the hypothesis that prenatal DHA supplementation among women living in stressful environments would lead to reductions in perceived stress and greater modulation of the activation of the HPA axis in response to stress.

Materials and Methods

Pregnant women were recruited from Obstetrics Clinics within the University of Pittsburgh Medical Center from 2010–2012. Sample size was determined based on power calculations using existing data on perceived stress and cortisol response to the Trier Social Stress Test (TSST) among pregnant women. Given the goals of the study, only demographically eligible women were approached for screening. Demographic eligibility included: Medicaid insurance or Medicaid eligible, African American race, age between 20 and 30 years, and 16–21 weeks of gestation.

In addition to African American women being disproportionately represented among families living in inner-city poverty in the U.S. and having higher rates of suboptimal birth outcomes,[19] there are race differences in cortisol reactivity to stress and pregnancy.[24–26] In order to control for these group differences either adequate numbers of participants of different races would need to be included or the study would have to be limited to a single race. Given the scope of the study, we chose to study African Americans only. We limited the sample to ages 20–30, which comprises over 60% of pregnancies among African American.[27] This range excluded younger and older ages of mothers during which the risk for suboptimal pregnancy outcomes increases.

Power calculations were computed using published data: the standard deviation (SD) of perceived stress using the Perceived Stress Scale was estimated to be 7.4.[28] Forty participants in the supplement group and 20 in the placebo group yielded 80% power to detect a difference in stress levels of approximately 4 between the two groups using a two-sided test at the 0.05 significance level. The SD of the cortisol response to the TSST at 20 minutes was estimated to the equal to 8 nmol/L.[29] Forty participants in the supplement group and 20 in the placebo group yielded 80% power to detect a difference in peak response of approximately 6 nmol/l between the two groups using a two-sided test at the 0.05 significance level.

Women were recruited using two methods. First, research assistants attended obstetric clinics and provided fliers to patients that listed the demographic inclusion criteria (i.e., African American race, Medicaid insurance, gestational age between 16 and 21 weeks, and maternal age between 20 and 30 years) asking those eligible to complete the screening. Second, demographically eligible patients identified through electronic medical records were contacted by mail and phone to assess interest in the study. Telephone screenings were then conducted to assess eligibility. Exclusion criteria included2 or more servings of sea fish per week, known medical complications (gestational diabetes, pre-eclampsia, subchorionic hematoma), regular use of steroid medications, regular alcohol use, cigarettes or use of illegal substances (by maternal report), use of blood thinners or anti-coagulants, use of psychotropic medications, BMI >40, and allergy to iodine or soy. One hundred forty-six participated in screening for eligibility. Of those screened, 26 were ineligible, 64 were eligible and enrolled, 48 were eligible at time of screening but could not be enrolled prior to the enrollment window (i.e., 16–21 weeks of gestation), and 8 refused to participate (see Figure 1).

Participants were reimbursed on an accelerated schedule with $40 for their first visit and an increase in payments of $10 for each subsequent visit. The Institutional Review Boards of the University of Chicago and University of Pittsburgh approved all study procedures. This study also was a registered clinical trial (NCT01158976).

Once enrolled, women were randomly assigned on a 2:1 ratio to receive the omega-3 nutritional supplement (n = 43) or a corn and soybean oil placebo (n = 21) beginning at enrollment and up through the end of pregnancy. We expected greater variability in the dependent measures (e.g., stress reactivity) among the experimental participants than the control patients. Thus, in order to optimize power to test the hypotheses, we enrolled a higher number of participants in the experimental group to adequately capture that variability. Women randomized to the study group received the supplement via two gel capsules providing: 450 mg of DHA; 40 mg of docospentaenoic acid and eicosatetranoic acid; 90 mg of eicosapentaenoic acid; and 15 IU of vitamin E,, supplied by Nordic Naturals. Women receiving the placebo received two capsules supplied by Nordic Naturals that were matched in size, color, and smell to the study drug. The pharmacist at the University of Pittsburgh used computer generated random assignment of ID numbers to active supplement or placebo in blocks of nine: six ID numbers were randomized to active supplement and three to placebo. In order to maintain the double-blind, the pharmacist divided each randomization block of nine ID numbers into groups of three: three ID numbers were

assigned to group A (placebo) and the remaining six were assigned to either group Bor C, both of which received identical doses of active supplement. This approach allowed the pharmacist to randomize on a 2:1 ratio without having the unbalanced design break the blind. Once the study was complete, the blind was broken and the two groups receiving identical doses of active supplement were combined for analyses.

Cortisol response to a social evaluative stressor was measured at baseline, 24 and 30 weeks of gestation. Participants completed questionnaires covering stressful life events, perceived stress, and symptoms of depression at baseline, 24, and 30 weeks of gestation. Research assistants contacted participants by phone 3 times per week to ask the time of day that the supplement was taken, and gathered data on perception of taste, and possible gastrointestinal side effects to increase compliance.

Symptoms of depression were assessed using the *Edinburgh Postnatal Depression Scale*,[30] a 10-item measure designed to assess pre- and postnatal depression without confounding the assessment of depression with somatic symptoms of pregnancy (e.g., weight gain, loss of energy). Internal consistency of in this sample was high: alpha = .88 at baseline; .86 at 24 weeks; .87 at 30 weeks. The *Difficult Life Circumstances Scale*[31] is a set of 28 yes-no questions about difficult circumstances at home or work that may be a problem for the primary caregiver. The measure was designed to include items that would be applicable to women living in poverty such as difficulty with finances and housing. The internal consistency of the scale as measured by alpha ranged from .73 to .80. The *Perceived Stress Scale*[32] is a 14-item scale designed to measure the degree to which situations in one's life are appraised as stressful. The internal consistency of the scale as measured by alpha was high: .85 at baseline; .88 at 24 weeks;.87 at 30 weeks.

Following published recommendations for assessing physiological stress reactivity during pregnancy,[33] the Trier Social Stress Test (TSST)[34] was used as a psychological stressor. The TSST procedure consists of a two-minute preparation time, followed by a 5-minute speech (as if for a job interview), and then a 5-minute mental arithmetic task. The latter two tasks are performed in front of a video camera and an audience. The TSST typically elicits individual differences in cortisol reactivity, even during pregnancy.[29,35]

Saliva was collected at three time points at the baseline, 24 and 30 week assessments: 20 minutes following arrival to the lab, and 20, 45 minutes post-TSST. To collect saliva samples, an absorbent, unflavored dental roll was applied to the tongue, cheek, and gums for several minutes. The dental roll was then placed in a labeled plastic salivette. Samples were immediately transferred to a freezer and stored at −20°C until assayed. On the day of testing, samples were thawed, centrifuged at 3,000 rpm for 10 minutes allowing for a clear sample to be pipetted into appropriate test wells. All samples from each subject were assayed in the same batch to minimize variability, and assayed with reagents from the same lot. Samples with sufficient saliva were assayed in duplicate using the Salimetrics HS Salivary Cortisol EIA Kit for unbound cortisol. This assay has a lower limit of sensitivity from .007 to 1.2 μg/dL. The average between-assay variance is 3.9% and 7.1%, and the average within-assay variance is 6.7% and 6.9% for high and low concentrations, respectively. The correlation between saliva and serum using the Salimetrics HS Salivary Cortisol EIA Kit and the Coat-

a-Count Serum Cortisol RIA kit is .96, $p < .0001$. Analyses were conducted with $\log_{10}$-transformed cortisol values, but are presented as untransformed μg/dL for ease of interpretation.

Results

Of the 64 participants who completed the baseline assessment, four (6.3%) withdrew from the study: two participants from each study arm. Two participants randomized to placebo withdrew due to adverse events: one miscarried and the other withdrew due to mood changes. Two participants randomized to active supplement withdrew due to adverse events: one reported headaches and the other upset stomach. Forty-seven participants (73.4%) attended all three sessions. Descriptive statistics are presented in Table 1 for depression, negative life events and perceived stress at baseline, 24, and 30 weeks of gestation. Data collected from all participants, including those who withdrew, are included in the analyses. Baseline self-report data for one participant in the active group was lost. One participant, with undetectable cortisol values for five of the nine samples, was not included in the analyses of cortisol response.

The first goal was to examine group differences in level of depression, negative life events, and perceived stress by conducting analyses of variance for each measure, controlling for the other two measures. At baseline and 24 weeks of gestation, there were no group differences in any of the three measures. At 30 weeks, perceived stress was significantly lower among the participants receiving supplementation (mean = 27.4) compared to those receiving placebo (mean = 29.5), controlling for negative life events and depression scores at 30 weeks (F [3, 47] = 5.06, p = .029, *cohen's d* = .65 (Figure 2), a difference of more than half of a standard deviation for the sample.

Cortisol response to the TSST was examined as a function of group status (supplementation versus placebo) at baseline, 24 and 30 weeks of gestation by repeated measures analysis of variance, using a Greenhouse-Geisser correction to account for lack of sphericity. Time of day was significantly associated with initial cortisol levels at baseline, but not at 24 and 30 weeks; time of day was controlled in analyses involving cortisol levels at baseline.

Cortisol levels before and after the TSST did not vary as a function of supplementation at baseline or at 24 weeks. At 30 weeks, cortisol levels over time significantly differed as a function of supplementation (F [1.78, 83.85] = 6.24, p = .004, *cohen's d* = .76). As shown in Figure 3, women who received placebo had higher levels of cortisol upon arrival to the lab compared to women receiving omega-3 supplementation. Levels for women receiving placebo were characterized by a relatively steep decline, whereas levels for women receiving omega-3 supplement evidenced a slight increase in response to the stressor, on average, followed by decline during the period of recovery. To further probe the differences in levels upon arrival to the laboratory, the two groups were compared at baseline, 24 weeks, and 30 weeks of gestation. As shown in Figure 4, cortisol levels upon arrival to the lab were similar for the two groups at baseline, but diverged over time such that by 30 weeks of gestation women who received placebo had levels that were on average 20% higher than

women receiving supplement (mean = 0.35 versus 0.27) (F [1.74, 74.63] = 3.51, p = .041, *cohen's d* = .56).

Discussion

The present study was conducted from the perspective of the prenatal programming hypothesis.[1] Specifically, a primary hypothesized mechanism by which prenatal stress affects offspring development is through exposure of the fetus to high levels of glucocorticoids released by the mother. This exposure, in turn, affects the fetal stress architecture in part by adjusting the set point for mounting a stress response and interfering with the feedback mechanisms for maintaining homeostasis. Although the postpartum environment continues to affect brain development, it is plausible that this initial insult sets the stage for a poor developmental trajectory that begins with poorly modulated response to stress in infancy.

Results from the present study provide preliminary evidence that changes in prenatal nutrition may be one way to interrupt the suboptimal prenatal programming of the fetus developing in the context of high levels of maternal stress. Pregnant women living in high stress, low-income, urban environment who received a fatty acid supplement reported lower levels of perceived stress than women who received placebo, despite a lack of change in exposure to stressors. Moreover, the reduction of perceived stress was independent of depression, which was not associated with fatty acid supplementation. The lack of an association between DHA supplementation and depression symptoms is consistent with the literature on perinatal depression. In several randomized controlled trials, DHA was not associated with depression scores,[36–37] but supplementation did appear to enhance the efficacy of psychopharmacologic treatment for depression,[38] suggesting that systems associated with depressed mood and affect neurotransmission, such as the HPA-axis, are impacted by fatty acid supplementation.

In fact, evidence from the present study suggests that DHA supplementation does affect the functioning of the HPA-axis by modulating response to a social stressor. This was demonstrated primarily via a lower cortisol response to arriving at the laboratory. By 30 weeks of gestation, participants who received DHA supplementation had lower levels of cortisol upon arrival to the laboratory, and on average demonstrated a slight increase in cortisol in response to the stressor, a pattern typically observed late in pregnancy.[29,33] In contrast, participants who had received placebo evidenced high levels of cortisol upon arrival to the laboratory, followed by a steep decrease in cortisol levels during and after exposure to the TSST. One interpretation of this pattern is that an exaggerated response to anticipatory stress leads to a less flexible response to the stressor. The high levels of cortisol upon arrival to the lab followed by a lack of responsiveness to the stressor has been described as a pattern of dysregulated HPA axis functioning associated with depression both in adults[39] and children.[40] Lack of cortisol response to a social stressor also has been observed among adults reporting high levels of early life adversity, especially females.[41] DHA supplementation appears to protect pregnant women living in low-income environments from manifesting this type of dysregulation.

Demonstrating associations between of fatty acid supplementation and lower prenatal stress, however, is not sufficient. The next step will be to test the hypothesis that alterations in maternal stress levels via fatty acid supplementation improves developmental outcomes in the offspring. Data from other studies support the testing of this hypothesis. For example, Bolten and colleagues[35] reported that cortisol reactivity to the TSST at 32–34 weeks of gestation, but not basal cortisol levels, was associated with infant self-regulation. Similarly, Werner and colleagues[42] found that infants of women with high levels of maternal cortisol 25 minutes after arrival to the lab during late gestation were more likely to be classified as "high reactive"(i.e. more motor activity and crying) in response to novel stimuli.

Thus, several important links among prenatal stress, cortisol levels, and infant neurodevelopment have been demonstrated. Data from our preliminary study are compelling in terms of the potential impact on health disparities in maternal and infant health, a significant public health problem that is poorly understood. The findings, however, are in need of replication. In addition, a number of limitations should be noted. First, the small sample size and attrition of participants over time likely impact the reliability of the findings, and thus replication and timing of effects need to be explored in a larger study population. Although retention across study visits was comparable for the two groups, there may have been some differential selection bias across the two groups. Second, given the preliminary nature of the study, we did not control for multiple testing, and of the 13 tests of significance, we could expect that one may be due to chance. The effect sizes for the significant results were medium to large, however, providing some indication that the results are not spurious. Third, we relied on maternal report of uptake as opposed to a rigorous assessment of blood levels of fatty acids across pregnancy. In addition to the possibility that uptake varied, blood levels may have varied among individuals with the same level of supplementation due to other dietary factorsor individual differences in metabolism. Fourth, although we did not re-administer the TSST after 30 weeks, it would be important to examine whether differences in cortisol levels as a function of supplementation are maintained. Moreover, in the context of improving infant outcomes, it may be important to supplement earlier in gestation. The third trimester is a period of fetal development that may be particularly sensitive to prenatal stress given the increase in maternal cortisol and decrease in placental 11β-HSD2, yet exposure earlier in gestation may also confer risk.[43]

In conclusion, the results reported here extend earlier work on the association between fatty acid supplementation and improved obstetric outcomes, and complement existing research on prenatal stress and offspring neurodevelopment by providing preliminary evidence for nutritional moderation of prenatal stress.

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Abbreviations

DHA	docosahexaenoic acid
TSST	Trier Social Stress Test
HPA-Axis	hypothalamic pituitary adrenal axis

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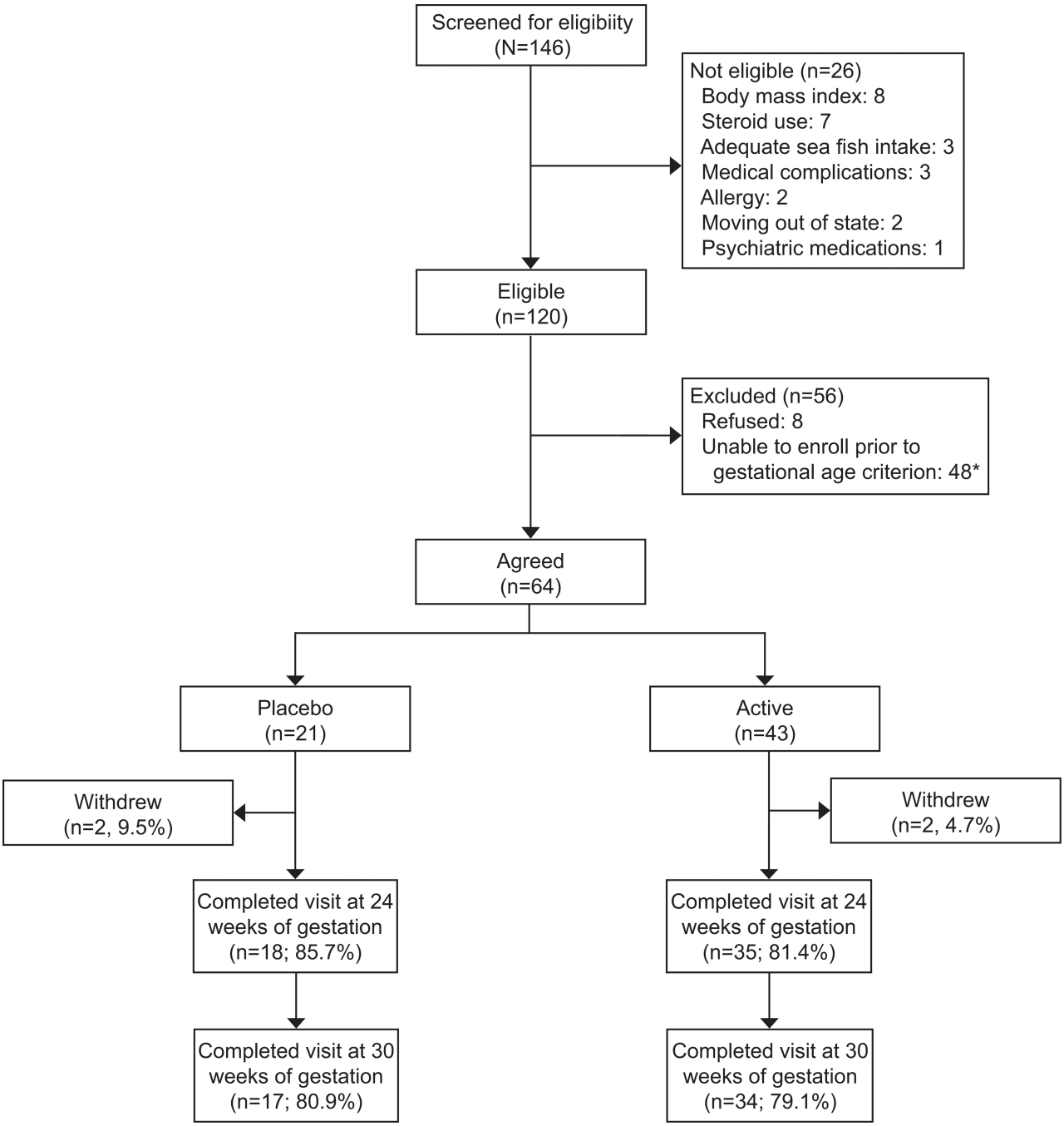

Figure 1.

Nutrition and Pregnancy Study participation rates. *Eligible at time of screening; but, unable to schedule baseline within gestational age criterion.

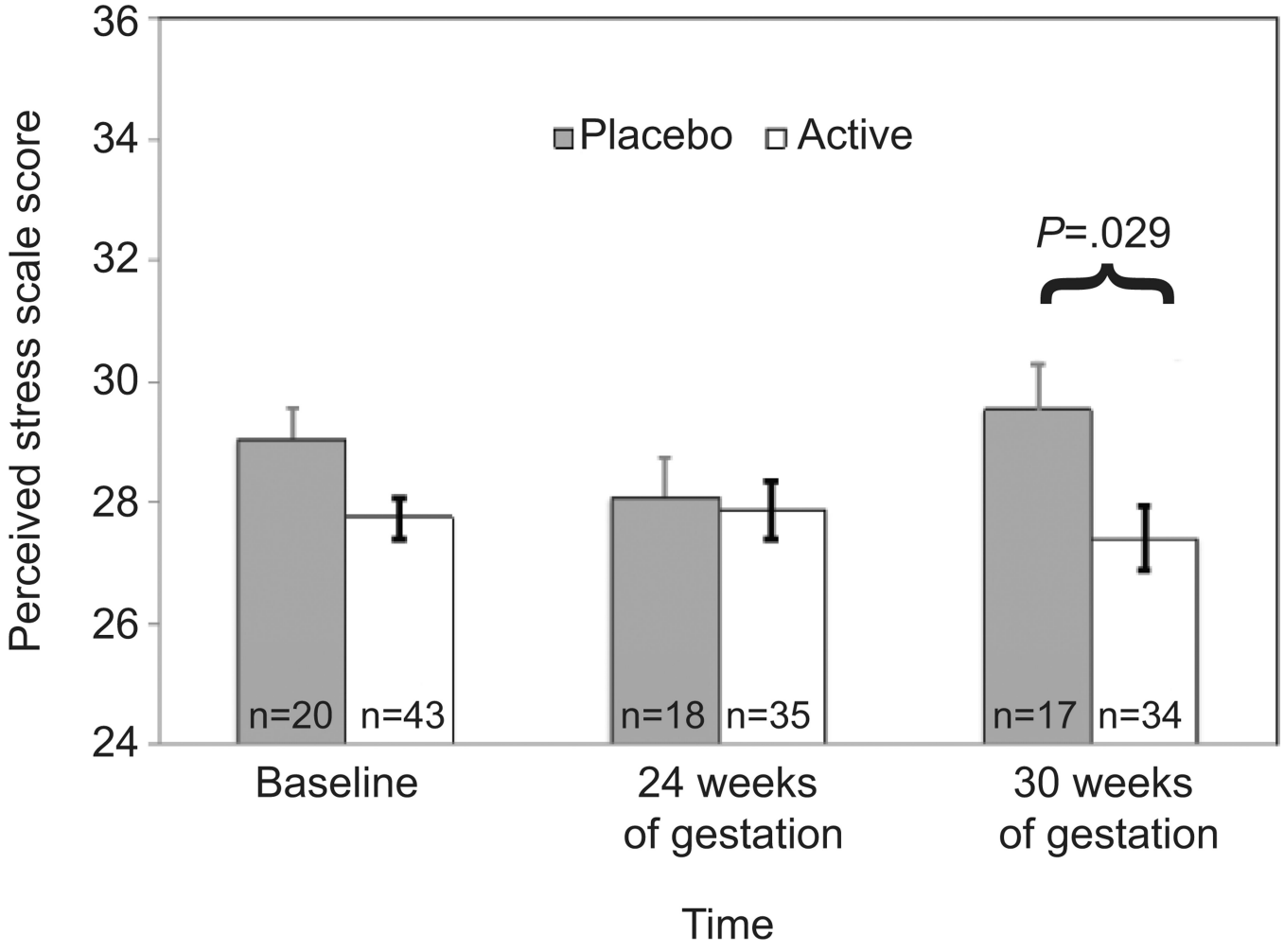

Figure 2.
Effect of omega-3 supplementation on perceived stress score, controlling for negative life events and depression scores.

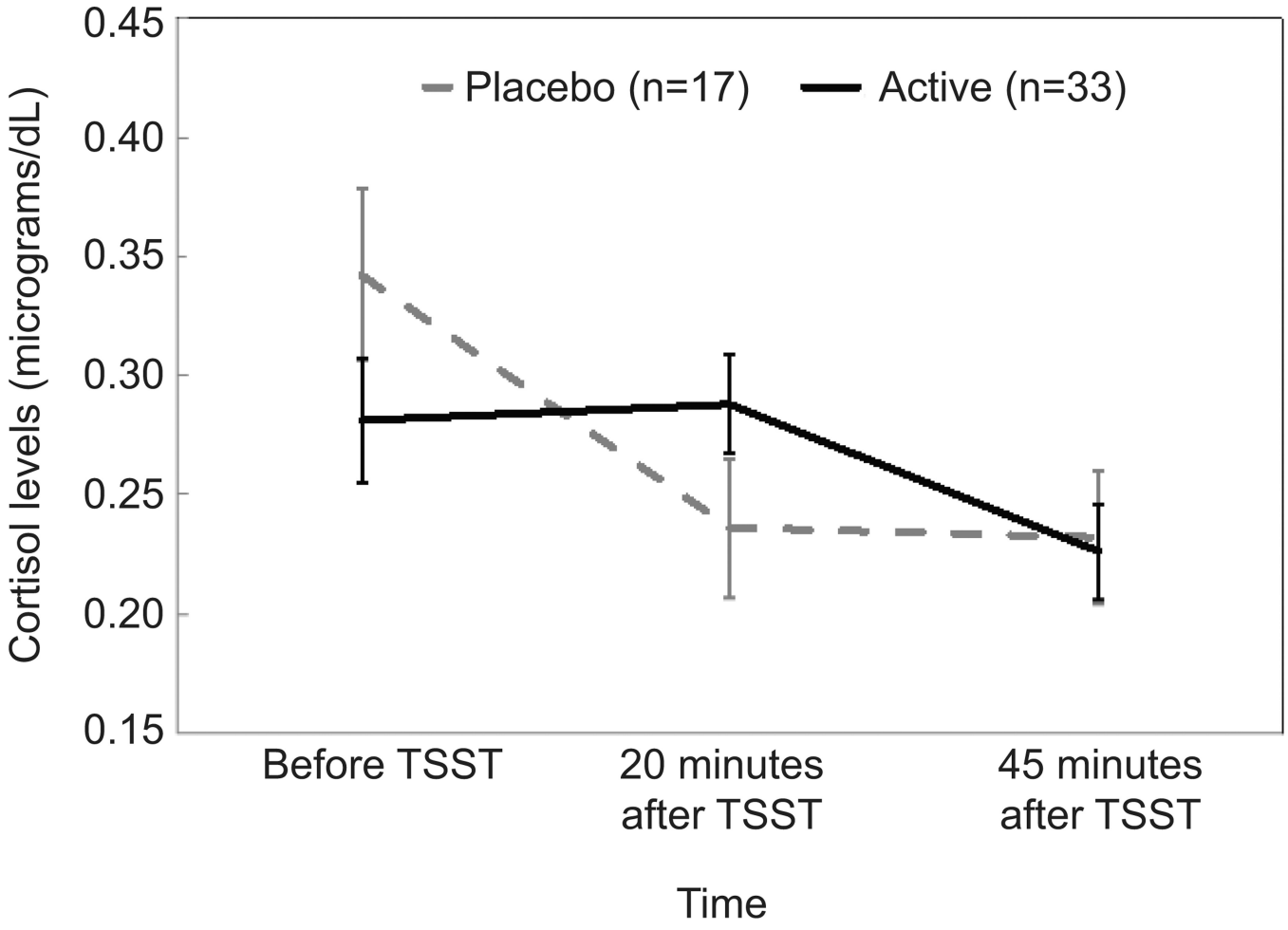

Figure 3.

Cortisol levels before and after the Trier Social Stress Test (TSST) at 30 weeks of gestation. $F(1.78, 83.85) = 6.24$, $P = .004$; *cohen's d* = .76; error bars indicate standard error at each time point within each group.

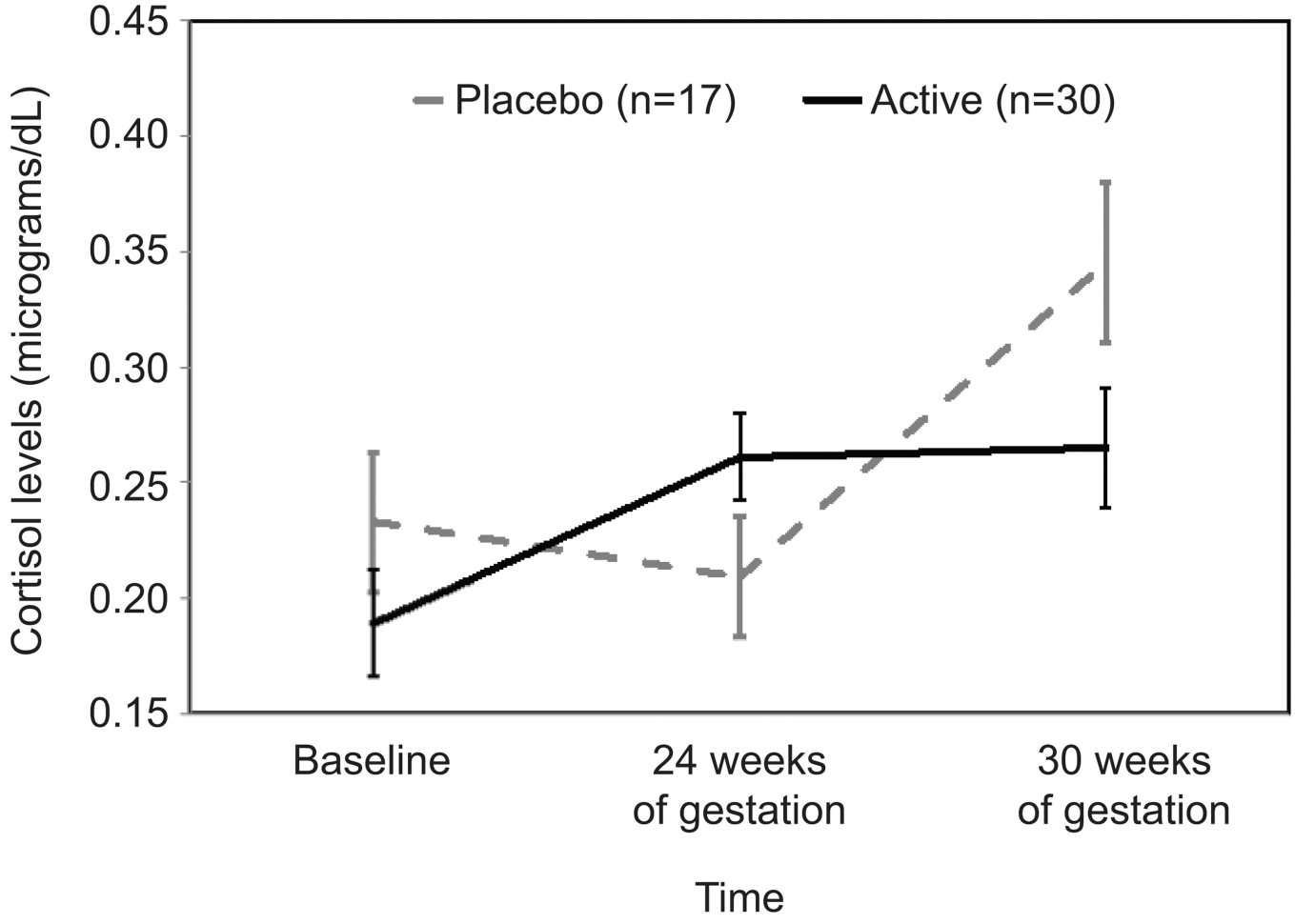

Figure 4.

Cortisol levels 20 minutes following arrival to laboratory at baseline, at 24 weeks of gestation, and at 30 weeks of gestation. $F(1.74, 74.63)$, $P=.041$; *cohen's d*=.56; error bars indicate standard error at each time point within each group.

Table 1

Descriptive statistics for depression, negative life events, and perceived stress scores at baseline, 24 weeks, and 30 weeks for total sample and the active and placebo groups

	Baseline			24 weeks			30 weeks		
	Total N = 63 Mean (SD)	**Active N = 20 Mean (SD)**	**Placebo N = 43 Mean (SD)**	**Total N = 53 Mean (SD)**	**Active N = 35 Mean (SD)**	**Placebo N = 18 Mean (SD)**	**Total N = 51 Mean (SD)**	**Active N = 34 Mean (SD)**	**Placebo N = 17 Mean (SD)**
Depression	12.97 (4.9)	13.05 (5.3)	12.80 (4.2)	12.92 (4.8)	13.3 (5.1)	12.11 (4.1)	11.86 (4.9)	12.12 (4.8)	11.35 (5.1)
Negative Events	5.03 (3.4)	5.16 (3.4)	4.75 (3.6)	4.08 (3.5)	4.57 (3.9)	3.11 (2.4)	3.80 (0.51)	3.91 (0.63)	3.59 (0.89)
Perceived stress	28.16 (2.4)	27.77 (2.2)	29.00 (2.7)	27.94 (3.0)	28.06 (3.5)	27.72 (2.0)	28.08 (3.4)	27.47 (3.4)	29.29 (3.1)